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Abbreviations and Acronyms

ACIAR — Australian Centre for International Agricultural Research
ACMV — African cassava mosaic virus
ACP-EC — African, Caribbean and Pacific-European Community
ADB — Asian Development Bank
ADF — acid detergent fiber
AGS — AVRDC *Glycine max* selection
a.i. — active ingredient
AMV — alfalfa mosaic virus
ANOVA — analysis of variance
ARC — AVRDC Asian Regional Center
ARP — AVRDC African Regional Program
ARTT — advanced soybean rust tolerance trials
ASET — AVRDC Soybean Evaluation Trials
ATER — area time equivalency ratio
AUDPC — Area under disease progress curve
Avg. — average
AVNET — Collaborative Vegetable Research Program for Southeast Asia
AVSET — AVRDC Vegetable Soybean Evaluation Trials
AYT — advanced yield trial
BAW — beet armyworm
BePMV — bell pepper mottle virus
BER — blossom end rot
BLM — black leaf mold
BMZ — German Ministry for Economic Cooperation
BP — bacterial pustule
Bt — *Bacillus thuringiensis*
BW — bacterial wilt
CAMV — cowpea aphid-borne mosaic
cfu — colony-forming units
CGIAR — Consultative Group on International Agricultural Research
CGR — crop growth rate
CHC — cabbage head caterpillar
CLS — cercospora leaf spot
cM — centimorgan
CMS — cytoplasmic male sterility
— cell membrane stability
CMV — cucumber mosaic virus
CONVERDS — Collaborative Network for Vegetable Research and Development in Southern Africa
CPA — para-chlorophenoxy acetic acid
CR — common region
CSIRO — Commonwealth Scientific Industrial Research Organization (Australia)
CT — cherry tomato
CTA — Technical Centre for Agricultural and Rural Co-operation (EC)
CTL — control threshold level
cv. — cultivar
CV — coefficient of variation
CVMV — chili veinal mottle virus
DAC — double antigen coating
DAE — days after emergence
DAI — days after inoculation
DAIS — District Agricultural Improvement Station (Taiwan)
DAS — days after sowing
— double antibody sandwich
DAT — days after transplanting
DBM — diamondback moth
diam — diameter
DM — downy mildew
DMRT — Duncan's multiple range test
DNA — deoxyribonucleic acid
DSR — disease severity ratings
DTF — days to flowering
DTM — days to maturity
DWB — dry weight basis
EC — electric conductivity
EDTA — ethylenediaminetetraacetic acid
EEC — European Economic Community
ELISA — enzyme-linked immunosorbent assay
EVT — elite variety trial
FLSD — Fisher's least significant difference
FMTT — fresh market tropical tomato
FMV — feathery mottle virus
GCLV — garlic common latent virus
GET — germplasm evaluation trials
GIS — Geographic Information System
GRSU — Genetic Resources and Seed Unit
GTZ — German Agency for Technical Cooperation (Germany)
GYT — general yield trial
HDM — high dry matter
HER — heading efficiency ratio
HPLC — high performance liquid chromatography
HS — heat-sensitive

HSI — head shape index
HSP — heat shock protein
HT — heat-tolerant
IARC — international agricultural research center
IBPGR — International Board for Plant Genetic Resources (CGIAR)
IBSRAM — International Board for Soil Research and Management (IARC)
IBWDN — International Bacterial Wilt Disease Nursery
ICARDA — International Center for Agricultural Research in the Dry Areas (CGIAR)
ICIMOD — International Centre for Integrated Mountain Development (IARC)
ICIPE — international Centre of Insect Physiology and Ecology (IARC)
ICLARM — International Center for Living Aquatic Resources Management (IARC)
ICMV — Indian cassava mosaic virus
ICRAF — International Council for Research in Agroforestry (IARC)
ICRISAT — International Crops Research Institute for the Semi-Arid Tropics (CGIAR)
ICW — imported cabbageworm
ID — internal diameter
IDRC — International Development Research Centre (Canada)
IEM — immunoelectron microscopy
IFDC — International Fertilizer Development Center (IARC)
IFPRI — International Food Policy Research Institute (CGIAR)
IIMI — International Irrigation Management Institute (IARC)
IITA — International Institute for Tropical Agriculture (CGIAR)
ILCA — International Livestock Centre for Africa (CGIAR)
ILRAD — International Laboratory for Research on Animal Diseases (CGIAR)
IMN — International Mungbean Nursery (AVRDC)
IMPMN — International Mungbean Powdery Mildew Nursery (AVRDC)
IMYMVN — International Mungbean Yellow Mosaic Virus Nursery (AVRDC)
INTHOPE — International Hot Pepper Trial Network (AVRDC)
IPM — integrated pest management
IRRI — International Rice Research Institute (CGIAR)
ISEM — immunosorbent electron microscopy
ISNAR — International Service for National Agricultural Research (CGIAR)
IYT — intermediate yield trial
LAD — leaf area duration
LAI — leaf area index
LAR — leaf area ratio
LI — light interception
LSD — least significant difference
LYSV — leek yellow stripe virus
MAb — monoclonal antibody
m asl — meters above sea level
MC — moisture content
MDS — multidimensional scaling
MPC — membrane protein complex
MYMV — mungbean yellow mosaic virus
NAR — net assimilation rate
NARS — national agricultural research systems
NC — nitrocellulose
NGO — Nongovernmental organization
NIRS — near-infrared reflectance spectroscopy
OP — open-pollinated
OPC — Office of Publications and Communications
OYDV — onion yellow dwarf virus
OYT — observational yield trial
PBNV — peanut bud necrosis virus
PCR — polymerase chain reaction
PDA — potato dextrose agar
PE — polyethylene
PeMV — pepper mottle virus
PLRV — potato leaf roll virus
PMMV — pepper mild mottle virus
POME — palm oil mill effluent
ppm — parts per million
PVE — potato virus E
PVMV — pepper veinal mottle virus
PVX — potato virus X
PVY — potato virus Y
PYT — preliminary yield trial
RAPD — random amplified polymorphic DNA
REDCAHOR — Collaborative Network for Vegetable Research and Development in Central America
RFLP — restriction fragment length polymorphism
RGR — relative growth rate
RH — relative humidity
ROC — Republic of China
ROI — return on investment
RTI — rust tolerance index
RYT — regional yield trial
SAI — stem area index
SAVERNET — South Asian Vegetable Research Network
SD — standard deviation

SDC — Swiss Development Council
SDI — selective dissemination of information
SEC — standard error of calibration
SED — standard error of deviation
SEP — standard error of prediction
SLV — shallot latent virus
SPFMV — sweet potato feathery mottle virus
SPLCV — sweet potato leaf curl virus
SPLV — sweet potato latent virus
SPV II — sweet potato virus II
SPYDV — sweet potato yellow dwarf virus
SRI — soybean rust infection
SSD — single seed descent
STI — stress tolerance index
TA — technology adaptaiion
TAV — tomato aspermy virus
TBSV — tomato bushy stunt virus
TEV — tobacco etch virus
TFW — tomato fruitworm
TLCV — tobacco leaf curl virus
TLCV Tai — Taiwan tomato leaf curl virus
TMGMV — tobacco mild green mosaic virus
TMV — tobacco mosaic virus
TOA — tomato oatmeal agar
ToMV — tomato mosaic virus
TSS — total soluble solids
TSWV — tomato spotted wilt virus
TTC — triphenyl tetrazolium chloride
TTLCV — Taiwan tomato leaf curl virus
TuMV — turnip mosaic virus
TVDF — Tropical Vegetable Data File (AVRDC)
TVMV — tobacco vein mosaic virus
TYLCV — tomato yellow leaf curl virus
TYTV — tomato yellow top virus
UNDP — United Nations Development Programme
UNICEF — United Nations International Children's Educational Fund
USAID — United States Agency for International Development
YVMV — yellow vein mosaic virus

Crop Improvement Program

Allium Breeding

Evaluation for Onion Yield Performance During Different Seasons

Summary

Ten germplasm evaluation trials (GET) involving more than 450 onion entries were conducted during winter, spring, and summer. Yield levels were high during the November to December plantings. Bulb yield of later plantings decreased gradually. Planting dates after February lead to drastic reduction in bulb yield. During summer, most of the lines did not survive. However, a few tropical summer stress-tolerant lines with fairly high bulb yield (25 t/ha) were identified.

Introduction

The main goal of the onion breeding program is to develop varieties suitable for tropical regions. The requirement for such varieties will be tolerance to biotic and abiotic stresses which are mainly responsible for low productivity in the tropics. The mechanism of these stresses at a single location situation can be understood by taking advantage of seasonal variations. Taking this into consideration, 10 evaluation trials were conducted on more than 500 entries collected from different parts of the world mainly to (1) identify the yield potential of various lines at different planting dates, and (2) to select promising entries for further testing.

Materials and Methods

A total of 453 entries were included in nine GETs conducted at seven different planting dates between November 1993 to July 1994 (table 1). Popular commercial varieties Granex 429 and Texas Early Grano 502 were used as checks in all these trials.

GETs were planted in a randomized complete block design (RCBD) with three replications. Each plot consisted of a 1.5-m-long and 0.6 m-wide bed. Spacing between two rows in a bed was 15 cm and 10 cm between plants in a row.

Results and Discussion

Bulb yield levels were high during the November planting dates (GET 1 to GET 3) with more than 50% of the lines yielding over 50 t/ha. Bulb yields of later plantings (GET 4 and GET 5) gradually decreased (<50 t/ha). The spring planting (GET 6) lead to drastic reduction in bulb yield (<15 t/ha). During the summer trials (GET 7 and GET 8) further reduction in bulb yield was observed (<11 t/ha). In the summer trial (GET 9) which included lines with possible heat stress tolerance, performance of some of the lines was very good with bulb yield up to 25 t/ha. These tropical summer stress-tolerant lines have been identified for use in the breeding program.

Table 1. Performance of onion lines for various yield categories during different planting dates

Trial	Date of transplanting	Total no. of lines evaluated	Percent of total lines with bulb yield >50 t/ha	40-50 t/ha	30-40 t/ha	<30 t/ha
GET-1	10 Nov.	73	58.9	10.9	9.6	20.6
GET-2	16 Nov.	32	53.1	18.8	15.6	12.5
GET-3	30 Nov.	25	64.0	12.0	16.0	8.0
GET-4	21 Dec.	66	7.5	24.2	22.7	45.6
GET-5	28 Dec.	40	0.0	32.5	45.0	22.5
GET-6	5 Feb.	22	0.0	0.0	0.0	100.0[b]
GET-7	25 June	96	0.0	0.0	0.0	100.0[c]
GET-8	6 July	36	0.0	0.0	0.0	100.0[d]
GET-9[a]	26 July	63	0.0	0.0	0.0	100.0[d]
						100.0[e]

[a] Also includes heat-tolerant lines
[b] Bulb yield <15 t/ha
[c] Bulb yield < 6 t/ha
[d] Bulb yield up to 11 t/ha
[e] Bulb yield up to 25 t/ha

Development of Tropical Summer Stress-tolerant Onion Lines

Summary

The prevailing summer stress situation in the tropics is one of the major factors responsible for low productivity in onion. Developing summer stress-tolerant lines can be a viable solution. About 200 onion lines, four *Allium fistulosum* accessions, and three F_1 hybrids between *A. cepa* and *A. fistulosum* were evaluated during the summer (temperature up to 33°C). Six onion lines were identified as having a high level of summer stress tolerance with bulb yield ranging from 15.0 to 25.9 t/ha. All the four *A. fistulosum* accessions as well as all the interspecific F_1 hybrids had high levels of summer stress tolerance.

Introduction

Tropical onions in general suffer from low productivity because of biotic and abiotic stresses prevailing in the tropics. Among the abiotic stresses, sensitivity to heat and flooding stresses at long daylength in the summer is the major factor. Development of onion lines with tolerance to tropical summer stress may possibly lead to the improvement of productivity in the tropics.

Materials and Methods

About 200 onion lines, including a few lines tentatively identified previously for summer stress tolerance, four *A. fistulosum* accessions, and three F_1 hybrids between *A. cepa* and *A. fistulosum* were evaluated during the summer (June-September) in an observational row trial. Observations were recorded for survival rate, plant growth, and bulb yield.

Results and Discussion

The majority of the onion lines had high sensitivity to summer stress leading to poor survival and growth. Plant survival rate varied from 0 to 91.4%. Some of the promising check varieties like Granex 429, Texas Early Grano 502, and Superex had extremely low survival rates; lines AC 443, AC 425, AC 444, TA 262, AC 47, and AC 325 had the best survival rates (>80%). Six onion lines, AC 21, AC 325, AC 412-1, AC 444, TA 267, and TA 270, were identified as having a high level of summer stress tolerance with bulb yield ranging from 15 to 25.9 t/ha (table 2).

Table 2. Promising tropical summer stress-tolerant onion lines identified from summer trials in 1994

Entry	Source	Yield (t/ha)	Bulb wt (g)	Survival rate (%)	Bulb color	Bulb shape
AC 425	Holland	10.5	39.2	82.2	White	Flat globe
AC 426	Holland	11.9	44.5	74.4	Red	Flat globe
AC 443	India	13.8	51.6	91.1	Red	Flat globe
AC 444	India	17.8	66.8	83.3	Red	Flat globe
TA 262	Nigeria	12.9	48.2	82.2	Red	Globe
TA 267	Israel	18.6	69.8	36.7	Red	Globe
TA 270	India	22.4	84.0	34.2	Red	Globe
TA 268	India	16.9	63.4	73.3	Red	Globe
AC 48	India	11.4	42.8	60.0	Red	Globe
AC 412-1	India	15.6	58.4	56.7	Red	Globe
AC 21	India	15.3	57.3	66.7	Red	Globe
AC 47	India	13.2	49.6	82.0	Red	Globe
AC 325	Holland	25.9	97.2	81.0	Yellow	High globe

All the four *A. fistulosum* accessions (TA 104, TA 106, TA 108, and TA 198) were summer stress-tolerant with excellent survival rate and plant growth. Similarly, the three F_1 hybrids, viz., TA 198 x AC 50, TA 198 x TA 70, and TA 108 x AC 50, were also highly tolerant to summer stress indicating the possible dominance of the gene for this trait. These summer stress-tolerant lines are under further investigation.

Breeding for Improved Onion Bulb Storage Quality

Summary

Storage studies with onion were conducted under ambient temperature (28-32°C) as well as under low temperature (10°C) conditions. Out of 130 onion lines evaluated for 5 months under ambient conditions, 10 lines had good bulb storage qualities with low storage losses (14-37%), compared to check varieties which had more than 60% losses. At low temperature storage, out of 43 lines evaluated for 4 months, two lines — AC 325 and AC 429 — were found highly promising with only 4% storage losses.

Introduction

Existing onion varieties, particularly those grown in the tropics, have poor bulb storage life leading to huge losses during storage. The need for developing onion lines with long bulb storage life has been given priority in AVRDC's onion improvement program.

Materials and Methods

Well-cured bulbs from 130 onion lines were stored under ambient conditions in a well-ventilated room in perforated plastic crates. Each entry was replicated three lines. The temperature during the 5-month storage period ranged from 28 to 32°C. Observations on sprouting, rotting, and weight loss were taken at monthly intervals.

At low temperature, storage bulbs of 43 lines were stored in perforated plastic crates for 4 months at 10°C. Monthly observations were taken for sprouting, rotting, and weight loss.

Results and Discussion

Ambient storage

Of 130 onion lines evaluated, 10 lines, viz., AC 141, TA 239, 358, 264, 266, 377, 378, 382, 386, and 387, had good bulb storage qualities (table 3). Total cumulative losses in these lines due to sprouting, rotting, and weight loss ranged from 14.0 to 37.5%. In comparison the commercial check varieties Granex 429 Superex and Texas Early Grano 502 had more than 60% losses. The best storage lines—TA 382 (14% losses) and TA 364 (19% losses)—also had high dry matter content indicating the positive correlation between these two traits. Sprouting losses in general were very low (0.0 to 1.5%), which may be due to the high temperatures prevailing during the storage.

Table 3. Performance of selected onion lines for bulb storage at ambient conditions[a]

Entry	Variety name	Source	Bulb yield (t/ha)	Rotting loss (%)	Sprouting loss (%)	Physiol. wt. loss (%)	Total loss (%)	Dry matter (%)	TSS (°Brix)	Pungency[b]
AC 141	Oignon Rouge	France	23.8	3.0	17.0	17.5	37.5	16.0	15.3	5.9
TA 239	Galil/Grandstand	Israel	31.4	20.2	1.3	10.8	32.3	6.6	6.6	6.6
TA 266	Ram 710 (H)	Israel	28.6	27.6	0.0	5.4	33.0	8.2	7.9	4.5
TA 358	PSX 2789 (H)	USA	36.1	16.9	1.5	7.6	26.0	6.8	7.3	4.8
TA 364	Creole Red	USA	23.4	4.8	1.5	13.0	19.3	12.2	10.7	4.7
TA 377	XP 8403 (OP)	USA	33.5	7.3	0.0	13.0	20.8	9.5	9.4	2.9
TA 378	XPH8407 (H)	USA	39.9	17.6	1.3	10.9	29.8	7.9	7.9	3.7
TA 382	Dehydrator no. 3	USA	24.7	1.0	1.4	11.6	14.0	13.1	11.7	5.7
TA 386	H-675 (H)	Israel	30.6	10.8	0.0	10.0	20.8	11.4	10.9	3.1
TA 387	H-817 (H)	Israel	36.5	18.7	0.0	13.3	32.0	7.9	8.4	3.5
Check	Granex 429	USA	42.3	52.6	3.2	18.7	74.5	6.7	6.7	4.4
Check	TEG 502	USA	33.8	35.0	2.2	26.3	63.5	6.4	6.5	5.0
Check	Superex	Japan	32.2	27.8	19.2	19.8	66.8	7.1	6.5	4.4
Mean				20.6	3.2	12.7	36.6			
CV				52.9	60.3	72.6	27.4			

[a] Temperature range: 28 - 32°C.

[b] Unit of pungency is micromole pyruvic acid per gram fresh weight

Low temperature storage

At low temperature storage (10°C), 43 onion lines were evaluated for 4 months (table 4). Sprouting losses were very high in most of these lines ranging from 18 to 100%. Two lines, AC 325 and AC 429, were identified as highly promising, as these had only 2.0% sprouting losses, combined with very low rotting and weight loss. After storing for 4 months these lines had only 4% total losses, compared to check variety Granex 429 which had 95.8% losses. These lines seem to maintain high dormancy even at low temperature conditions, which may be an interesting trait for breeding onions with long storage life.

Table 4. Promising onion lines identified for low temperature storage[a]

Entry	Bulb yield (t/ha)	Rotting loss (%)	Sprouting loss (%)	Physiol. wt loss (%)	Total loss (%)
AC 169	58.9	6.5	40.0	2.5	49.0
AC 325	70.0	0.0	2.0	2.0	4.0
AC 326	56.0	2.1	33.6	4.5	59.1
AC 429	62.0	0.0	0.0	3.0	3.0
AC 446	82.5	11.3	16.8	6.7	34.8
TA 211	55.7	0.5	43.0	6.5	50.0
TA 212	72.0	0.0	20.0	3.0	23.0
TA 236	68.8	0.0	18.0	5.0	23.0
TA 239	32.3	2.2	19.0	4.8	26.0
Granex 429 (ck)	71.9	0.0	88.2	7.6	95.8

[a] Storage duration: 4 months at 10 C

Breeding for Stemphylium Leaf Blight Resistance in Onion

Summary

Resistant sources have been identified in *A. fistulosum* accessions for stemphylium leaf blight resistance under naturally epiphytotic conditions in the field. Crosses have been made successfully between *A. fistulosum* and *A. cepa* lines to transfer the disease-resistant trait to onion lines. Initial observations indicate the possible dominant nature of genes for resistance.

Introduction

Among the prevalent diseases causing major damage to onion crops in the tropics, stemphylium leaf blight caused by *Stemphylium vesicarium* ranks at the top along with purple blotch disease caused by *Alternaria porri*. Very little work has been carried out so far on host-plant resistance for these diseases. Priority has been given to developing resistant onion lines for these diseases at AVRDC.

Materials and Methods

A. fistulosum lines (TA 104, TA 108, and TA 198) identified for resistance to stemphylium leaf blight during the previous year were crossed successfully with several onion genotypes. F_1 hybrids thus produced were evaluated for disease reaction under naturally epiphytotic conditions in the field. Studies were also conducted on the fertility behavior of these crossed plants for generation advancement.

Results and Discussion

Sources of resistance to stemphylium leaf blight identified by our pathologist in five accessions of *A. fistulosum* and some of their crosses with *A. cepa* lines were evaluated during the year. Out of 15 crosses thus made, three crosses, TA 198 x AC 50, TA 198 x TA 70, and TA 108 x AC 50, evaluated for disease reaction under natural epiphytotic condition in the field were found resistant in the initial observations, indicating the possible presence of a dominant gene for resistance. These hybrids had a high level of sterility leading to serious problems in seed-set. Meiotic studies indicated normal meiosis in most of the cases with few heteromorphic bivalents, occasional univalents, and lagging chromosomes. Variation was noticed for pollen fertility in different crosses ranging from 15 to 45%. In the plants having higher pollen fertility, occasional fruit-set was observed after selfing, with one or two seeds per fruit. A program is under way to identify crosses with better fertility to advance the generations for further evaluation and selection.

Onion Heterosis Breeding

Summary

Development of F_1 onion hybrids is one of the major activities in onion improvement at AVRDC. The prime requisite for exploiting heterosis in this crop is the availability of male sterility. The tropical germplasm line AC 26 with cytoplasmic male sterility identified during the last year was evaluated for its stability in different seasons at AVRDC and found highly stable for the male sterility trait. This trait will be transferred to different genetic backgrounds for use in heterosis breeding.

Introduction

Hybrids generally have yield and adaptability advantage over open-pollinated varieties. There are several hybrid varieties commercially cultivated in very large areas in temperate countries, but very few are available and are grown only in a limited area in tropical countries. One of the main reasons for the lack of F_1 hybrids in the tropics is that male sterility is not widely available and very limited research work, so far, has been conducted on heterosis breeding in the tropics. A stable male sterility system, preferably using tropical male sterility, is the basic requirement for developing F_1 hybrids suitable for the tropics.

Materials and Methods

The male-sterile line AC 26 was evaluated during winter, spring, and summer for the stability of the male sterility trait. Plants were grown from the bulbs, kept in the vernalization room, and planted at different dates to get flowering during different seasons of the year. Flowers were observed for pollen sterility using acetocarmine stain. The pollen grains which did not stain were considered sterile. Crosses were also made between 10 selected onion lines (AC 2, AC 9, AC 8, AC 47, AC 50, TA 4, TA 59, TA 69, TA 70, and AC 32) and the male-sterile line AC 26 to transfer the male sterility trait to different genetic backgrounds.

To develop inbred populations, selfing was carried out in 150 plants from 30 onion accessions for further evaluation.

Results and Discussion

The male-sterile line AC 26 was found highly stable for the male sterility trait as the pollen sterility was 100% in all the plants evaluated during winter, spring, and summer. Crosses were made between 10 different onion lines and the male-sterile line AC 26 to transfer male sterility to different genetic backgrounds. All the hybrids thus produced were evaluated and found male-sterile, indicating a possibly strong cytoplasmic control of male sterility or the widespread availability of recessive male sterility genes in homozygous condition with fertile cytoplasm (*R ms ms*) in all these lines. Backcrosses were also made in most of the lines to transfer male sterility to different genetic backgrounds for possible use in producing F_1 hybrids with high heterosis.

One hundred fifty lines were produced from 30 onion accessions. Selections were made for uniformity and bulb yield characters. Twenty-five such inbred lines were selected for further evaluation.

Development of Early Maturing Onion Lines and Lines with High Dry Matter

Summary

Twenty-seven onion lines have been identified for early bulb maturity (<100 days after transplanting). Of these, 10 lines were selected for high bulb yield and quality traits. To improve storage quality these lines are now being used in the crossing program.

Five lines with high dry matter (HDM) content ranging from 15.9 to 17.2% have been identified. Bulb yield in these HDM lines ranged from <8 to 23.8 t/ha. Two of the HDM lines, TA 363 and TA 382, had white bulbs and thus may be useful for the processing industry.

Introduction

Early maturing onion lines can easily fit in cropping systems, can escape major disease problems, and also help in setting up early markets. Similarly, lines with high dry matter may be useful in developing lines which can produce high dry matter per unit area. There is an established correlation between high dry matter and long storage life of bulbs.

Materials and Methods

More than 400 onion lines were evaluated for bulb maturity during winter. Bulb maturity was measured as the time it takes from transplanting to the harvesting of bulbs.

For dry matter evaluation, the bulbs were dehydrated after making uniform slices. TSS was determined by using a refractometer. Pungency was determined by using the pyruvic acid method.

Results and Discussion

Bulbs which matured in less than 100 days after transplanting were considered as early maturing lines. Of 400 lines evaluated during winter, 27 were identified as early maturing. Of these, 10 lines, TA 172, 175, 180, 183, 186, 188, 192, 201, 229, and 231, were selected for high bulb yield (>30 t/ha) and other quality characters (table 5).

Table 5. Performance of promising onion lines identified for earliness towards bulb maturity

Entry	Variety name	Source	Days to maturity[a]	Bulb yield (t/ha)	Bulb color
TA 172	ELAD (H)	Israel	95	34.7	Yellow
TA 175	Numex BR-1	USA	95	58.3	Yellow
TA 180	Rio Plata (H)	USA	88	56.2	White
TA 183	Rio Unico (H)	USA	88	40.7	Yellow
TA 186	Rio Rings (H)	USA	88	61.7	Yellow
TA 188	Rio Blanco Grande	USA	100	52.2	White
TA 192	Gladalan Brown	Australia	100	84.3	Yellow
TA 201	Baia Periforme	Brazil	100	41.3	Yellow
TA 229	Early Lockyer Brown	Australia	95	38.6	Yellow
TA 231	Early Lockyer White	Australia	85	36.7	White
Check	Granex 429	USA	110	71.9	Yellow

[a] Bulb maturity after transplanting

TA 192 had a bulb yield of 84.3 t/ha in 100 days. Other highly promising lines were AT 186 (61.7 t/ha), AT 175 (58.3 t/ha), AT 180 (56.2 t/ha), and AT 188 (52.2 t/ha). Since all these lines have poor storage quality, work is in progress to improve this trait.

In a separate experiment, 190 germplasm lines were evaluated for high dry matter, TSS, and pungency. Five lines, AC 141, TA 263, TA 264, TA 363, and TA 382, were selected for high dry matter content ranging from 15.9 to 17.2% (table 6). Compared to these, check varieties Granex 429 and Texas Early Grano 502 had only 6.7 and 6.4% dry matter, respectively. The line TA 363 had high dry matter (17.1%) and TSS (16.3%), but very low pungency compared to TA 382 which had a very high pungency of 9.4 m/g and high TSS (16.5%) and dry matter (16.5%). This line was also identified as having the longest bulb storage life. Bulb yield in these lines ranged from <8 (TA 263, TA 264) to 24.7 t/ha (TA 382).

Table 6. Performance of selected onion lines identified for high dry matter, TSS, and pungency

Entry	Variety name	Source	Dry matter (%)	TSS (°Brix)	Pungency[a]	Bulb yield (t/ha)	Bulb color
Ac 141	Oignon Rouge ∂	France	16.0 a	15.3 a	5.9	23.8	Dark red
TA 263	A.H.I.	Pakistan	16.5 a	13.3 a	-	[b]	Red
TA 264	Phulkara	Pakistan	15.9 b	12.5 ab	4.3	[b]	Red
TA 363	PSR 11390 (H)	USA	17.2 a	16.4 a	4.6	20.3	White
TA 382	Dehydrator no. 3	USA	16.5 a	11.8 ac	9.4	24.7	White
Check	Texas Early Grano 502	USA	6.4 c	6.5 c	5.0	58.7	Yellow
Check	Granex 429	USA	6.7 a	6.7 c	4.4	71.9	Yellow
Mean			12.8	11.3	5.1		
CV (%)			5.9	22.8	-		

[a] Unit of pungency is micromole pyruvic acid per gram fresh weight

[b] Bulb yield less than 8 t/ha

Garlic Germplasm Evaluation Trials (GET)

Summary

A total of 188 garlic entries were evaluated during the year for yield and quality traits in seven different trials (GET 1-GET 7). Bulb yield in these lines ranged from 1.5 to 14.6 t/ha. Fourteen lines have been identified for high bulb yield (>10 t/ha).

Introduction

Garlic germplasm evaluation remains a major activity to identify useful genotypes with high bulb yield and quality traits. Many of the accessions (188 lines) collected mainly from Taiwan were evaluated in seven replicated yield trials to identify high-yielding genotypes.

Materials and Methods

A total of 188 garlic entries were evaluated in seven different trials conducted during winter at different planting dates from September to November. The trials were planted in RCBD with three replications. Each plot consisted of a 1.5-m-long and 0.6-m-wide bed. Spacing between two rows in a bed was 15 cm and 10 cm between the plants in a row. Observations were taken during the crop growth stage and, finally, at harvest stage for bulb yield and quality traits.

Results and Discussion

Among different trials conducted, bulb yield ranged from 1.5 to 14.6 t/ha in different garlic lines. Fourteen lines were identified for high bulb yield (>10 t/ha), and of these, the six best lines were selected, GL 62 (17.3 t/ha), GL 59 (14.6 t/ha), GL 51 (13.9 t/ha), GL 50 (13.2 t/ha), GL 28 (13.2 t/ha), and GL 40 (12.2 t/ha) (table 7). GL 62 had the largest bulb (avg. bulb wt 115.2 g) followed by GL 59 (104.2 g), and GL 57 (104.0 g). These lines also had large cloves. Further evaluation of these lines will be carried out to identify superior lines for yield and quality traits.

Table 7. Performance of promising garlic lines identified for bulb yield

Variety	Yield (t/ha)	Avg. bulb wt (g)	Bulb diameter (mm) Equatorial	Polar	Neck
GL 28	13.2	65.6	54.6	43.0	8.7
GL 40	12.4	69.9	59.2	40.0	8.4
GL 50	13.2	61.4	57.5	41.1	8.2
GL 51	13.9	70.0	61.2	44.6	8.4
GL 57	8.8	104.0	70.2	46.4	10.9
GL 59	14.6	103.9	68.3	42.8	13.9
GL 62	17.3	115.2	72.3	43.2	8.9
Hsi-lo (ck)	14.1	52.8	60.0	42.2	9.9
FG 1 (ck)	13.9	78.7	61.3	48.9	8.7
Mean	5.1	65.0	58.1	41.9	9.2
CV	35.7	15.9	5.7	10.4	24.8

Garlic Clonal Selections

Summary

Clonal selections carried out in 125 individual bulbs obtained from 14 garlic accessions led to the identification of superior clones with high bulb yield and uniformity. Six clones were selected for high yield (>20 t/ha) and high bulb uniformity. The yield increase in these lines was about 80% more than their respective bulk checks.

Introduction

Garlic is propagated only by vegetative means because of its obligate apomixis nature, which makes conventional crossbreeding for varietal improvement almost impossible. Clonal selection is considered one of the best methods for varietal improvement in this crop by utilizing the available variability within the lines.

Materials and Methods

Ten bulbs were selected from 11 different accessions of garlic; similarly, five bulbs were selected from three other accessions. In all, 125 individual bulbs (clones) were selected from 14 accessions of garlic mainly collected from Taiwan. These selections were based on bulb size, weight, and clove size and number. Planting was done separately for all clones of a single bulb during the winter and the crop was observed during the growth period for variations in disease incidence and other characteristics. Finally, selections were made at harvest based on the yield and uniformity of bulbs. Selected clones were stored for multiplication.

Results and Discussion

Out of 125 individual bulbs (clones) selected from 14 accessions grown during winter, 10 clones were identified for their superiority in bulb yield and other major traits (table 8). The highest bulb yield (22.7 t/ha) was observed in the clone GL 76-7 showing a 95% increase over the bulk check of the same variety. This clone also exhibited the highest bulb weight (85.0 g), average clone weight (7.2 g), equatorial bulb diameter (62.2 mm), and polar bulb diameter (50.2 mm).

Table 8. Promising individual clonal selections in garlic

Entry	Bulb yield (t/ha)	Avg. bulb wt (g)	Avg. clove no./bulb	Avg. clove wt (g)	Bulb diameter (mm) Equatorial	Polar
GL 76-7	22.7	85.0	9.2	7.2	62.2	50.2
GL 68-1	21.3	80.0	7.9	6.8	61.8	46.4
GL 79-1	21.0	78.9	9.6	6.6	62.0	48.6
GL 68-2	20.7	77.8	9.9	5.2	60.4	47.6
GL 68-4	20.6	77.1	8.8	4.4	59.6	43.2
FG 1-9	20.3	76.0	12.6	5.4	59.8	40.2
GL 65-10	19.2	72.0	9.8	5.5	50.6	48.6
GL 79-2	18.0	67.5	7.5	6.0	58.6	48.2
GL 77-5	18.0	67.5	9.0	5.6	59.2	44.8
GL 77-4	17.5	65.7	9.0	5.7	61.2	43.6
GL 65 (ck)	10.3	56.0	-	-	55.1	44.8
GL 68 (ck)	11.6	54.7	-	-	55.1	42.5
GL 68 (ck)	11.6	66.7	-	-	59.3	45.9
GL 76 (ck)	11.6	66.7	-	-	59.3	45.9
GL 77 (ck)	9.4	56.0	-	-	56.3	41.1
GL 79 (ck)	10.7	63.0	-	-	57.7	44.0
FG 1 (ck)	11.5	70.3	-	-	58.5	43.2

Other high-yielding clones with more than 20 t/ha bulb yield were GL 68-1, GL 79-1, GL 68-2, GL 68-4, and FG 1-9. In these clones bulb yield increase was more than 80% compared to their respective bulk checks. Thus, clonal selection was found to be a very effective way of improving yield and quality in garlic.

Development of Long-storage Garlic Lines

Summary

Storage studies conducted separately for bulb and clonal selections at ambient condition (28-32°C) for 5 months lead to the identification of superior lines with very low storage losses. In the bulk selections, four lines (GL 66, GL 71, GL 73, and GL 74) with very low storage losses (4-6.4%) have been identified.

Introduction

Garlic storage losses in the tropics remains a major concern. At AVRDC, development of garlic lines with long storage bulbs is given major attention. Preliminary evaluation trials have lead to promising results.

Materials and Methods

Well-cured garlic bulbs from 53 bulk and 50 clonal selections were stored in hanging net bags in a well-ventilated room with three replications each. Evaluations were carried out at monthly intervals for losses due to sprouting, rotting, and weight loss. The temperature during the storage period (5 months) ranged from 28 to 32°C.

Results and Discussion

Out of 53 lines evaluated in the bulk selection group, four (GL 71, GL 66, GL 74, and GL 73) have been selected for their long storage qualities (table 9). The line GL 71 was highly promising with only 6.4% losses during the 5-month storage period. Other lines showing good promise were GL 66 (9.6% losses), GL 74 (10.7%), and GL 73 (11.4%). Storage losses in most of the other lines ranged from 19.7 to 65.0%.

In the clonal selection group, the total losses during the 5-month storage period ranged from 1.8 to 35.5%. The clone GL 77-7 was the best with the least storage losses (1.8%) and high bulb yield (16.9 t/ha). Other promising clones with very low storage losses were GL 67-7 (5.6%), GL 80-7 (5.4%), GL 80-6 (6.1%), and GL 79-3 (6.2%) (table 10). In these clones bulb yield ranged from 10.3 to 15.7 t/ha. Some of the high-yielding clones, GL 68-1 (21.3 t/ha), GL 68-2 (20.7 t/ha), and GL 76-7 (22.7 t/ha), had comparatively high storage losses ranging from 22 to 35%.

Table 9. Evaluation of garlic lines for storage quality[a]

Entry	Marketable bulbs (%)	Rotting loss (%)	Physiol. wt loss (%)	Total loss (%)	Bulb yield (t/ha)
GL 65	63.8 e-f	25.1 a	11.1 b-d	36.2 ab	10.3
GL 66	90.4 ab	5.6 ef	4.0 fg	9.6 ef	10.5
GL 67	61.3 g	19.0 a-c	19.7 a	38.7 a	8.4
GL 71	93.6 a	3.7 f	2.7 g	6.4 f	7.2
GL 73	88.6 ab	6.7 d-f	4.8 e-g	11.4 ef	9.1
GL 74	89.2 ab	4.3 f	6.4 d-g	10.7 ef	7.6
GL 76	65.3 d-f	23.2 ab	11.4 b-d	34.6 a-c	11.6
GL 77	80.3 bc	15.1 bc	4.6 e-g	19.7 de	9.5
GL 79	71.8 c-e	18.3 a-c	9.9 b-e	28.2 a-d	10.6
GL 80	77.4 c	13.1 c-e	9.4 b-f	22.6 d	11.5
FG 1	70.6 c-f	14.4 cd	15.0 ab	29.4 a-d	8.9
Hsi-lo	73.7 c-e	17.8 a-c	8.6 c-f	26.3 b-d	10.8
Ho-Mei	75.8 cd	11.3 c-f	12.9 bc	24.2 cd	8.0
Mean	77.1	13.7	9.3	22.9	9.6
CV	7.3	32.8	32.5	24.9	18.4

[a] Bulbs were stored for 5 months at ambient condition (28- 32 C)

Table 10. Results of storage studies in the clonal selection of garlic

Entry	Bulb yield (t/ha)	Total losses (%)[a]
GL 65-3	17.3	31.7
GL 65-9	15.4	22.5
GL 65-10	19.2	25.4
GL 67-6	13.3	12.2
GL 67-7	10.3	5.6
GL 68-1	21.3	33.3
GL 68-2	20.7	33.3
GL 68-4	20.6	35.5
GL 76-7	22.7	22.3
GL 76-9	14.5	15.1
GL 77-4	17.5	17.2
GL 77-5	18.0	19.7
GL 77-7	16.9	1.9
GL 78-6	13.7	12.8
GL 79-1	21.0	28.3
GL 79-2	18.0	22.9
GL 79-3	13.9	6.2
GL 80-6	15.7	6.1
GL 80-7	12.6	5.4
FG 1-9	20.3	18.7

[a] Total losses after 5-month storage at ambient temperature (28-32°C)

Garlic Flowering and Fertility Studies

Summary

During the year, 24 flowering garlic lines were collected and evaluated for flowering and fertility behavior. Of these, 10 lines were identified as promising for these traits. Variations were recorded for number of flower stalks, number of topsets and flower per umbel, pollen fertility, and percent fruit-set. Variations for pollen fertility and fruit-set were noted between lines as well as within lines. Plants with high pollen fertility and fruit-set were selected in GL 61, FG 2, GL 35, and GL 107.

Introduction

Garlic, because of sterility, is propagated using vegetative planting material. This has led to several production problems such as severe yield losses caused by viral diseases, including vulnerability to several other diseases carried by the vegetative planting material. These diseases create a serious bottleneck in the movement of germplasm because of quarantine regulations. Garlic sterility has also hampered genetic manipulation. All these factors necessitate the development of fertile garlic lines.

Materials and Methods

A total of 30 lines were evaluated for flowering and fertility behavior. The lines were planted in an observation row trial and observations were recorded for number of flowering stalks, and presence and number of topsets and flowers. Topsets were removed from all the umbel heads using fine forceps to allow flowers to develop. Meiotic studies were conducted using young flower buds, and pollen fertility was determined by using acetocarmine staining method. Hand-pollination was carried out to ensure pollination.

Results and Discussion

All the plants in all 30 lines produced flowers from February to March. Detailed studies related to flowering and fertility led to the identification of 10 promising lines (table 11). In all these lines there was normal flower development after the removal of topsets. Variations towards pollen fertility (0-34%) among the plants within the line were noted in all the lines. Plants were selected for the high level of pollen fertility. Hand-pollination resulted in the development of fruits in all these lines.

Table 11. Flowering and fertility traits in selected promising lines of garlic

	No. of flower stalks/ plant	No. of topsets/ umbel	No. of flowers/ umbel	Length of flower stalk (cm)	Pollen fertility (%)	Flower color	Fruit-set (%)
G 27	1	25 (15-40)	21 (13-28)	53.3	0-28	Purple	10.3
G 30	1	18 (15-32)	21 (13-32)	56.0	4-25	Purple	3.7
G 31	1	40 (30-55)	32 (25-40)	50.3	0-15	Purple	10.3
G 33	1	25 (15-30)	18 (15-30)	55.8	0-24	Purple	3.0
G 53	1	22 (15-33)	25 (18-38)	66.3	0-5	Purple	5.2
G 61	3	28 (20-40)	20 (11-32)	70.2	7-22	Purple	21.0
G 62	1	54 (35-62)	38 (20-50)	52.3	5-20	Purple	6.3
G 84	1	15 (10-35)	18 (15-20)	73.0	22-32	Purple	30.3
G 86	1	21 (12-35)	12 (10-15)	75.2	15-30	Purple	22.0
FG-2	1	38 (28-60)	30 (22-40)	55.1	0-34	Purple	25.2

Fruit development in all these lines was normal in the initial stage, and well developed growing embryos were observed inside the fruit. Fruit-set varied from 3.0 to 30.3% in different lines. However, fruit development was adversely affected at the later stage, which resulted in the premature drying of the fruit within 25 days, thus hampering seed development. Taking these observations into consideration, future programs have been planned to study the fertility behavior in all these lines in detail, possibly using embryo rescue techniques.

Breeding for True Seed Shallot

Summary

Sixteen lines collected from Taiwan, Indonesia, India, and Thailand had good flowering and seed-setting ability. Seeds of these lines were produced in net cages using houseflies as pollinators. Seeds produced from each line are being used for evaluation and selection for bulb yield and quality traits.

Introduction

Presently, shallot is cultivated by using vegetative planting material. Because of this the crop suffers from problems such as (1) viral and other diseases and insect pests carried by vegetative planting material, and (2) higher production cost. Consequently, problems are also encountered in the movement of germplasm. Taking these into consideration, priority has been given to the development of true seed shallot varieties at AVRDC.

Material and Methods

Shallot bulbs produced through seeds in 16 varieties were vernalized for about 6 weeks at 5-10°C and planted in October. Flowering started in 10 of these lines after 50 days, and all plants of each variety were covered with net cages. When the flowers were in full bloom, houseflies were released for effective pollination. Seeds were collected from 10 lines for further evaluation.

Results and Discussion

Out of 16 lines planted after vernalization treatment, 11 had good flowering, viz. S 24 (Local-Taiwan), S 31 (Local-Philippines), S 32 (Bima), S 34 (Somdeng Local), S 35 (Bilijo), S 38 (Kunning Tablet), S 39 (Kramat Tablet), S 40 (local-Yoga-Yakarta), S 43 (Local-Thailand), S 44 (Sisaket), and S 78 (Local-India). Seeds produced from these lines are being used for evaluation of bulb yield and quality traits during winter, and further selections will be made for bulb yield, quality, and seed production efficiency in these lines.

Allium Pathology

Virus Elimination and Indexing of Garlic and Shallot

Summary

AVRDC has adopted a virus elimination and indexing scheme that uses meristem tip (0.3 mm) culture for virus elimination. The meristem-derived plants are observed for two growth cycles and subject to three ELISA tests for onion yellow dwarf virus (OYDV), garlic common latent virus (GCLV), shallot latent virus (SLV), and leek yellow stripe virus (LYSV). The objective of this subproject is to improve the efficiency of this scheme while continuing routine virus elimination and indexing of all incoming garlic and shallot germplasm. Two hundred and four garlic and 19 shallot lines were subjected to the scheme in 1994. The basic scheme adopted in 1993 was slightly modified with an additional virus elimination step, i.e., growing the meristems on medium containing the antiviral compound, virazole. By doing so, virus infection in meristem-derived plants was reduced by 20% in the first ELISA test (E1) and by 74% in the second ELISA (E2) test.

We have also added three more antisera in the last ELISA test (E4) conducted after the meristem-derived plants have reached the second full-maturity cycle in the screenhouse: tobacco mosaic virus (TMV), miteborne filamentous virus (MbFV), and a general monoclonal antibody for many members of the potyvirus group. There has been no positive reaction with these so far.

Twenty-five virus-free garlic and six virus-free shallot line have been obtained so far by the virus elimination scheme. Another 40 garlic and 10 shallot lines have already finished the first growth cycle.

In a related study, two virus-free and field propagated lines of a local cultivar were grown and compared. The yield of one of the meristem-derived line was 76% higher than that of the field-propagated line. This indicates that meristem culture not only eliminates viruses but also increases yield considerably.

Introduction

Last year we introduced a virus elimination and indexing scheme that used meristem tip (0.3 mm) culture of 24-h phostoxin (for insect elimination) fumigated cloves and five ELISA tests of the meristem-derived plantlets at various times during plant development up to the second maturity cycle. Ten virus-free garlic and two virus-free shallot lines were obtained by this method by the end of 1993. Improvement of this method was thought necessary, particularly to further increase virus elimination and reduce the number of ELISA tests. The incorporation of virazole, an antiviral chemical in the tissue culture medium, was tested to increase virus elimination efficiency. Furthermore, a hot water treatment of bulbs was tried to prevent possible contamination of the tissue culture and growth room facilities with stem and bulb nematodes, which are often found between the outer scales of the bulbs. The effect of virus elimination on yield and virus reinfection was also investigated.

Materials and Methods

Routine virus elimination and virus indexing

A total of 204 garlic and 19 shallot lines were subjected to virus elimination/indexing in 1994 by a method slightly modified from the one described in the AVRDC 1993 Progress Report. Meristems of 0.3 mm were placed first on MS medium and 10 days later transferred to MS medium amended with 50 mg/l virazole (MSV medium). After 6 weeks on MSV medium, a 0.1- to 0.2-cm-long shoot was reexcised and placed on MS medium. Five indirect dot blot ELISA tests (E0, E1, E2, E3, E4) using antisera to four viruses, i.e., onion yellow dwarf virus, garlic common latent virus, shallot latent virus, and leek yellow stripe virus, were performed as previously described. One ELISA test (E0) was conducted prior to meristem excision to investigate the status of virus infection in nature. The fifth ELISA (E4) test, which was done at the end of the second full maturity cycle, was done with antisera to the four viruses mentioned earlier, and, in addition, with antisera to miteborne filamentous virus, tobacco mosaic virus and with a monoclonal antibody that detects most viruses of the potyvirus group.

Hot water treatment of garlic and shallot cloves

Three garlic lines (Taiwan local, China Pei Suan, China Tsang Suan) and two shallot lines (Taiwan local and Thailand local) were used. Twenty bulbs of each line were subjected to heat treatment which consisted of submerging the cloves in tap water for 20 h, and then placing them for 2 h in a waterbath where the temperature was set at 50 °C. After the treatment, meristems were excised and further processed by the method described earlier.

Yield comparison of field-derived and meristem-derived garlic lines

The yield of two meristem-derived lines of the local garlic cultivar Black Leaf was compared with that of the original field-propagated line of the same cultivar. Fifteen cloves per line were used in a nonreplicated observational trial in Ching Jing, a highland area in Taiwan. The cloves were planted on 17 May in a 20 °C insectproof growth room and transplanted to the field on 31 May in autoclaved AVRDC soil mixture (soil : compost : sand : rice husks = 3 : 1 : 1 : 1) and Known-You plant medium (2 : 1). This soil mixture was placed in a plastic-lined (1.40 x 0.75 x 0.30) hole, the bottom of which was covered with a 10-cm layer of autoclaved sand. Plant-to-plant distance was 12 cm and between-plant distance 15 cm. The distance between the lines was 20 cm. Both meristem-derived and field-propagated plants were covered with a net cage of 1.5 x 1.0 x 1.5 m and 40 mesh/cm^2. Indirect dot ELISA tests (for OYDV, SLV, GCLV, and LYSV) were conducted at 1, 2, and 3 months after planting. Bulbs were harvested 92 days after planting and yields were recorded.

Results and Discussion

Routine virus elimination

A detailed list of all shallot and garlic lines that have gone through and are currently undergoing the process is shown in tables 1a and 1b. We now have 25 virus-free garlic and six virus-free shallot lines. Another 40 garlic and 10 shallot lines have already finished the first growth cycle. Meristem survival for both garlic and shallot was high, with 92 and 98%, respectively. ELISA tests (E0) taken before meristem excision clearly show that virus infection in incoming materials is high in garlic, with 71% and somewhat lower (43%) in shallot. This clearly shows the necessity for implementing a virus elimination/indexing scheme that will prevent the introduction of new viruses or strains.

By subjecting these virus-infected cloves to meristem tip culture virus incidence could be reduced by approximately 90%. Thus, only 6.6% of the garlic and 4% of shallot plantlets grown from meristems of virus-infected cloves were still found to carry virus in ELISA 1.

Only the ELISA 1-negative plantlets were allowed to mature fully (first maturity cycle). When subjected to a second ELISA test (E2) at the end of the first maturity cycle, none of the shallot and only 3% of the garlic plants were found virus-infected. When cloves of these E3-negative plants were grown to the second maturity cycle, all plants were found free of virus. This indicates that our virus elimination and indexing procedure is effective in removing viruses.

Since we now routinely grow out the meristems in virazole containing Murashige and Skoog medium (MSV), the number of virus-positive plants could be further reduced (table 2). The use of virazole will also reduce the presence of viruses for which antisera are not yet available and for which, consequently, no serological tests could be performed.

Yield comparison of field and meristem-derived garlic

The field-propagated plants were 100% virus-infected, whereas the meristem-derived plants of the same line remained free of virus until harvest (table 3) when planted in the farmers' field under protective net cover. The yield of one of the meristem-derived lines was 11% lower and that of the other meristem-derived line 76% higher than that of the field-propagated line of the same cultivar. This shows that meristem culture of virus-infected lines not only eliminates virus, but also contributes to a substantial increase in yield. The lower yield of the M8 line is most likely due to the fact that cloves of this line were still quite small after the second growth cycle in pots in the greenhouse following meristem culture. This low yield of meristem-derived plants in the initial growth cycles in the field has also been observed in other crops such as sweet potato. However, yields were observed to increase in succeeding field plantings as such plants adjust to the field conditions.

Hot water treatment

The hot water treatment generally did not result in lower meristem survival in garlic. However, a reduction of 30% in meristem survival was noted in one of the two shallot cultivars tested. There was no notable change in virus infection in E0 and E1. Thus, this treatment can be used when needed only, i.e., when incoming bulbs are found infested with bulb nematodes.

Conclusion

The previously adopted virus elimination scheme is more effective with the incorporation of the antiviral compound virazole in the scheme. When meristem-derived plantlets were grown in the field, considerable yield increases were obtained in some lines. When such plantlets were protected by insect-proof netting, virus reinfection did not occur.

We will continue routine virus elimination and indexing of new accessions using this improved scheme. We also plan to improve the process by further characterization of new viruses and different serotypes of known viruses, and the development of antisera and/or probes with low specificity. Studies will also be continued on the long-term effect of meristem culture on yield and on how reinfection with viruses takes place.

Table 1a. Virus elimination and indexing of shallot as of 1994

Item[a]	Before meristem culture	After meristem culture 1[b]	2[c]	3[d]	4[e]
Thailand II					
Total no. of lines	1				
No. of meristems excised	9				
No. of meristems survived	9 (100%)				
No. of cloves tested	5				
No. of plantlets tested		8	5	5	5
No. of positive cloves	0				
No. of positive plantlets		0	0	0	0
Positive for OYDV+SLV	0	0	0	0	
OYDV+GCLV +LYSV+SLV	0	0	0	0	0
Poty					0
MbFV					0
TMV					0
No. of virus-free lines	1				
Thailand III					
Total no. of lines	3 (100%, 3 lines)				
No. of meristems excised	33				
No. of meristems survived	33 (100% 3 lines)				
No. of cloves tested	13 (3 lines)				
No. of plantlets tested		29 (3 lines)	15 (3 lines)	15 (3 lines)	
No. of positive cloves	0				
No. of positive plantlets		0	0	0	
Positive for OYDV+SLV	0	0	0	0	
OYDV+GCLV +LYSV+SLV	0	0	0	0	
Poty				0	
MbFV				0	
TMV				0	
No. of virus-free lines					
AVRDC Acc. I					
Total no. of lines	1				
No. of meristems excised	13				
No. of meristems survived	12 (92.3%)				
No. of cloves tested	6				
No. of plantlets tested		12	6		
No. of positive cloves	0				
No. of positive plantlets		1 (8.3%)	0		
Positive for OYDV+SLV	0	1 (100%)	0		
OYDV+GCLV +LYSV+SLV	0	0	0		
Poty					
MbFV					
TMV					
No. of virus-free lines					
AVRDC Acc. II					
Total no. of lines	3				
No. of meristems excised	31				
No. of meristems survived	31 (100% 3 lines)				
No. of cloves tested	11 (3 lines				
No. of plantlets tested		31 (3 lines)	16 (3 lines)	16 (3 lines)	
No. of positive cloves	0				
No. of positive plantlets		0	0	0	
Positive for OYDV+SLV	0	0	0	0	
OYDV+GCLV +LYSV+SLV	0	0	0	0	
Poty				0	
MbFV				0	
TMV				0	
No. of virus-free lines					

Table 1a. continued.

Item	Before meristem culture	After meristem culture 1[a]	2[b]	3[c]	4[d]
Indonesia I					
Total no. of lines	3				
No. of meristems excised	39				
No. of meristems survived	39 (100%, 3 lines)				
No. of cloves tested	17 (3 lines)				
No. of plantlets tested		35 (3 lines)	16 (3 lines)	12 (3 lines)	
No. of positive cloves	8 (47.1%, 3 lines)				
No. of positive plantlets		3 (8.6%, 1 line)	0	0	
Positive for OYDV+SLV	7 (87.5%, 3 lines)	3 (100%, 1 line)	0	0	
OYDV+GCLV +LYSU+SLV	1 (12.5%, 1 line)	0	0	0	
Poty				0	
MbFV				0	
TMV				0	
No. of virus-free lines					
Indonesia II					
Total no. of lines	5				
No. of meristems excised	69				
No. of meristems survived	67 (97.1%, 5 lines)				
No. of cloves tested	36 (5 lines)				
No. of plantlets tested	36 (5 lines)	27 (5 lines)	25(5 lines)		
No. of positive cloves	4 (66.7%, 5 lines)				
No. of positive plantlets		0	0	0	
Positive for OYDV+SLV	21 (87.5%, 4 lines)	0	0	0	
OYDV+GCLV +LYSV+SLV	15 (62.5%, 5 lines)	0	0	0	
Poty				0	
MbFV				0	
TMV				0	
No. of virus-free lines					

[a] Thailand I: Ja 92-246 (1 line); Thailand II: TA 169 (1 line); Thailand III: Burma, Sri Saket, Bang Chang (3 lines); AVRDC Acc. I: Philippines TA 297 (1 line); AVRDC Acc. II: Thailand (TA 316), Thailand (TA 329), Philippines TA 331; Indonesia I: Bima Brejes, Sumenep, Kuning (3 lines); Indonesia II: Sumenep, Bima, Philippines, Balijo, Kuning Tablet (6 lines)

[b] ELISA test conducted on meristem-derived plantlet (when leaf approximately 5 cm long) while still in the test tube

[c] ELISA test conducted on plants grown in soil for 2 months

[d] ELISA test conducted before harvest of plants grown in soil in insectproof screenhouse (approximately 0.5 month)

[e] ELISA test conducted on second generation of plant grown in soil in insectproof screenhouse (approximately 5 months)

[f] Dot ELISA on NCM membranes

Table 1b. Virus elimination and virus indexing of garlic as of 1994

Item	Before meristem culture	After meristem culture 1[a]	2[b]	3[c]	4[d]
Thailand I					
Total no. of lines	7				
No. of meristems excised	277				
No. of meristems survived	261 (94.2%)				
No. of cloves tested	262 (7 lines)				
No. of plantlets tested		159 (7 lines)	38 (7 lines)	38 (7 lines)	38 (7 lines)
No. of positive cloves	151 (57.6%, 7 lines)				
No. of positive plantlets		11 (6.9%, 5 lines)	0	0	0
Positive for OYDV+SLV	94 (62.3%, 6 lines)	2 (18.1%, 2 lines)	0	0	0
OYDV+GCLV +LYSV+SLV	130 (86.1%, 7 lines)	9 (81.8%, 3 lines)	0	0	0
SLV	0				
GCLV	0				
TMV	0				
No. of virus-free lines	7				
Thailand II					
Total no. of lines	1				
No. of meristems excised	20				
No. of meristems survived	20 (100%)				
No. of cloves tested	20				
No. of plantlets tested		19	3		
No. of positive cloves	8 (40%)				
No. of positive plantlets					
Positive for OYDV+SLV	3 (37.5%)	0	0		
OYDV+GCLV +LYSV+SLV	7 (87.5%)	0	0		
Poty					
MbFV					
TMV					
No. of virus-free lines					
Thailand III					
Total no. of lines	3				
No. of meristems excised	52				
No. of meristems survived	52 (100%)				
No. of cloves tested	72				
No. of plantlets tested		50 (3 lines)	9 (3 lines)		
No. of positive cloves	46 (63.9%, 3 lines)				
No. of positive plantlets		1 (2%, 1 line)	0		
Positive for OYDV+SLV	24 (52.2%, 1 line)	1 (100%)	0		
OYDV+GCLV +LYSV+SLV	23 (50%, 3 lines)	0	0		
Poty					
MbFV					
TMV			0		
No. of virus-free lines					
France					
Total no. of lines	10				
No. of meristems excised	34 (10 lines)				
No. of meristems survived	34 (100%, 10 lines)				
No. of cloves tested	32 (10 lines)				
No. of plantlets tested		24 (9 lines)	17 (8 lines)	16 (3 lines)	12 (8 lines)
No. of positive cloves	19 (59.4%, 8 lines)				
No. of positive plantlets		8 (33.3%, 4 lines)	0	0	0
Positive for OYDV+SLV	5 (26.3%, 4 lines)	7 (87.5%, 4 lines)	0	0	0
OYDV+GCLV +LYSV+SLV	19 (100%, 8 lines)	1 (12.5%, 1 line)	0	0	0
Poty				0	
MbFV				0	
TMV				0	
No. of virus-free lines	8				

Table 1b. continued.

Item	Before meristem culture	After meristem culture 1[a]	2[b]	3[c]	4[d]
Local Hua Suan					
Total no. of lines	1				
No. of meristems excised	15				
No. of meristems survived	15 (100%)				
No. of cloves tested	15				
No. of plantlets tested		14	11	10	6
No. of positive cloves	7 (46.7%)				
No. of positive plantlets		3 (21.4%)	0	0	0
Positive for OYDV+SLV	6 (85.7%)	3 (100%)	0	0	0
OYDV+GCLV +LYSV+SLV	7 (100%)	1 (33.3%)	0	0	0
Poty					0
MbFV					0
TMV					0
No. of leaves tested	15				
No. of positive leaf	15 (100%)				
Positive for OYDV+SLV	15 (100%)				
OYDV+GCLV +LYSV+SLV	15 (100%)				
No. of virus-free lines	1				
AVRDC Acc. I					
Total no. of lines	40				
No. of meristems excised	639 (40 lines)				
No. of meristems survived	563 (88.1%, 40 lines)				
No. of cloves tested	750 (40 lines)				
No. of plantlets tested		483 (40 lines)	182 (40 lines)	25 (5 lines)	
No. of positive cloves	466 (62.1%, 38 lines)				
No. of positive plantlets		5 (1.0%)	5 (3.8%, 6 lines)		0
Positive for OYDV+SLV	445 (95.5%, 38 lines)	5 (100%, 4 lines)	5 (71.4%, 4 lines)		0
OYDV+GCLV +LYSV+SLV	370 (79.4%, 35 lines)	0	2 (28.6%, 2 lines)		0
Poty					0
MbFV					0
TMV					0
EM (poty)			10 (7 lines)		
No. of virus-free lines					
AVRDC Acc. II					
Total no. of lines	11				
No. of meristems excised	161 (11 lines)				
No. of meristems survived	117 (72.7%, 11 lines)				
No. of cloves tested	174 (11 lines)				
No. of plantlets tested		114 (11 lines)	63 (11 lines)	10 (7 lines)	
No. of positive cloves	121 (69.5%, 11 lines)				
No. of positive plantlets		9 (7.9%, 5 lines)	5 (7.9%, 2 lines)	0	
Positive for OYDV+SLV	91 (75.2%, 11 lines)	8 (88.9%, 4 lines)	3 (60%, 2 lines)	0	
OYDV+GCLV +LYSV+SLV	97 (80.2%, 11 lines)	1 (11.1%, 1 line)	2 (40%, 1 line)	0	
Poty				0	
MbFV				0	
TMV				0	
No. of virus-free lines					

Table 1b. continued.

Item	Before meristem culture	After meristem culture 1[a]	2[b]	3[c]	4[d]
AVRDC Acc. III					
Total no. of lines	10				
No. of meristems excised	113 (10 lines)				
No. of meristems survived	112 (99.1%, 10 lines)				
No. of cloves tested	126 (10 lines)	109 (10 lines)			
No. of plantlets tested					
No. of positive cloves	52 (41.3%, 7 lines)				
No. of positive plantlets		0			
Positive for OYDV+SLV	50 (96.2%, 7 lines)	0			
OYDV+GCLV +LYSV+SLV	31 (59.6%, 6 lines)	0			
Poty					
MbFV					
TMV					
No. of virus-free lines					
Local (DAIS)					
Total no. of lines	31				
No. of meristems excised	478 (31 lines)				
No. of meristems survived	461 (96.4%, 31 lines)				
No. of cloves tested	501 (31 lines)				
No. of plantlets tested		403 (31 lines)	134 (31 lines)	11 (6 lines)	
No. of positive cloves	462 (92.2%, 31 lines)				
No. of positive plantlets		6 (1.5%, 5 lines)	6 (4.5%, 4 lines)	0	
Positive for OYDV+SLV	437 (94.6%, 31 lines)	6 (100%, 5 lines)	6 (100%, 4 lines)	0	
OYDV+GCLV +LYSV+SLV	399 (86.4%, 31 lines)	0	1 (1.7%, 1 line)	0	
Poty				0	
MbFV				0	
TMV				0	
No. of virus-free lines					
Indonesia I					
Total no. of lines	1				
No. of meristems excised	15				
No. of meristems survived	15 (100%)				
No. of cloves tested	23	15			
No. of plantlets tested					
No. of positive cloves	5 (21.7%)				
No. of positive plantlets		1			
Positive for OYDV+SLV	4 (80%)	1 (100%)			
OYDV+GCLV +LYSV+SLV	5 (100%)	0			
Poty					
MbFV					
TMV					
No. of virus-free lines					
Indonesia II					
Total no. of lines	9				
No. of meristems excised	130 (9 lines)				
No. of meristems survived	130 (100%, 9 lines)				
No. of cloves tested	168 (9 lines)				
No. of plantlets tested		126 (9 lines)			
No. of positive cloves	117 (69.6%)				
No. of positive plantlets		37 (29.4%, 7 lines)			
Positive for OYDV+SLV	112 (95.7%, 91 lines)	30 (81.1%, 7 lines)			
OYDV+GCLV +LYSV+SLV	56 (47.9%, 9 lines)	18 (48.6%, 6 lines)			
Poty					
MbFV					
TMV					
No. of virus-free lines					

Table 1b. continued.

Item	Before meristem culture	After meristem culture 1[a]	2[b]	3[c]	4[d]
Dominican Republic					
Total no. of lines	11				
No. of meristems excised	114 (11 lines)				
No. of meristems survived	111 (97.4%,11 lines)				
No. of cloves tested	114 (11 lines)				
No. of plantlets tested		57 (11 lines)			
No. of positive cloves	104 (91.2%, 11 lines)				
No. of positive plantlets		17 (29.8%, 8 lines)			
Positive for OYDV+SLV	103 (99.0%, 10 lines)	10 (58.8%, 5 lines)			
OYDV+GCLV +LYSV+SLV	102 (98.1%, 11 lines)	14 (82.4%, 7 lines)			
Poty					
MbFV					
TMV					
No. of virus-free lines					
Breeding I					
Total no. of lines	8				
No. of meristems excised	109 (8 lines)				
No. of meristems survived	109 (100%, 8 lines)				
No. of cloves tested	116 (100%, 8 lines)	82 (8 lines)			
No. of plantlets tested		82 (8 lines)			
No. of positive cloves	116 (100%, 8 lines)				
No. of positive plantlets		5 (6.1%, 3 lines)			
Positive for OYDV+SLV	116 (100%, 8 lines)	5 (100%, 3 lines)			
OYDV+GCLV +LYSV+SLV	115 (99.1%, 8 lines)	1 (20%, 1 line)			
Poty					
MbFV					
TMV					
No. of virus-free lines					
Breeding II					
Total no. of lines	4				
No. of meristems excised	53 (4 lines)				
No. of meristems survived	53 (100%, 4 lines)				
No. of cloves tested	53 (4 lines)				
No. of plantlets tested		21 (4 lines)			
No. of positive cloves	53 (100%, 4 lines)				
No. of positive plantlets		0			
Positive for OYDV+SLV	53 (100%, 4 lines)	0			
OYDV+GCLV +LYSV+SLV	53 (100%, 4 lines)	0			
Poty					
MbFV					
TMV					
No. of virus-free lines					

Table 1b. continued.

Item	Before meristem culture	After meristem culture 1[a]	2[b]	3[c]	4[d]
Breeding III					
Total no. of lines	17				
No. of meristems excised	238 (17 lines)				
No. of meristems survived	230 (96.6%, 17 lines)				
No. of cloves tested	273 (17 lines)				
No. of plantlets tested					
No. of positive cloves	271 (99.3%, 17 lines)				
No. of positive plantlets					
Positive for OYDV+SLV	238 (87.8%, 17 lines)				
OYDV+GCLV +LYSV+SLV	244 (90%, 17 lines)				
Poty					
MbFV					
TMV					
No. of virus-free lines					

[a] ELISA test conducted on meristem-derived plantlet (leaf approximately 5 cm long) while still in the test tube
[b] ELISA test conducted on plants grown in soil for 2 months
[c] ELISA test conducted before harvest of plants grown in soil in insectproof screenhouse (approximately 5 months)
[d] ELISA test conducted on second generation of plant grown in soil in insectproof screenhouse (approximately 0.5 month)
• Dot ELISA on NCM membranes

Thailand I:	7 lines	TOP 92-67, Ja 92-118, Ja 92-383, Ja 92-395, Ja 92-419, Ja 92-423
Thailand II:	1 line	TA 168 (Var. Sisaket)
Thailand III:	3 lines	Ratchaburi, cv. Chiang Mai, cv. Sri Saket
France:	9 lines	Niger TA 127, Gandiolais TA 138, Guinee 91 TA 142, Meneses TA 145, Mallorquat TA 149, Ti Vacoa TA 132, Jamaique TA 158, Senegal TA 159, Martinique TA 151
AVRDC Acc. I:	40 lines	Philippines TA 275, TA 276, TA 277, TA 278, TA 279, TA 280, TA 281, TA 282, TA 283, TA 284,TA 285, TA 286, TA 287, TA 288, TA 289, TA 290, TA 292, TA 293, TA 294, TA 295, TA 296,TA 298, TA 299, TA 300, TA 301, TA 302,TA 303, TA 304, TA 305, TA 306, TA 307, TA 308, TA 309, TA 310, TA 311, TA 312, TA 313, TA 314, TA 315
AVRDC Acc. II:	11 lines	Philippines TA 318, TA 319, TA 320, India TA 322, TA 323, TA 325, TA 326, TA 327, TA 328, Thailand TA 324
AVRDC Acc. III:	10 lines	Indonesia TA 336, TA 342, TA 345, TA 346, TA 347, TA 351, Thailand TA 324
Indonesia I:	1 line	Lumbu Putih
Indonesia II:	9 lines	Angkrik, Jatibarang (LEHRI)C, Lumbu Kuning (LEHRI), Bagor (LEHRI) Sanur (LEHRI), Layur (LEHRI)D, Ampenan Lumbu Hijau, Angkriks (small bulbs)
Breeding I:	8 lines	G 65, G 66, G 72, G 74, G 76, G 77, G 80
Breeding II:	4 lines	G 125 ~ G 128
Breeding III:	17 lines	G 129 ~ G 145
Dominican: Republic	11 lines	Katin-R, Katin-M, Hsueh-chia, Katio-B, Taiwan-B, Taiwan Top sets, Morado-M, Katin-F, Katin-2G, Morado-R, Morado Top sets

Table 2. Effect of virazole on virus contamination of garlic

ELISA	Treatment	No. plants tested	ELISA positive (%)
E1	No virazole	234	3.6
	Virazole	193	3.0
E2	No virazole	111	5.4
	Virazole	73	1.4

Table 3. Comparison of yield and virus reinfection in field-propagated[a] and meristem-derived plants of the cultivar Black Leaf

Line[b]	Total yield (g)[c]	Virus infection (%)[d]
$M_8GH_2F_1$	151.8 (11)	0
$M_{12}GH_2F_1$	246.2 (+ 45)	0
F (ck)	170.0	100

[a] All plants were protected by a 40 mesh/cm² net cage

[b] $M_8GH_2F_1$ = virus-free cloves originating from meristem no. 8, grown to two maturity cycles in the greenhouse

$M_{12}GH_2F_1$ = virus-free cloves originating from meristem no. 12

F = Farmers' field-propagated cloves (ck)

[c] Number in parentheses is % yield increase over the farmer's field-propagated line

[d] Measured by DAS ELISA at 1, 2, and 3 months after planting; antisera used for the ELISA tests: OYDV, GCLV, SLV, OYDV, TMV, MbFV

Evaluation of Allium Accessions for Resistance to Stemphylium Leaf Blight

Summary

A total of 565 allium accessions representing 28 *Allium* spp. were evaluated in the field at AVRDC with natural inoculum for their stemphylium leaf blight reactions. Eighty-one accessions were rated resistant. Among the resistant accessions were 13 *A. ampeloprasum*; 10 each of *A. cepa* and *A. fistulosum*; 9 *A. sativum*; 7 *A. schoenoprasum*; 4 each of *A. porrum*, *A. ramosum*, and *A. tuberosum*; 2 each of *A. angulosum*, *A. ledebourianum*, *A. pskemense*, and *A. senescens*; and 1 each of *A. albidum*, *A. ascalonicum*, *A. cernum*, *A. dictyoprasum*, *A. drobovii*, *A. lineare*, *A. oreoprasum*, *A. prostratum*, *A. pyrenaicum*; and 3 unidentified *Allium* spp. None of the accessions were completely free of stemphylium leaf blight symptoms.

Introduction

Stemphylium leaf blight, caused by *Stemphylium vesicarium* (Wallr.) F. Simmons, was found to be the predominant foliar disease of allium crops in Taiwan during a year-round survey in 1993. Spot surveys conducted during 1993 and 1994 suggest that it also may be the predominant disease of allium crops in other Asian countries. It occurs on most allium crops including onion, garlic, shallot, Welsh onion, and leek. Stemphylium leaf blight can cause severe losses and even complete crop failure when conditions are highly conducive for disease development unless it is managed by regular fungicide applications. To address this problem, AVRDC initiated a program to identify sources of resistance to stemphylium leaf blight among *Allium* spp. The objective of this study was to evaluate the stemphylium leaf blight responses of the accessions included in the allium germplasm evaluation field trials at AVRDC during spring 1994.

Materials and Methods

A total of 565 *Allium* spp. accessions representing 28 *Allium* spp. were grown during winter 1993 through spring 1994 in AVRDC fields and evaluated for their reactions to stemphylium leaf blight. The accessions included: 1 *A. albidum*, 5 *A. altaicum*, 15 *A. ampeloprasum*, 2 *A. angulosum*, 16 *A. ascalonicum*, 290 *A. cepa*, 1 *A. cernuum*, 2 *A. dictyoprasum*, 1 *A. drobovii*, 12 *A. fistulosum*, 1 *A. fuscoviolaceum*, 2 *A. ledebourianum*, 1 *A. lineare*, 1 *A. oreoprasum*, 1 *A. oschaninii*, 4 *A. porrum*, 1

A. prostratum, 1 *A. pseudoampeloprasum*, 2 *A. pseudocepa*, 3 *A. pskemense*, 1 *A. pyrenaicum*, 4 *A. ramosum*, 178 *A. sativum*, 7 *A. schoenoprasum*, 2 *A. senescens*, 4 *A. tuberosum*, 3 *A. vavilovii*, and 4 unidentified *Allium* sp. Young plants were transplanted during November and December in the fields where they were arranged in a randomized complete block design with 26 to 60 plants per replication and three replications of each entry. Disease development was from natural inoculum. Disease ratings were made from early March to mid-April by visual observation as the accessions approached maturity. Disease reactions were rated on a scale of 1 to 5 in which 1 = very resistant, no symptoms; 2 = resistant, < 5% of leaf area affected; 3 = moderately susceptible, 6-25% of leaf area affected; 4 = susceptible, 26-50% of leaf area affected; and 5 = very susceptible, > 51% leaf area affected.

Results and Discussion

Mean disease severity ratings among accessions within each *Allium* spp. are summarized in table 4. It is clear from these results that most accessions of *A. cepa* (onion and shallot) and *A. sativum* (garlic) are very susceptible to stemphylium leaf blight, although various levels of resistance do occur in a few accessions of both species.

Table 4. Field reactions[a] among *Allium* spp. to stemphylium leaf blight, spring 1994

	No. of accessions					
	VR	R	MR	S	VS	Total entries
A. albidum	0	1	0	0	0	1
A. altaicum	0	0	5	0	0	5
A. ampeloprasum (great-headed garlic)	0	13	1	0	1	15
A. angulosum	0	2	0	0	0	2
A. ascalonicum	0	1	2	6	7	16
A. cepa (onion)	0	10	22	49	209	290
A. cernuum	0	1	0	0	0	1
A. dictyoprasum	0	1	0	1	0	2
A. drobovii	0	1	0	0	0	1
A. fistulosum (Welsh onion)	0	10	1	1	0	12
A. fuscoviolaceum	0	0	1	0	0	1
A. ledebourianum	0	2	0	0	0	2
A. lineare	0	1	0	0	0	1
A. oreoprasum	0	1	0	0	0	1
A. oschaninii	0	0	0	1	0	1
A. porrum (leek)	0	4	0	0	0	4
A. prostratum	0	1	0	0	0	1
A. pseudoampeloprasum	0	0	1	0	0	1
A. pseudocepa	0	0	2	0	0	2
A. pskemense	0	2	1	0	0	3
A. pyrenaicum	0	1	0	0	0	1
A. ramosum	0	4	0	0	0	4
A. sativum (garlic)	0	9	17	31	121	178
A. schoenoprasum (chive)	0	7	0	0	0	7
A. senescens	0	2	0	0	0	2
A. tuberosum (Chinese chive)	0	4	0	0	0	4
A. vavilovii	0	0	0	3	0	3
Allium spp.	0	3	1	0	0	4
Total	0	81	54	92	338	565

[a] (VR) = No symptoms; (R) ≤5% leaf area affected; (MS) = 6-25% leaf area affected; (S) = 26-50% leaf area affected; and (VS) = ≥51% leaf area affected

A useful level of resistance was identified among accessions of other cultivated *Allium* spp. such as *A. ampeloprasum* (great-headed garlic), *A. fistulosum* (Welsh onion), *A. porrum* (leek), *A. schoenoprasum* (chives), and *A. tuberosum* (Chinese chives). Resistant sources were also observed in several other *Allium* spp., but only small numbers of accessions were available for testing.

Among the 565 accessions evaluated, 81 expressed good levels of resistance (table 5). These resistant accessions received mean severity ratings of 2 which places them in the resistant category with less than 5% of their leaf areas affected.

At this stage we are encouraged by the number of accessions that possess high levels of resistance to stemphylium leaf blight, and consider the outlook promising for the development of stemphylium leaf blight-resistant onion and garlic lines and cultivars. Our objective in 1995 is to develop a useful laboratory/greenhouse disease screening technique and to determine whether resistance screening can be done in the seedling stage.

Table 5. Allium accessions that possess resistance[a] to stemphylium leaf blight identified in the field, spring 1994

Allium species	AVRDC accession numbers
A. albidum	AC 180
A. ampeloprasum	AC 189, AC 190, AC 196, AC 214, AC 226, AC 234, AC 243, AP 123, AC 203, AC 209, AC 218, AC 231, AC 219
A. angulosum	AC 244, AC 245
A. ascalonicum	S 19
A. cepa	TA 69, TA 70, TA 357, TA 363, TA 364, TA 367, TA 370, AC 134, AC 324, AC 345
A. cernuum	AC 248
A. dictyoprasum	AC 249
A. drobovii	AC 251
A. fistulosum	AC 117, AC 439, TA 104, TA 105, TA 106, TA 108, TA 204, TA 209, TA 214, TA 218
A. ledebourianum	AC 255, AC 256
A. lineare	AC 257
A. oreoprasum	AC 259
A. porrum	AC 208, AC 120, TA 208, TA 222
A. prostratum	AC 261
A. pskemense	AC 253, AC 269
A. pyrenaicum	AC 270
A. ramosum	AC 118, AC 130, AC 271, AC 273
A. sativum	GL 60, GL 88, GL 89, GL 91, GL 99, GL 106, FG 1, FG 2, AC 274
A. schoenoprasum	AC 115, AC 277, AC 279, AC 283, AC 286, AC 289, AC 303
A. senescens	AC 296, AC 297
A. tuberosum	AC 116, AC 305, AC 306, AC 307
Allium sp.	AC 299, AC 300, AC 301

[a] **All accessions received a mean disease severity rating of 2, i.e., resistant with less than 5% of their leaf area affected**

Prevalence of Foliar Diseases Affecting Allium Crops at AVRDC

During November 1993 to October 1994, monthly plantings of garlic, onion, shallot, and Welsh onion were made at AVRDC and monitored for fungal diseases. The purpose of the study was to assess the relative importance of fungal foliar diseases of allium crops throughout the year. Diseased leaf samples, 100 or more, were collected at random at monthly intervals from 3-month-old plantings of each crop and examined microscopically for diagnostic spores. Among the 7,559 diseased leaves collected, 76% were infected by *Stemphylium vesicarium*, 9% by *Colletotrichum gloeosporioides*, 7% by *Alternaria porri*, and 6% by *Puccinia allii*. Stemphylium leaf blight, *S. vesicarium*, was present throughout the year, but it was most severe from November through June occurring on all the allium crops (fig. 1). Anthracnose, *C. gloeosporioides*, and purple blotch, *A. porri*, were most severe from June through September occurring on all the crops. Rust, *P. allii*, was present from February through June, but it occurred only on Welsh onion and garlic.

These results further illustrate the potential threat of stemphylium leaf blight to allium production in Taiwan. It occurs with a very high incidence throughout the major allium production period from November to March. Purple blotch and anthracnose occur mainly on the late planted crops that mature during the warmer and wetter season. The outbreak of rust on garlic and Welsh onion in 1994 was the first observed occurrence of this disease on allium crops at AVRDC in recent years.

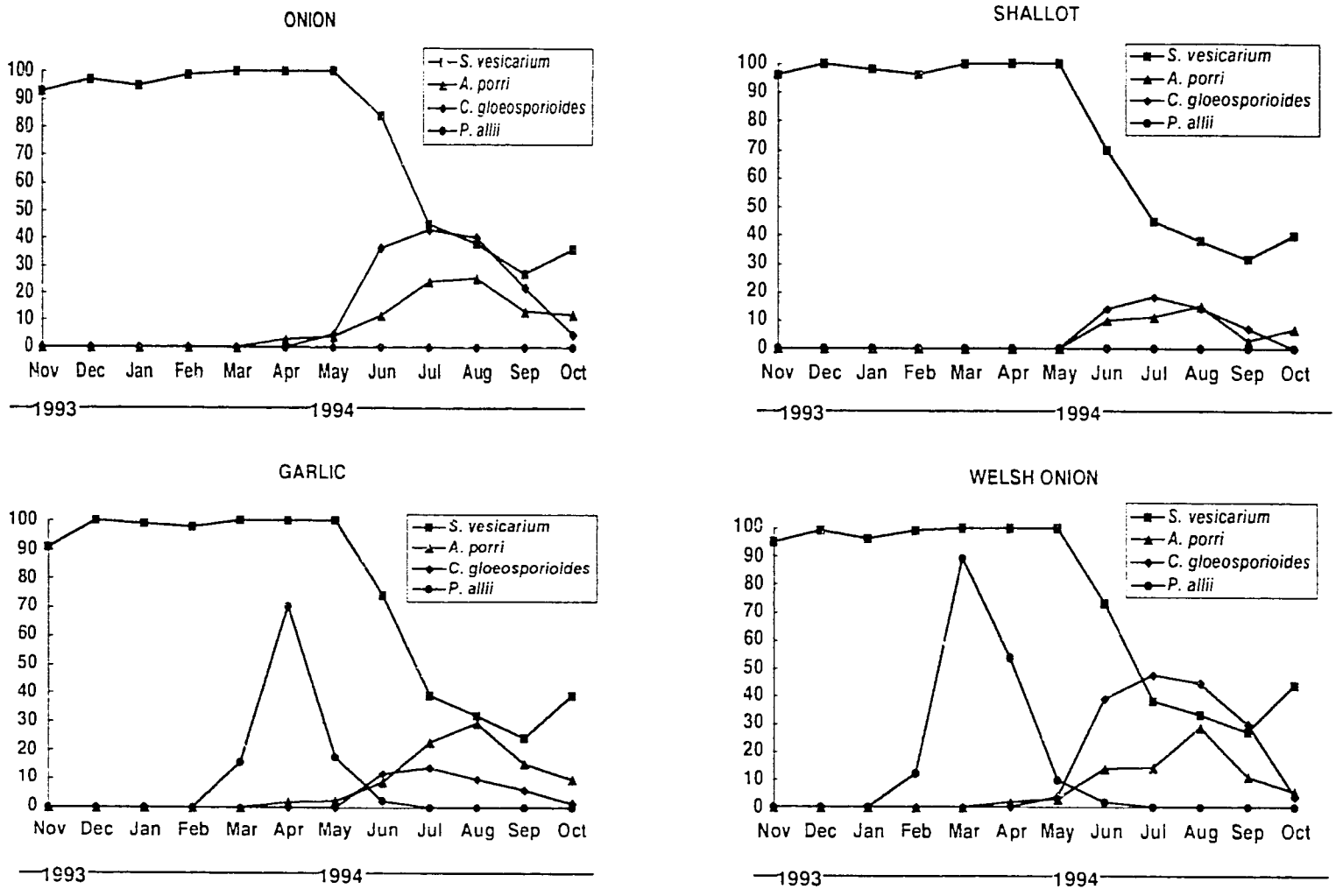


Fig. 1. Incidence of foliar fungal diseases on allium crops throughout the year on the AVRDC farm

Allium Physiology

Characterization of Garlic Clones Using Random Amplified Polymorphic DNAs

Random amplified polymorphic DNA (RAPD) analysis was applied to a garlic germplasm collection to assess the degree of variation within the collection and to investigate if this approach was suitable for clonal identification. Eighty-four clones from Argentina, Brazil, China, India, Indonesia, Philippines, Taiwan, and Thailand were evaluated using a set of 180 random 10-mer primers. Eighteen of the 180 primers revealed scorable polymorphisms among tested clones. Variations in banding profiles between clones were observed with almost all the 18 primers.

These profiles were assessed for use in systematic studies within the collection. Seventy-three band positions were scored (+/-) for all 84 clones studied. Cluster analysis was used to generate a dendrogram showing phylogenetic relationships between the clones. A multidimensional scaling (MDS) plot (fig. 1) indicated that these 84 clones were clustered into three distinct groups with very little overlap. The similarity levels among the three groups were 0.561 to 0.598, respectively. Group I clones have light-green leaves, and groups II and III dark-green leaves. The similarity levels within each group were higher than 0.780. This high similarity level within the same group indicates that clones of the same group were likely obtained from a narrow geographical area. Clones from Argentina and Philippines belong to group III. Those from Taiwan belong to groups I and II. Only three clones originated from Brazil, and clones from Thailand fall into group I. Clones from India and Indonesia belong to groups II and III. All flowering clones belong to group I.

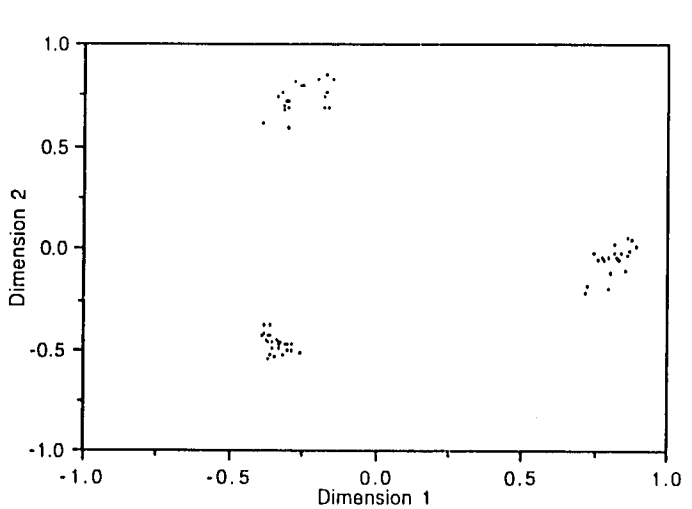


Fig. 1. Plot of first and second dimensions of multidimensional scaling analysis of genetic similarity matrix of 84 garlic clones (Individual dot stands for one clone)

Current theory suggests that there are three basic ecogeographic regions where garlic evolved as a semidomesticated plant. Since the historical movement of the tested clones in this study is hard to trace, it is not yet possible to tell whether the three clustered groups correspond to these three ecogeographical regions.

Based on RAPD data, some of our garlic collections are genetically similar. Therefore, results indicate that RAPD could be a useful tool for garlic germplasm management; it can eliminate potential duplicates in the collection. Studies on RAPD variations in: (1) plants derived from different bulbs of the same clone, (2) plants of selected and unselected bulbs of the same clone, and (3) plants of the same clone subjected to and not subjected to virus elimination, are still in progress.

Agrobacterium-mediated Transformation of Garlic

Summary

Plasmid pBI121 carrying NPTII and GUS marker genes were introduced into garlic via the *Agrobacterium*-mediated transformation method. Shoot tips and halved shoot tips of garlic, cv. Black Leaf, were excised and precultured for 2 weeks and 3 days, respectively, on modified B5 shooting medium. And thereafter, explants were inoculated with transformed *A. tumefaciens* on modified B5 media with 50 mg/l kanamycin. A number of regenerated shoots were obtained with the system. Polymerase chain reaction (PCR) and dot blot analyses of the regenerated shoots showed that the 3 kb GUS fragment was successfully integrated into the garlic. Root regeneration in transformed shoots is still being investigated, and stability of introduced foreign genes in transgenic garlic plants will be further examined.

Introduction

Obligate apomixis causes garlic to be propagated by asexual means and renders conventional cross breeding for varietal improvement impossible. Because of lack of cross breeding activities for garlic in the tropics and subtropics, unimproved clones are usually grown locally. At present, AVRDC breeders use clonal selection to improve garlic. In this study, we tried to establish the gene transformation system for garlic, which may serve as an alternative method of varietal improvement.

Materials and Methods

Cloves of sprouting and dormant bulbs of cv. Black Leaf were collected separately and their protective membranes removed. The peeled cloves were dipped in 70% ethanol and framed for 30 sec. After storage, leaves were carefully removed and 1- to 2-mm-long shoot tips and halved shoot tips with basal plate were dissected as explants for transformation.

Agrobacterium tumefaciens solution

Plasmid pBI121 carrying NPTII and GUS marker genes were transferred into *Agrobacterium tumefaciens* LBA4404 strain by freeze-thaw method. The concentration of *A. tumefaciens* for transformation was 2.4×10^8 to 4×10^8 cells/ml in MSO medium.

Transformation

Tow modified B5 media (i.e., B5 basal medium + 0.1 mg/l NAA + 0.5 mg/l 2ip + 2% sucrose, or B5 basal medium + 0.5 mg/l NAA + 2 mg/l 2ip + 3% sucrose) were used as shooting media. Explants were precultured in test tubes containing 10 ml of medium under 25°C and 12 h 3000 lux light intensity. Three days of preculture is optimum for explants of halved shoot tips, and 2 weeks for those of shoot tips. Explants were then immersed in *A. tumefaciens* solution for a few seconds, blotted dry with sterile filter paper, and cocultured on the shooting media for 0 to 14 days. Thereafter, explants were transferred to selection shooting media with 50 mg/l kanamycin and 500 mg/l carbenicillin. Kanamycin was used for the selection of the presence of NPTII gene, a kanamycin-resistant gene, and carbenicillin for growth inhibition of *A. tumefaciens*.

Dot blot analysis

Genomic DNA, extracted from regenerated shoots by a modified protocol, was dotted onto Amersham Hybond-N membrane, and UV crosslinked for 5 min at 254 nm wavelength. A 3 kb *Eco*RI/*Hin*dIII fragment of CaMV35S-GUS gene was labeled with DIG-dUTP and hybridizied onto the membrane. Signals were detected by exposing to X-ray film.

PCR reaction and Southern analysis

Polymerase chain reaction (PCR) was performed according to the manufacturer's instruction in 25 ml volume containing 0.66 unit *Taq* polymerase (Perkin Elmer Cetus) and 50 ng of plant DNA as template. For control PCR reaction mixture, 0.05 ng pBI121 DNA was added as template. Amplifications were performed in a Perkin Elmer Cetus DNA Thermal Cycler model 480 for 10 min at 94°C, followed by 30 cycles (1 min at 94°C, 1 min at 55°C, 2 min at 72°C), and further extension at 72°C for 7 min. PCR products were electrophoresed in 1.4% agarose gels run at 100 V in TBE buffer for approximately 3 h and blotted to Amersham Hybond-N membrane for Southern analysis. The pBI121 was digested with *Sst*I and *Sph*I and resulted in a 3 kb GUS and 1.76 kb NPTII fragments. Both fragments were labeled and used as probes for Southern analysis.

Results and Discussion

Explants of halved shoot tips from sprouting cloves expressed higher regeneration rate after inoculation: seven out of eight explants regenerated shoots on selection shooting media with 0.1 mg/l NAA + 0.5 mg/l 2ip + 2% sucrose, and four out of eight explants regenerated shoots on selection shooting media with 0.5 mg/l NAA + 2 mg/l 2ip + 3% sucrose. Shoots regenerated on the latter media were more vigorous. Explants of shoot tips from either dormant or sprouting cloves were able to regenerate shoots on selection shooting media with 0.1 mg/l NAA + 0.5 mg/l 2ip + 2% sucrose only; six out of nine explants from dormant cloves regenerated shoots, and 5 out of 11 explants from sprouting cloves regenerated shoots.

Transformation was determined by dot blot analysis. DNAs extracted from transformed shoots originated from different cloves were hybridized with a 3 kb GUS fragment probe. An alternative determination was made using PCR. The 785 bp NPTII and 1097 bp GUS genes were detected using genomic DNA from transformed shoots as template, and NPTII and GUS gene specific primers. These PCR products were able to hybridize with the NPTII and GUS fragments. The results confirmed that NPTII and GUS genes had been integrated into some regenerated shoots; however, root regeneration in transformed shoots is still being investigated. The transformation system could be an effective alternative method for garlic varietal improvement.

Environmental Effects on Growth, Development, and Yield of Bulb Alliums

The goal of bulb allium improvement is high yield of high quality produce. The attainment of this goal is the end result of many processes of growth and development as affected by various environmental factors. Research on fundamental principles and environmental factors as they affect yield of bulb alliums in the tropics and subtropics is scarce. In this study, various experiments were conducted to discern basic principles of growth, development, and yield formation in bulb alliums.

Monthly Planting

Two entries each of garlic (Black Leaf 1 and Black Leaf 2 from the local market) and onion (Granex 429 and Yellow Granex), and one entry of shallot (Light Red from the local market) were planted at the AVRDC experimental field from July 1992 to October 1993 for a total of 16 plantings. An RCBD with four replicates (1.5 x 2.5 m) was used for this experiment. Canopy light penetration and soil temperature were measured throughout the experimental period every 2 weeks. Three plants each were randomly sampled from each replicate at 60, 90, and 120 days after sowing (DAS) and at final harvest (about 150 DAS) and investigated for various growth parameters. Harvested bulbs from the final harvest, after curing and drying, were stored under ambient conditions to determine their storability. Detailed growth analyses are still in progress.

Preliminary results indicated that yields of garlic, onion, and shallot planted in September to December, September to January, and September to December, respectively, reached targeted levels of 10 t/ha (garlic), 25 t/ha (onion), and 15 t/ha (shallot). Yields of the tested entries planted in other months did not reach these targeted levels, mainly due to early bulb initiation resulting in arrested overall growth. Garlic bulbs harvested from the good yielding months could be stored longer than the target period of 5 months, whereas, none of the onion and shallot bulbs from the good yielding months could be stored for more than 3 months under ambient conditions.

Effects of Daylength

Bulb alliums develop bulbs in response to long daylength, and cultivars or clones grown in tropical regions require the ability to bulb under daylength less than 13.5 hours, the maximum natural daylength in the tropics. This study was done to investigate the daylength response of selected bulb alliums.

Twenty onion cultivars were tested for bulb development in three daylengths (10, 12, and 14 light h). None of the 20 onion cultivars developed bulbs at 10-h daylength 80 days after emergence. Bulb formation was promoted by 14-h daylength, in some cases resulting in leaf senescence (e.g., AC 326, AC 380-1, AC 384-2, AC 429, TA 215, TA 358, and Suprex). AC 36, AC 325, TA 232, TA 246, and Granex 429 developed bulbs without sacrificing overall growth under 12- and 14-h daylengths.

In a separate experiment, two clones each of local garlic (dark-green leaf and flowering types from the local market) and shallot (light red and red bulb types from the local market), and three onion cultivars (Granex 429, TA 188, and TA 207) were tested for bulbing response to different combinations of daylength, i.e., 40 days each of 10-10-10, 12-12-12, 14-14-14, 10-12-14, and 14-12-10 light h. Onions were extremely sensitive to constant 14 and 14-12-10 daylengths in terms of bulbing, resulting in minimum overall growth. Garlic and shallot had similar trends as onions but in a smaller extent. Higher onion and shallot yields were obtained with increasing daylength from 10 to 14 h. For all three bulb alliums, the seedling stage was receptive to daylength.

These results suggest that the timing of bulb initiation in relation to leaf development, and thus, the overall growth, is critical in determining yields in bulb alliums. Under the condition of progressively increasing daylength in September to December, test entries (i.e., so-called short-day bulb alliums) could produce sufficient leaf growth before bulb initiation to produce large bulbs.

Leaf Photosynthetic Capacity

Leaf photosynthetic capacity varies greatly among species under specific conditions optimum for each. An experiment was set up to investigate how varying irradiance levels affect single-leaf photosynthetic rates of two entries each of garlic (Black Leaf 1 and Black Leaf 2), onion (Granex 429 and Yellow Granex), and shallot (Light Red and Red). Measurements of the net photosynthetic rates for intact leaves were determined on a closed gas-exchange system. An infrared gas analyzer, LI-6200 Portable Photosynthesis System (LI-COR, Inc., Lincoln, NE, USA) was used for differential CO_2 measurements. Results show that maximum leaf photosyntheses were in the range of 7 to 10 CO_2 fixed $\mu M/m^2$ per sec, which are significantly lower than those of other AVRDC mandate crops. Light saturation (i.e., increasing light no longer increases photosynthesis) occurred in the range of 150 to 200 $\mu M/m^2$ per sec photosynthetic photon flux (PPF), which are about 1/10 of clear summer sunlight. Low photosynthetic capacity and light saturation may be responsible for the slow growth rate in bulb alliums.

Crucifer Breeding

Development of Heat-tolerant Chinese Cabbage Composite Populations

Summary

During the 1993-94 cool season, the composite was produced by randomly intercrossing within populations in an isolated screenhouse. Mass-pollinated seeds of B_5 composite populations were bulked in equal proportion. These bulked composite populations were grown in the field in 1994 hot season to evaluate their heat tolerance level. Differences among composite populations were not significant except that Composite III had a significantly higher head shape index. All composites outyielded the control variety, ASVEG No. 1, and had higher head weight and comparable solidity, indicating high levels of heat tolerance. The populations will be further selected for heat tolerance and composites to increase the frequency of heat tolerance genes.

Introduction

Since the main goal of tropicalizing Chinese cabbage with an economically reasonable yield level has already been attained, the Center decided to scale down the research activity to a maintenance level. Thus, the development of a few genetically diverse heat-tolerant composites was adopted as a general strategy. This strategy was designed to maximize the utilization of previously developed genetic materials and to preserve the materials for further research.

In 1993-94, we continued to work on the six composite populations which were developed earlier. These composite populations were evaluated for their general performance during the hot season in 1994. The feasibility of selection procedures to improve the populations was also investigated.

Materials and Methods

In midsummer 1993, selfed (S_1) families from the fourth composite cycle (B_3S_1/S1 and B_3PTS_1/S_1) were sown on 5 June and transplanted to the field on 5-7 July to observe heat tolerance performance. In the 1993 hot season, three bulked, bee-pollinated composite (B_5, B_3S_1/B_1, and B_3PTS_1/B_1) populations were compared to the standard heat-tolerant check, ASVEG No. 1. They were sown on 29 July and transplanted to the field on 24 August.

In late summer 1993, new populations were obtained from selected heat-tolerant plants of the B_3S_1 cycle (to be designated as cycle B_3S_2) and from selfed seeds (B_3PTS_1) of families with a high frequency of heat tolerance (to be designated as cycle B_3PTS_2) which means they are derived through a progeny

test using S_1 families) for observation and selection. Three new B_3S_2 populations were sown on 6 August and transplanted on 26 August. Each population consisted of 600 plants. Selfed seeds of B_3PTS_2 families were sown on 10 August and transplanted on 30 August. Composite I (Chang Puh type), Composite II (ASVEG No. 1 type), and Composite III (semitropical type) contained 26, 35, and 25 B_3PTS_2 populations, respectively. ASVEG No. 1 was used as the check. Each plot unit consisted of two 1.5-m-wide, 4-m-long, and double-row beds. The spacing used was 50 cm between rows and 40 cm between plants.

The procedures in developing composites included selection, flower induction, interbreeding in an isolated screen cage, bulking of seeds, and field evaluation. The progeny test and selection were also applied during the development cycle. The criteria for selection were: (1) frequency of heat-tolerant plants, (2) rate of bolting, (3) incidence of diseases (such as soft rot, downy mildew, Alternaria, and tipburn), and (4) other horticultural traits (e.g., vigor, maturity, and head shape). During the 1993-94 cool season, synchronization of flowering among the selected components was carried out in the three B_3S_2 composite populations and several families of three B_3PTS_2 groups. After selfing by hand, groups were randomly arranged in an isolated screen cage for mass pollination by honeybees. Seeds from each selection were harvested individually and composited in an equal proportion to create new base populations of the composite. These new base populations were evaluated for heat tolerance in the 1994 hot season.

In winter 1993, another three batches from mass-pollinated seeds of B_3 groups were sown in December. They were transferred to the vernalization chamber (5°C) for inducing flowers after germination. Seedlings were transplanted to clay pots after the treatment. Each group was moved to an isolated screen cage for mass pollination by honeybees. Seeds from each plant were collected individually and composited in an equal proportion to create new populations. These new base populations were also evaluated for heat tolerance in the 1994 hot season.

In the 1994 hot season trial, a randomized complete block design with three replications was employed. A unit plot consisted of two 4-m-long, double-row beds. Spacing was 150 cm from center to center of two adjacent beds, 50 cm between rows on the bed, and 40 cm between plants within the row. They were sown on 12 August and transplanted to the field on 31 August. The composite populations were evaluated for heat tolerance and other major horticultural traits.

Results and Discussion

Selections were chosen from the three B_3S_2 composites: 89 plants from Composite I, 86 from Composite II, 80 from Composite III. However, only 68 plants of Composite I, 51 of Composite II, and 40 of Composite III survived. Several selections were also made from three other B_3PTS_2 composites: 85 plants from Composite I, 89 from Composite II, and 75 from Composite III. Only 67 plants of Composite I, 46 of Composite II, and 56 of Composite III survived. The selections that survived were induced to flower synchronously. Seeds that either selfed or intercrossed were harvested separately from individual plants to produce new bases of populations.

In the 1994 hot season trial, differences in yields among the nine composites were not significant except for the long head conformation. Three Composite III selections appeared to have elongated heads with head-shape indices of 2.0-2.2. All composites outyielded the control variety, ASVEG No. 1, and had higher head weights and comparable solidity (table 1). Thus, a high level of heat tolerance frequency could be selected from the composites. Further selection and improvement of the heat tolerance of composites will be continued in 1995.

Table 1. Performance of composites[a] compared to the heat-tolerant check, ASVEG No. 1, at AVRDC, 1994[b]

Entry	Yield (t/ha)	Maturity (DAT)[c]	Harvest rate (%)	Head weight (kg)	Heading efficiency ratio	Head shape index	Solidity (g/cc)	Heading rate (%)	Soft rot (%)
Composite I (B_0)	37.6 ab	44 ab	89 bcd	1.3 abc	1.11 bc	1.5 cd	0.70 ab	100 a	12 abc
Composite I (B_1S_2/B_1)	38.1 ab	34 d	98 ab	1.2 c	1.13 b	1.6 cd	0.69 ab	100 a	2 cd
Composite I (B_1PTS_2/B_1)	36.4 ab	43 abc	88 cde	1.2 abc	0.99 c	1.7 bc	0.69 ab	100 a	9 a-d
Composite II (B_0)	39.3 ab	44 ab	89 bcd	1.4 abc	1.17 b	1.4 d	0.68 ab	100 a	11 abc
Composite II (B_1S_2/B_1)	43.2 a	41 c	92 abc	1.4 ab	1.16 b	1.5 cd	0.62 b	100 a	8 bcd
Composite II (B_1PTS_2/B_1)	33.4 bc	42 bc	83 cde	1.2 bc	1.11 bc	1.7 bc	0.69 ab	100 a	17 ab
Composite III (B_0)	38.4 ab	46 a	80 de	1.3 a	0.98 c	2.0 ab	0.77 ab	92 b	12 abc
Composite III (B_1S_2/B_1)	37.6 ab	46 a	78 e	1.4 a	1.03 bc	2.1 a	0.68 ab	97 a	20 a
Composite III (B_1PTS_2/B_1)	40.1 ab	45 a	85 cde	1.4 ab	1.13 b	2.2 a	0.81 a	99 a	14 ab
ASVEG No. 1 (ck)	28.9 c	33 d	100 a	0.87 d	1.4 a	1.5 cd	0.65 ab	100 a	0 d
CV (%)	9.8	3.3	6.0	9.3	6.5	9.7	13.9	2.2	54.4

[a] Mean separation within columns at 5% level (DMRT)
[b] Sown on 12 August and transplanted on 31 August 1994
[c] Days after transplanting

Development of Heat-tolerant Chinese Cabbage Populations with Long Head Shape

Summary

Selected families with the desired long head shape and dense head formation (indication of heat tolerance) were taken from four segregating populations. Most of the selected families were medium-early to medium-late maturing with compact, medium-long to long heads. Some F_6 families from an intercross population were medium maturing with very long heads which were the typical bamboo-type heads of B 61. Several S_1 families derived from the maternal line selection (MLS) of 77M(3)-27 were medium-early maturing with long and large heads. Selections with the longest head types were taken for intrafamily intercrossing with the aim of recapturing the head conformation of B 61 and the heat tolerance of the tropical parents in the subsequent generation.

Introduction

One of the major hindrances in popularizing AVRDC's tropical Chinese cabbage in some countries is its round to near-round head conformation. Consumers are apparently familiar with cultivars with an elongated head shape, particularly Wong Bok which is popular in the tropical highland production regions. A breeding project was initiated in 1985 to develop heat-tolerant varieties with cylindrical or elongated head shape to meet the consumer preference in the tropics. In 1994, several populations developed from various sources were planted for further selection.

Materials and Methods

Four batches of segregating populations selected in fall 1993 and screened against heat tolerance in summer 1994 season were planted for further selection. Batch 1 consisted of six F_6 families from intercrosses between promising and cylindrical-headed inbred lines of the backcross program. Batch 2 consisted of 12 F_5 families from triple crosses between promising, cylindrical-headed inbred lines with heat-tolerant inbred lines. Batch 3 included 40 S_1 families which were derivatives of the maternal line selections (MLS) from 77M(3)-27. Batch 4 included 22 S_1 families which were derived from MLS of 77M(3)-35. Forty individuals of each of the selected families were planted. In addition, 40 plants of ASVEG No. 1 were also planted in each batch as a general check for heat tolerance. The segregating families and the checks were planted to the field on 1.5-m beds. Unit plots consisted of double-row and 4-m-long beds. Spacing between rows within each bed was 50 cm and between plants within rows, 40 cm. Seeds were sown on 4 July and seedlings were transplanted to the field on 25 July. Selection of progenies emphasized the combination of compact head formation with elongated head shape.

Results and Discussion

Table 2 shows the general characteristics of the selected families from segregating populations possessing the combination of desired elongated and compact heads. The first two batches were related to promising cylindrical-headed inbred lines. The rest were selections from MLS of open-pollinated varieties. Most selected families tended to be medium-early to medium-late maturing with compact and medium-long to long heads. As expected, ASVEG No. 1 matured the earliest and had very compact round heads.

Table 2. Summary of selection for heat-tolerant families with elongated head shape from various segregating populations, AVRDC, summer 1994

Population	No. of families	General maturity[a]	Heat tolerance[b]	Head length[c]	Disease[b]
Batch 1	6	M–L	4 – 6	M–L	2 – 6
Batch 2	12	ME–L	4 – 8	M–ML	1 – 4
Batch 3	40	E–M	5 – 9	M–L	2 – 4
Batch 4	22	E–ML	4 – 7	M–ML	2 – 5
ASVEG No. 1 (ck)		VE	10	S	4

[a] E = early; ME = medium early; M= medium; ML = medium late; L = late; VE = very early
[b] Rating scale: 1 = low; 10 = high
[c] S = short; M = medium; ML = medium long; L = long

Some F_6 families of intercross population were medium maturing with very long heads, typical of the bamboo-type heads of B 61. These selections produced heads that were very tight on the bottom and middle parts, but partly loose at the top. Several S_1 families derived from MLS of 77M(3)-27 were medium-early maturing with long and large heads.

In general, the segregating materials deviated appreciably from the bamboo-type head conformation of B 61. Selections with the longest head types were taken for intrafamily intercrossing with the aim of recapturing the head conformation of B 61 and the heat tolerance of the tropical parents in the subsequent generation. These selections will be further tested and stabilized. Self-incompatibility of the selections will be examined during the selfing process to isolate potential inbreds for hybrid development.

Development of Heat-tolerant Common Cabbage

Summary

To develop heat-tolerant common cabbage lines/varieties, 99 varieties were grown in the field during the 1994 summer-wet season observation trial. The 77 headed varieties matured from 41 to 87 days after transplanting (DAT) with a mean of 68 days. Thirty-seven varieties (48% of the headed entries) had more than 90% heading rate, and only 10 entries had less than 50%. In contrast, only 19 varieties had harvest rates more than 90%, while 31 varieties had less than 50%. The distribution of head weight revealed that 56 varieties (73% of the headed entries) produced heads less than 500 g. The mean head weight was 432 g. The heaviest heads were obtained from KK Cross with an average head weight of 878 g. Among the 77 varieties, yields ranged from 0.4 to 21.1 t/ha with a mean of 7.4 t/ha. Again, the best yield of 21.1 t/ha was produced by KK Cross. The top five varieties were KK Cross, KS-Green, Delicious, Y-R Summer 50, and Shia Fong No. 1 (table 3). They yielded 18-21 t/ha, exhibited perfect heading and harvest rates, and were free from soft rot infection.

Eleven selected varieties were planted in a replicated elite variety trial in summer 1994. Significant differences in yield and some horticultural characters were observed among the varieties. The mean yield was 10.8 t/ha, while the average head weight was 542 g. Five varieties: Choon Chiou, Liang Guan, KK Cross, Y-R Summer 50, and Shia Chong, produced yields between 11.5 and 14.5 t/ha which were comparable to the yields of control varieties, Shia Fong No. 1 and KY Cross. In addition, an extraordinarily early maturing variety, Golden Cross 40, appeared to have potential value for use in tropical cabbage breeding. It had small compact heads, high heading ability, and is free from soft rot infection.

Introduction

Heat-tolerant common cabbage hybrids have been developed and released to cabbage growers in the tropics by several seed companies. However, seeds of these hybrids are inordinately priced. The quality of the seeds also tends to be lower than that grown in high elevations or during the cool season in the lowland. National programs clamor for less expensive seeds of tropical varieties. The development of heat-tolerant common cabbage for the tropics is a goal of the center's crucifer improvement program.

Materials and Methods

Observation trial

In the hot-wet monsoon season of 1994, 99 accessions were grown under hot and humid conditions to observe their heat tolerance, disease resistance, and general performance. They were collected from Taiwan, China, and commercial seed companies worldwide. These accessions were sown on 11 May and transplanted to the field on 14 June. Seedlings were nursed in polyethylene pots under screen net tunnels. Each unit plot consisted of one 5-m-long, double-row bed. The spacing was 150 cm between beds, 50 cm between rows, and 50 cm between plants within the row. There were 20 plants in each plot without replications. Beds were mulched with rice straw on the top to reduce weeds and disease infection by continuous heavy rains.

Elite variety trial

Eleven selected varieties were planted in a replicated elite variety trial in summer 1994 to evaluate their heat tolerance, disease resistance, and general performance. They were Shia Chong and Choon Chiou, F_1 hybrids from Noongsheng Seed Company of Taiwan; Liang Guan cabbage from China; and six F_1 hybrids from Takii Seed Co. of Japan: KK Cross, Golden Cross 40, Y-R Summer 50, Southern Treasure, Tight Globe, and Green Stone. These varieties were sown on 11 May along with two F_1 hybrids from Japan, namely, Shia Fong No. 1, and KY Cross, as checks. Seedlings were nursed in polyethylene pots under screen net tunnels. They were transplanted to the field on 14 June. A RCBD with two replications was used. Each unit plot consisted of 20 plants on a 5-m-long, double-row bed. The spacing was 150 cm between beds, 50 cm between rows, and 50 cm between plants within the row. Beds were mulched with rice straw on the top.

Results and Discussion

The 1994 summer trials were severely damaged by continuous heavy rains. There was a total of 1424 mm precipitation accumulated from 31 July till 16 August. Excess water retarded growth and promoted soft rot infection during the heading stage.

Observation trial

Among the 99 collections, 22 varieties did not head. The 77 headed varieties matured from 41 to 87 days after transplanting with a mean of 68 days (fig. 1). Thirty-seven varieties (48% of the total entries) had more than 90% heading rate, and only 10 entries had less than 50%. In contrast, 19 varieties had harvest rates more than 90%, while 31 varieties had less than 50%. Some varieties have high heading rate but low harvest rate due mainly to soft rot infection. Majority of the entries had less than 30% soft rot infection.

The distribution of head weight revealed that 73% of the entries produced heads less than 500 g. The mean head weight was 432 g. The heaviest heads were obtained from KK Cross, a heat-tolerant variety, with an average head weight of 878 g. In general, most of the varieties produced relatively small heads indicating a low level of heat tolerance.

Among the 77 varieties, yields ranged from 0.4 to 21.1 t/ha with a mean of 7.4 t/ha. Again, the best yield of 21.1 t/ha was produced by KK Cross. The top five varieties were KK Cross, KS-Green, Delicious, Y-R Summer 50, and Shia Fong No. 1 (table 3). They yielded 18-21 t/ha, exhibited perfect heading and harvest rates, and showed no soft rot infection.

Elite variety trial

Significant differences in yield and some horticultural characters were observed among the varieties. The yields of this trial were relatively low compared to those of the observation trial. The mean yield was 10.8 t/ha, while the average head weight was 542 g. Five varieties, Choon Chiou, Liang Guan, KK Cross, Y-R Summer 50, and Shia Chong, produced yields between 11.5 and 14.5 t/ha which were comparable to the yields of Shia Fong No. 1 and KY Cross (table 4). Apparently the low yields of the entries were attributed to small or loose heads and/or soft rot infection. An extremely early variety, Golden Cross 40, matured in 41 days after transplanting. Although Golden Cross 40 produced small compact heads (325 g), its earliness and uniform heading ability have potential value in the development of tropical cabbage varieties.

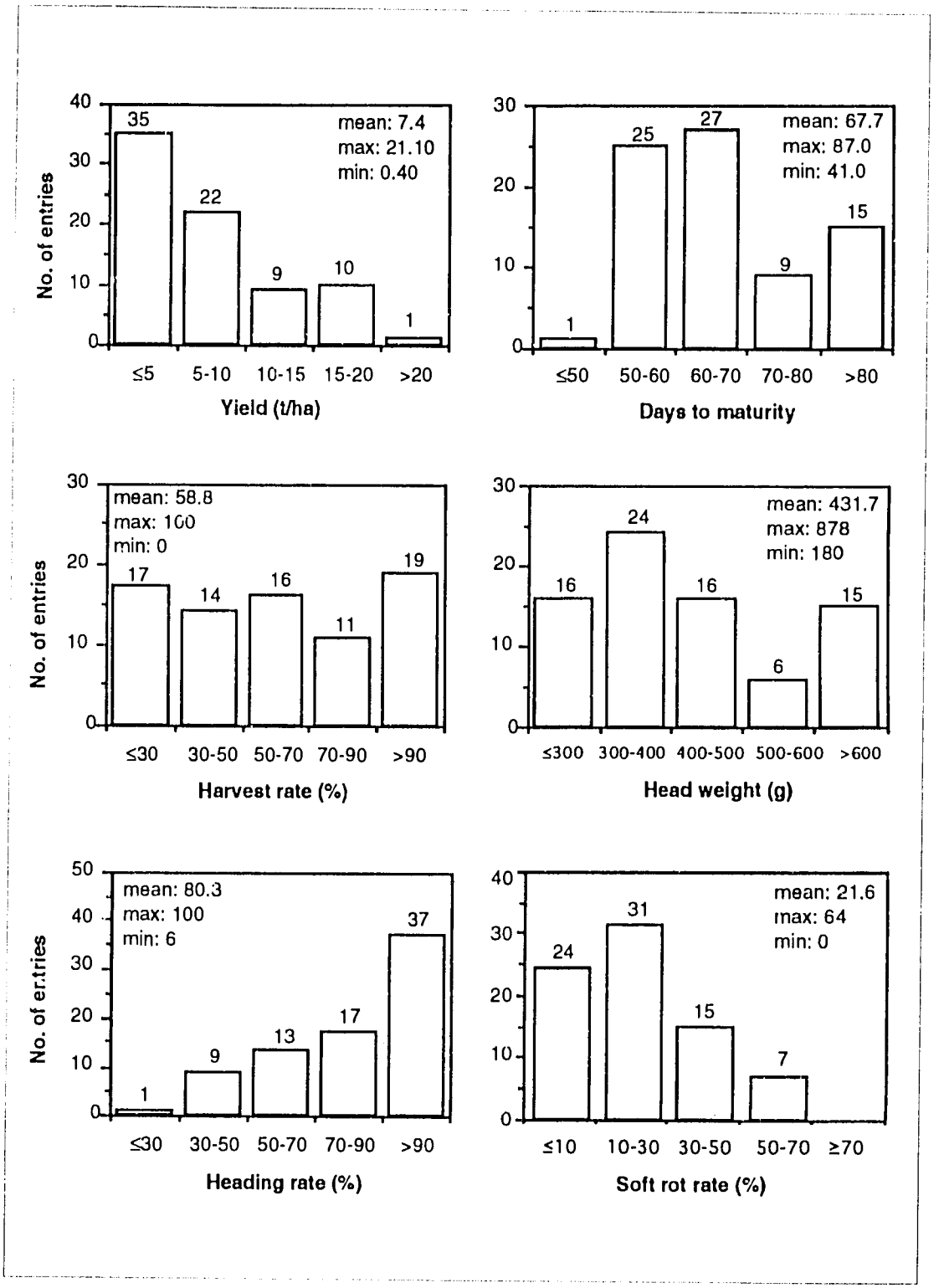


Fig. 1. Distribution of horticultural characteristics among 77 headed common cabbage accessions in a summer observational trial

Table 3. Yield and head weight of the top 10 varieties of common cabbage in the observation trial, summer 1994[a]

Variety	Source	Yield (t/h)	Head weight (g)	Head rate (%)	Harvest rate (%)	Soft rot (%)
KK Cross	Takii, Japan	21.1	793	100	100	0
KS-Green	Kaneko, Japan	19.5	733	100	100	0
Delicious	Watanabe, Japan	18.7	703	100	100	0
YR Summer 50	Takii, Japan	18.4	690	100	100	0
Shia Fong No. 1	Nongsheng, Taiwan	17.9	671	100	100	0
KK Cross	Fratelli Ingegnoli, Italy	17.6	878	100	75	25
Shih No. 1	TSS, Taiwan	17.2	645	100	100	0
Copenhagen Market	Petoseed, USA	16.6	665	100	94	6
Tropical Queen	Takayama, Japan	16.3	713	100	86	14
Shia Chong	Nongsheng, Taiwan	16.1	603	100	100	0
Mean of 77 varieties		7.4	432	80	59	22

[a] Sown on 11 May and transplanted on 14 June 1994

Table 4. Performance of common cabbage varieties[a] in the hot-wet season trial at AVRDC, 1994[b]

Entry	Variety	Yield (t/ha)	Maturity (DAT)	Harvest rate (%)	Head weight (kg)	Heading efficiency ratio	Head shape index	Solidity (g/cc)	Heading rate (%)	Soft rot (%)
BB 006	Shia Fong No. 1	13.2abc	63bc	83ab	0.61abc	0.99c	0.82ef	0.48ab	100a	18
BB 008	KY Cross	14.1ab	59c	80ab	0.66ab	1.01c	0.76fg	0.51ab	100a	21
BB 025	Shia Chong	11.5a-d	68b	94ab	0.46cd	0.72d	0 94c	0.36b	100a	7
BB 110	Choon Chiou	14.5a	63bc	84ab	0.65ab	1.01c	0.70g	0.47ab	100a	16
BB 111	Liang Guan	13.6abc	63bc	69b	0.75a	0.92c	0.75fg	0.49ab	81c	23
BB 112	KK Cross	13.3abc	63bc	79ab	0.65ab	1.21b	0.84def	0.45ab	100a	22
BB 113	Golden Cross 40	8.7de	41d	100a	0.33d	1.49a	1.38a	0.65a	100a	0
BB 114	Y-R Summer	12.1abc	66b	100a	0.45cd	0.78d	0.92cd	0.39b	100a	
BB 115	Southern Treasure	11.0bcd	63bc	76ab	0.55bc	0.98c	0.91cde	0.57ab	94b	19
BB 116	Tight Globe	6.8e	73a	82ab	0.31d	0.52e	1.12b	0.51ab	97a	16
BB 117	Green Stone	10.5cd	65b	74b	0.54bc	1.01c	0.86cde	0.56ab	94b	20
	CV(%)	11.8	3.3	12.5	13.7	6.5	4.5	17.4	1.3	

[a] Mean separation within columns at 5% level (DMRT)
[b] Sown on 11 May and transplanted on 14 June 1994

Flower Induction in Common Cabbage

Summary

A series of flower induction experiments were repeated under artificial conditions with combining vernalization (5°C for 5, 10, 20, 25, and 30 days) and daylength treatments (24 h of light or darkness). The results confirmed that there were no significant differences in days to flowering between light and dark treatments. On the other hand, 20 days of vernalization at 5°C was effective to induce flowering in common cabbage.

Introduction

Seed production of crucifers in the tropics is difficult due to inadequate winter chilling to induce bolting and flowering. High temperatures result in abnormal floral development: enlarged sepals, defective anthers, and low-viability pollen. Therefore, the seed-set is poor.

Many *Brassica* species are induced to flower by low temperatures. The promotive effect of low temperatures on flowering is called vernalization. Effects of low temperatures on common cabbage are apparent only when growing plants are treated. Common cabbage is considered as the green plant vernalization type. Many *Brassica* species are quantitatively long-day plants. The longer the daylength is, the more extensive and earlier the flowering becomes. Many *Brassica* species which respond to vernalization are also sensitive to long-day stimulation to flowering. The interaction of these two factors are either supplementary or complementary. Thus, long-day stimulation may quantitatively displace the low temperature requirement. A combination of vernalization and longer daylength may be required for the maximum flowering of common cabbage.

Mechanical treatment by cutting heads in the field was attempted to enhance bolting and flowering in selected common cabbage varieties during the 1992 winter. Cutting across the head was the most effective way to induce flowering and also producing more branches on the plants. In 1993, a vernalization experiment revealed that 25 days of vernalization (5°C) enhanced flowering in common cabbage. Long-day treatment did not affect vernalization. To confirm these results, a series of flower induction experiments were repeated under artificial conditions, combining vernalization and daylength treatments.

Materials and Methods

In the 1993 experiment exploring the relationship between duration of vernalization and daylength, two F_1 hybrids, Shiafong No. 1 and KY Cross, were sown on 8 September and transplanted to the field on 4 October. One hundred fifty plants of each variety with normal heads, were selected and trimmed on 25 November. One hundred twenty were induced to flower under different vernalization conditions, while the remaining 30 were used as checks and borders. The vernalization treatment began on 30 November. There were five plants for each treatment. Each plant was completely trimmed on the head and roots, leaving only a little root zone. The stumps were wrapped in newspapers and placed in plastic bags. Vernalization was under 5°C with 12 different combinations of low-temperature duration (i.e., 5, 10, 15, 20, 25, and 30 days) and light duration (24-h continuous light and complete darkness). After the vernalization treatment, the field experiment used a randomized complete block design with two replications. Data on days to flowering were collected.

Results and Discussion

In 1992-93, a vernalization experiment revealed that 25 days of vernalization (5°C) enhanced flowering in common cabbage. Long-day treatment did not affect vernalization. The experiment combining vernalization and long-day treatments was repeated in 1993-94. Although variety Shiafong flowered earlier than KY Cross, both responded to vernalization treatments similarly. Again, the results confirmed that there were no significant differences in days to flowering between light and dark treatments. Furthermore, the long-day requirement for flowering may be replaced by sufficient durations of low temperature treatment in common cabbage. On the other hand, 20 days of vernalization at 5°C is effective to induce flowering in common cabbage (fig. 2). The 25- and 30-day vernalization durations were as effective as 20 days to enhance flowering.

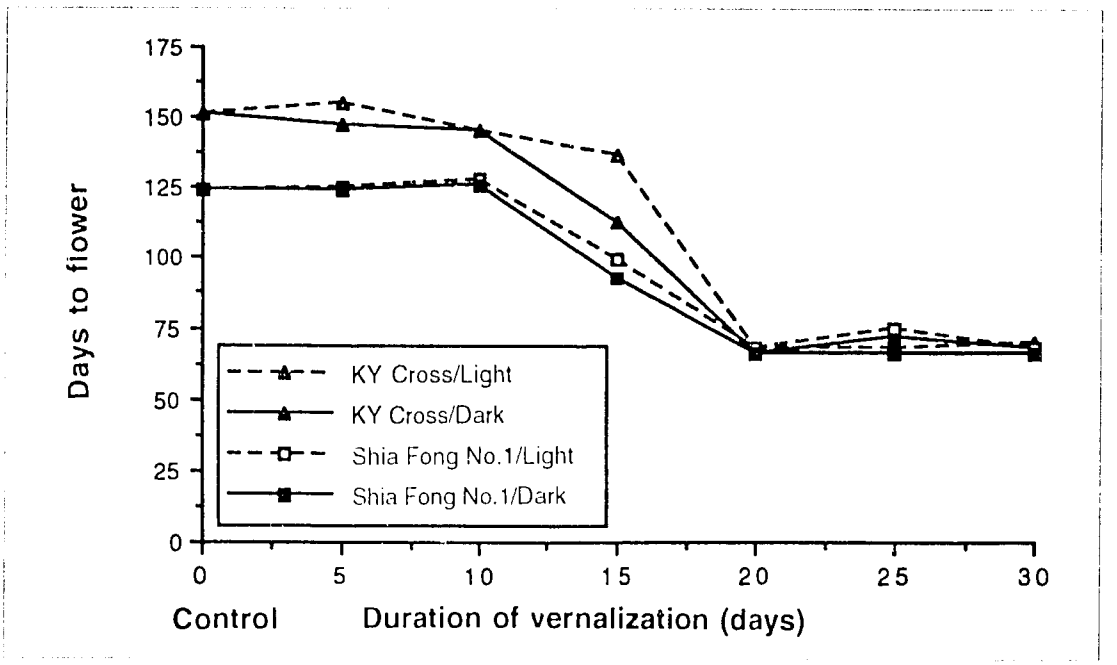


Fig. 2. Effect of duration of vernalization and daylength treatments (5°C) on flower induction of two common cabbage varieties

The 1993-94 field experiment suffered from devernalization due to the warmer winter after the treatment. Research on flower induction on plants derived from tissue culture and rooted cuttings produced from sprouts of common cabbage will be explored.

International Cooperation

Seed Distribution

In 1994, a total of 753 seed packets of cruciferous crops were distributed to 84 cooperators in 38 countries. Most of the seeds consisted of open-pollinated and/or F_1 hybrid cultivars, together with local checks and the standard AVRDC heat-sensitive check. A few shipments of parental lines of hybrids were also provided to national programs for in-country seed production experiments. Parents of AVRDC Hybrid No. 62 were sent to the Seed Company of Ho Chi Minh City for seed production. Over three-year trials, the seed company believes that the variety has great potential in Vietnam.

AVRDC Chinese cabbage varieties have created a summer Chinese cabbage industry in China since 1981. More than 20 varieties of Chinese cabbage using AVRDC lines as parents have been released to farmers. Seed requirement has increased tremendously year by year. Reports of promising performance of AVRDC breeding materials from various cooperators were received during the year.

China

A symposium on the research and promotion of heat-tolerant Chinese cabbage in China was held in Nanjing on 26-28 September 1994. Countrywide activities, accomplishments, and the benefits of using AVRDC-developed heat-tolerant Chinese cabbage in China were the major topics discussed. AVRDC Chinese cabbage varieties had created a summer Chinese cabbage industry in China since 1981. More than 20 varieties of AVRDC Chinese cabbage or derived from AVRDC-developed heat-tolerant materials have been released to farmers in China (table 5).

Table 5. AVRDC-improved Chinese cabbage varieties released in China

Local name	AVRDC ID	Releasing agency
ASVEG No. 1	Hybrid No. 62	CAAS
86-4	[a]	CAAS
Zhong Za No. 1	[a]	CAAS
Zhong Za No. 2	[a]	CAAS
146	[a]	Heilongjiang AAS
Jin Bai 45	[a]	Tianjin AAS
Shandong No. 19	[b]	Shandong AAS
Xia Bai 58	Hybrid No. 58	Sichuan AAS
Xia Bai 59	Hybrid No. 59	Sichuan AAS
Xia Bai 62	Hybrid No. 62	Sichuan AAS
Hwa Xiang No. 1	[a]	Hunan AAS
Hwa Xiang No. 3	[a]	Hunan AAS
Hwa Xiang No. 5	[a]	Hunan AAS
Fu Bao	Hybrid No. 58	Jiangsu AAS
Xia Feng	Hybrid No. 62	Jiangsu AAS
Shuong Guan	[a]	Jiangsu AAS
Nai Re No. 1	Hybrid No. 58	Guangdong AAS
Nai Re No. 2	Hybrid No. 62	Guangdong AAS
Nai Re No. 3	Hybrid No. 59	Guangdong AAS
Nai Re No. 4	77M(2/3)-41-0-2-0-0	Guangdong AAS

[a] Hybrids bred from one AVRDC inbred parent

[b] Hybrid bred from a selection of AVRDC heat-tolerant open-pollinated variety (Nai Re No. 4) as one of the parents

Philippines

Mr. Eugene C. Cahiles and Ms. Rizalina C. Guibao, researchers of the Vegetable Section of the Bohol Agricultural Production Center, conducted yield trials at Tubigon, Bohol, to evaluate four open-pollinated varieties and six hybrids of AVRDC with the standard heat-sensitive check, B 40. A dry season trial was sown on 21 October and transplanted on 11 November 1993. Results are shown in table 6. Varietal differences were significant between the heat-sensitive check and AVRDC heat-tolerant varieties in yield, head weight, and heading efficiency ratio, head shape index (HSI), and heading and harvest rates. The performance of all AVRDC varieties was comparable to the standard heat-tolerant check, Hybrid No. 62, which had been formally released in the Philippines as Reyna Elena. Two new open-pollinated varieties, 77M(3)-27 (MLS_2) and 77M(3)-35 (MLS_3), which were improved through maternal line selection, showed an outstanding long head shape with HSI of 2.13 and 2.62, respectively. They are expected to match Reyna Elena (HSI of 1.46), if not surpass local preference which is determined to some extent by head conformation.

Table 6. Yield and other characteristics[a] of AVRDC entries in a dry season trial at Bohol Agricultural Promotion Center, Tubigon, Bohol, Philippines, 1993[b]

Entry	Yield (t/ha)	Maturity (DAT)	Harvest rate (%)	Head-weight (kg)	Heading efficiency ratio	Head-shape index	Solidity (g/cc)	Heading rate (%)	Soft rot (%)
77M(3)-27(MLS$_7$)	18.5 ab	46	95 a	0.6 b	1.54 abc	2.13 b	0.65 ab	97 a	2
77M(3)-35(MLS$_7$)	18.8 ab	46	97 a	0.6 b	1.29 bc	2.62 a	0.73 ab	100 a	3
77M(3)-40	15.5 b	46	97 a	0.5 bc	1.25 c	1.50 d	0.56 ab	97 a	0
77M(2/3)-46	23.4 a	44	97 a	0.7 a	1.62 abc	1.77 c	0.84 a	98 a	0
Hybrid No. 82-156	19.1 ab	42	98 a	0.6 b	1.71 ab	1.45 d	0.65 ab	100 a	2
Hybrid No. 82-157	14.5 b	46	92 a	0.5 bc	1.37 bc	1.30 d	0.62 ab	93 a	2
Hybrid No. 83-20	19.5 ab	44	100 a	0.6 b	1.91 a	1.47 d	0.53 b	100 a	0
Hybrid No. 85202	17.3 b	46	95 a	0.6 b	1.68 abc	1.87 c	0.58 ab	95 a	0
Hybrid No. 85216	18.2 ab	46	95 a	0.6 b	1.92 a	1.77 c	0.69 ab	98 a	3
Hybrid No. 62	18.0 ab	44	98 a	0.6 b	1.66 abc	1.46 d	0.61 ab	98 a	0
B 40 (HS ck)[c]	9.1 c	46	77 b	0.4 c	0.78 d	–	–	77 b	0
CV (%)	16.4	4.8	8.4	12.4	14.9	7.3	22.7	6.8	224.7

[a] Mean separation within columns at 5% level (DMRT)
[b] Sown on 21 October and transplanted on 11 November 1993
[c] HS = heat-sensitive

Vietnam

After testing AVRDC heat-tolerant Chinese cabbage varieties, Ms. Tran Thi Anh Dao selected Hybrid No. 62 for its potential for production in Vietnam. Hybrid No. 62 had high heading rate, low soft rot incidence, and early maturity under hot-wet season conditions in the suburbs of Ho Chi Minh City. The farmers are being encouraged to grow this heat-tolerant variety and production areas are being developed for the crop. Parents of AVRDC Hybrid No. 62 were sent to the Seed Company of Ho Chi Minh City for in-country seed production.

Crucifer Entomology

Effect of Host-plant Age on the Parasitism of DBM by *Oomyzus sokolowskii*

Summary

A field experiment was conducted to study the influence of diamondback moth (DBM) host-plant (cabbage) age on the parasitism of DBM larvae by *Oomyzus sokolowskii*. There was no significant difference in parasitism when DBM was feeding on cabbage plants that were grown in the field 1 to 8 weeks after transplanting (5- to 9-week-old plants). *Oomyzus*, thus, can be introduced in the field at any time during the cabbage-growing season.

Introduction

Oomyzus sokolowskii, a larval parasite suitable for the control of DBM in the tropical lowlands, has been imported and studied at AVRDC since mid-1992. Initially, we developed a mass-rearing procedure which enabled us to rear this insect for our own study and introduction in Taiwan as well as AVNET and SAVERNET countries to control DBM. This parasite is now introduced in Thailand, Malaysia, and the Philippines. In the meantime, at AVRDC, we are gradually studying various biological and ecological parameters to understand the characteristics of its parasitism of DBM which will enable us to judge the suitability of this parasite under various agroecological conditions. In the first of a series of experiments, we studied the effect of DBM host-plant (cabbage) age on the parasitism of this pest by *O. sokolowskii*.

Materials and Methods

A parcel of land was rototilled and worked into 1.5-m-wide beds. The beds were further divided into 24 single 1.5 x 5.0-m beds. The plots were arranged in three rows of eight plots each. Each row constituted one replicate and each plot one treatment. A distance of 1.5 m was maintained between two adjacent plots and 1 m between two replicates. The whole area on four sides was confined by a fine mesh, 2-m-high nylon net, and the same mesh net was used to cover the top. Once every week for 8 consecutive weeks we transplanted 4-week-old cabbage seedlings in three randomly selected plots, with one plot per replicate. Plants were maintained DBM-free. When the cabbage plants in the last transplanted plot were in the field for 1 week, we introduced 10 third instar DBM larvae per plant on each of 10 plants selected at random in each plot. The plants that received DBM larvae were marked.

Immediately after the introduction of DBM, we released 5000 *Oomyzus* adults inside the cage. At 3 and 5 days after parasite introduction, we collected 30 larvae from each plot and reared them in the laboratory until pupation. We recorded the number of *Oomyzus* and DBM pupae that developed and computed the percent parasitism.

Results

Parasitism varied between 18 and 54% in the first observation (3 days) and 30 and 50% in the second observation (5 days). There was no statistically significant relationship between plant age and parasitism (fig. 1). *Oomyzus sokolowskii* parasitism of DBM is, thus, not affected by host-plant age. This parasite, therefore, can be introduced in the field for the biological control of DBM at any stage of plant growth.

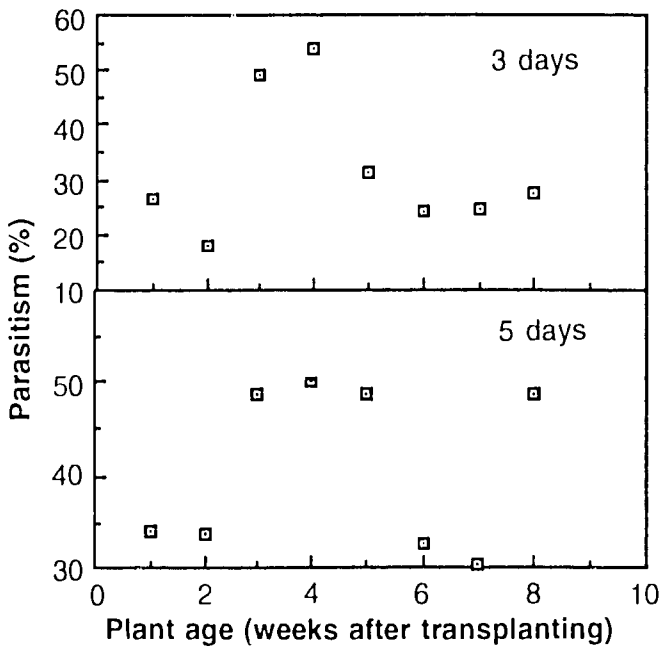


Fig. 1. Effect of host-plant age on the parasitism of DBM by *Oomyzus sokolowskii*

Upper frame: observations taken 3 days after parasite release; lower frame: 5 days after parasite release

Preference of *Oomyzus sokolowskii* for Various Stages of Diamondback Moth

Summary

In a study of preference of *Oomyzus sokolowskii* for parasitism of various immature stages of DBM, freshly formed DBM pupae and larvae of each of the four instars were exposed to parasitism by *O. sokolowskii*. The parasite failed to attack the pupae. It preferentially parasitized the third and fourth instar DBM larvae. Parasitism of the first two instars was significantly less.

Introduction

It has often been stated, but not clearly demonstrated, that *Oomyzus sokolowskii* is a pupal parasite of DBM. Based on these reports, in 1992, we studied preference of this parasite for pupae and third and fourth instar DBM larvae. We found tha this parasite does not parasitize DBM pupae and equally prefers third and fourth instar DBM larvae. Like *Cotesia plutellae*, *O. sokolowskii* is a larval parasite. Since *O. sokolowskii* and *C. plutellae* have similar temperature requirements for development, it is possible that these parasites could compete with each other for DBM larvae. This could affect overall biological control of DBM. In this experiment, therefore, we studied preference of *O. sokolowskii* for all four larval instars. Since *O. sokolowskii* is a much smaller insect than *C. plutellae*, it was thought that *O. sokolowskii* could parasitize early instar DBM larvae as well.

Materials and Methods

We placed 20 individuals each of fresh DBM pupae and larvae of the first, second, third, or fourth instars on a large detached cabbage leaf. The petiole of this leaf was wrapped in moist cotton to prevent drying. The cabbage leaf was then placed in an acylic cylinder containing 200-300 mixed adults of *O. sokolowskii*. We allowed the parasite to lay eggs in the different DBM stages for 24 h. Larvae and pupae were collected and each instar was maintained separately. Larvae were reared until pupation to find out whether they pupated in the DBM or *Oomyzus* pupae. Pupae exposed to parasitism were maintained until adult emergence to find out whether they could be parasitized by *Oomyzus*.

Results

As in past studies, *O. sokolowskii* failed to parasitize DBM pupae (table 1). Among the larval instars, the parasite equally preferred the third and fourth instars. The first and second instars were parasitized substantially, but the parasitism was significantly less than that of the older larvae. *Oomyzus* is a tiny parasite, smaller than the neonate larva of DBM, but its parasitism of the first two instars was less than the last two instars. For *Oomyzus* mass rearing, we will continue to use third and fourth instar DBM larvae. Since *Cotesia* also attacks second and third instars we will need to study competition between *Oomyzus* and *Cotesia*.

Table 1. Parasitism of various stages of DBM by *O. sokolowskii*

Instar	Parasitism (%)
First	25.0
Second	41.2
Third	73.7
Fourth	76.2
Pupae	0.0
LSD (5%)	20.8

Effect of Various Insecticides on the Survival of *Oomyzus sokolowskii*

Summary

Commercial formulations of seven commonly used insecticides at recommended rates were tested for their toxicity to pupae of *Oomyzus sokolowskii*, a parasite of diamondback moth. Bifenthrin, mevinphos, cartap, and profenofos were highly toxic to both stages of the parasite. *Bacillus thuringiensis* and abamectin were relatively less toxic to parasite pupae.

Introduction

Our experience at AVRDC indicates that, despite best efforts by researchers and extension authorities, some farmers continue to use insecticides in crucifer-growing areas where parasites are newly established and DBM is no longer a serious pest. Some of the insecticide used is for controlling insect pests other than DBM. It is possible that some of the insecticides used could adversely affect DBM parasites and thereby exacerbate the DBM problem. We, therefore, conducted a laboratory experiment to study the toxicity of various commonly used insecticides to *O. sokolowskii*, a larval parasite which has potential in controlling DBM in the tropical lowland.

Materials and Methods

Commercial formulations of seven insecticides and one commonly used sticker were diluted as directed on the insecticide container for use in the field. Freshly formed pupae of *O. sokolowskii* were then dipped in insecticide solution for 5 sec. Treated pupae were air-dried under a gentle flow of air. The dry pupae were maintained at 28±2°C until all emerged into *O. sokolowskii* adults. The pupae that failed to emerge were considered dead. The total number of adults that emerged from each pupae were recorded.

In a simultaneous test with adults, the insecticide solutions were sprayed on fresh cabbage leaves. The treated leaves were placed in a 15-cm-diam acylic cylinder. Twenty *O. sokolowskii* adults were released inside each cylinder and insect mortality was recorded 72 h after treatment.

Results

The survival rate and the number of *O. sokolowskii* adults that emerged from surviving pupae are shown in fig. 2. Bifenthrin was the most toxic and *Bacillus thuringiensis* the least toxic insecticides. The order of toxicity was bifenthrin > mevinphos > cyromazine > cartap > abamectin > profenofos > *B. thuringiensis*. For adults, mevinphos and cartap were equally toxic while *B. thuringiensis* was the least toxic (fig. 3). The order of toxicity was mevinphos = cartap > profenofos > bifenthrin > abamectin = cyromazine > *B. thuringiensis*. The sticker was nontoxic to adults and its toxicity to pupae was lower than any insecticide included in the test. *Bacillus thuringiensis* was the least toxic to both adults and pupae. This biological insecticide is frequently used to control DBM. Abamectin, at present the most popular chemical for the control of DBM, was relatively less toxic to both stages of *O. sokolowskii*. Cyromazine is frequently used to control leaf miner. This chemical is relatively safer to *O. sokolowskii* adults but not to pupae. Mevinphos, an old broad-spectum organophosphorus insecticide, is often used in DBM control. This chemical is highly toxic to the parasite; so is bifenthrin, a newly introduced synthetic pyrethroid. *B. thuringiensis*, abamectin, and cyromazine seem to be acceptable for integrated pest management (IPM) in crucifer.

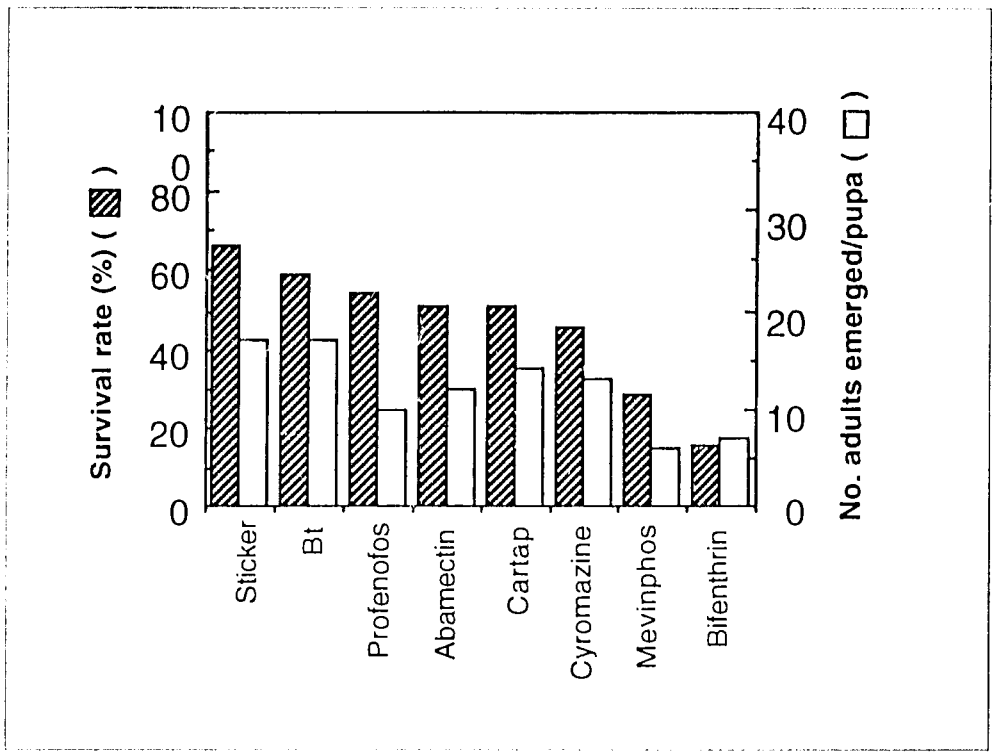


Fig. 2. Effect of various insecticides on the survival of pupae and emergence of *O. sokolowskii* adults

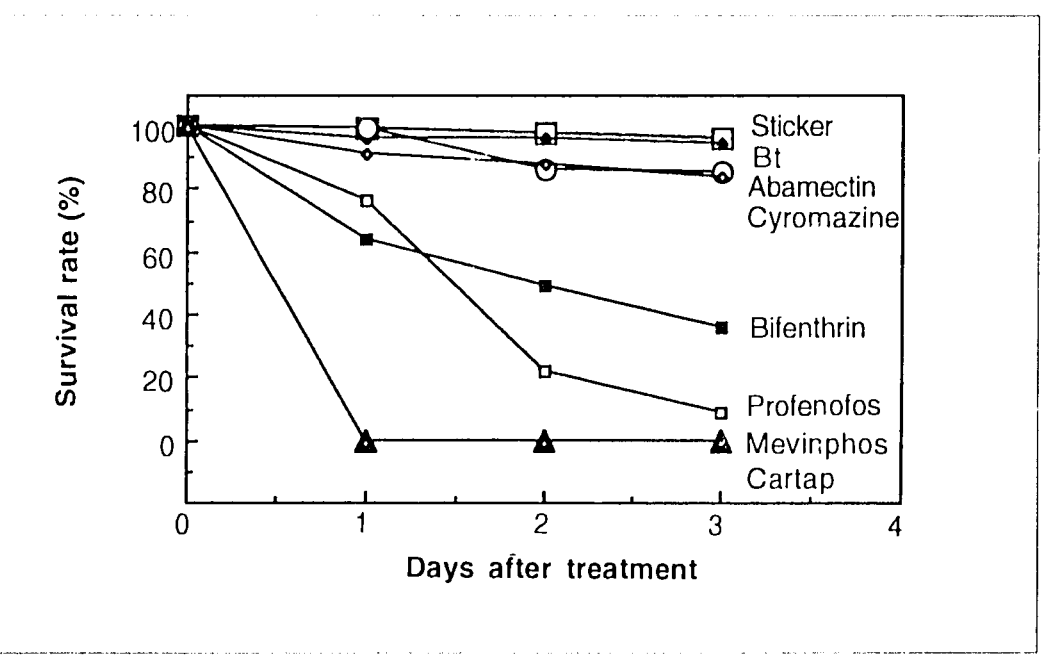


Fig. 3. Effect of various insecticides on the survival of adults of *O. sokolowskii*

Effect of Storage Temperature and X-ray Irradiation on Survival of *Oomyzus sokolowskii*

Summary

In a study of effect of storage temperature and storage duration on the viability of *Oomyzus sokolowskii* pupae, we observed much faster loss of viability at 0 and 10°C than at 5°C. In general, viability loss increased with temperature and storage duration between 15 and 60 days. The X-ray radiation used in airports to inspect baggage did not affect viability of pupae of *Diadegma semiclausum*, *Cotesia plutellae*, and *Oomyzus sokolowskii*.

Introduction

When shipping large numbers of parasites abroad, we are often required to store the parasite pupae produced daily until the required number is reached. The storage temperature and duration affects the survival of parasites. We either air-freight or hand-carry the parasites from Taiwan. Air-freighted goods are physically examined and are not, presumably, subject to X-ray radiation. However, hand-carried goods, including live specimens, passing through Taiwan's airports undergo X-ray irradiation whether specimens are placed in checked-in luggage, hand-carried briefcases, or carried in person. Since we frequently ship parasites to various destinations outside Taiwan, to improve efficiency of shipment, we studied the effect of storage temperature and duration and X-ray irradiation on the survival of *O. sokolowskii*.

Materials and Methods

In the first study, freshly formed *O. sokolowskii* pupae confined in plastic petri dishes, were stored at 0, 5, and 10°C for 15, 30, 45, and 60 days. At the end of each duration, we moved pupae to 28±2°C and recorded the number of those that emerged into adults.

In the second study, fresh pupae of *O. sokolowskii*, *Cotesia plutellae*, and *Diadegma semiclausum* placed in petri dishes were passed through an X ray source at the Kaohsiung International Airport hand baggage inspection belt. The duration of exposure of pupae to X-ray was three times longer than normally used for inspecting hand baggage. The irradiated pupae were then maintained at 28±2°C, and the number of pupae that emerged into adults were recorded.

Results

Results of effect of storage temperature and duration are summarized in table 2. Pupae stored at 0 and 10°C lost viability much faster than those stored at 5°C. In general loss of viability increased with time at each temperature.

Table 2. Effect of low temperature and length of storage on the emergence of *O. sokolowskii* adults

Temperature	Pupae emerged into adults (%) at			
(°C)	15 days	30 days	45 days	60 days
0	91.3	70.7	52.7	34.0
5	84.7	80.0	87.3	68.7
10	55.3	37.3	62.7	26.7
LSD (5%)	13.4	14.1	13.2	13.2

Data are means of four replicates

In the X-ray irradiation study, we did not observe any difference in the rate of pupal emergence into adults whether the pupae were irradiated or not. It was not possible to get quantitative measurement of X-ray radiation from the airport staff. The X-ray radiation at airports are, thus, not considered harmful to parasites and these insects can be safely sent via an airline flight.

Inundative Release of *Cotesia plutellae* and a Chrysopid Predator for the Control of Diamondback Moth in Lowlands

Summary

Attempts were made to control DBM and other crucifer pests in lowland areas with inundative releases of one parasite, *Cotesia plutellae*, and a chrysopid polyphagous predator, *Mallada basalis*. Cabbage was transplanted in four widely spaced 0.05-ha parcels in a 100-ha field. DBM was introduced 2 weeks after transplanting to initiate infestation and natural enemies 1 day later, and again once each at 4, 7, 10, and 13 weeks after transplanting. Once a week, DBM adult population was monitored by sex pheromone traps, and larval and pupal counts by observing 50 randomly selected plants in each parcel. Both adults and immature stages of DBM were substantially less in parcels where both natural enemies were used. *Mallada basalis* was less effective than DBM-specific parasite *C. plutellae* in controlling DBM. There was some synergism between the two natural enemies in controlling DBM larvae and pupae but not adults. *Spodoptera exigua* and *Spodoptera litura* infested cabbage during the season.

Introduction

Although *Cotesia plutellae*, a larval parasite of DBM, is well established in most lowland areas of tropical to subtropical Asia, it does not consistently control DBM there as *Diadegma semiclausum* does in the highlands. In many cases, *C. plutellae* occurs in adequate numbers only after DBM has caused substantial damage to the crop. It is possible that this parasite does not multiply rapidly or does not have enough alternate hosts during the off-season and must wait for substantial infestation of DBM for its multiplication. A field experiment was carried out at AVRDC wherein this parasite and a chrysopid predator, *Mallada basalis*, were released at regular intervals from the beginning of the crop season to study their control of DBM as well as other crucifer pests.

Materials and Methods

Four parcels of land, each measuring 0.05 ha, in four corners of a 100-ha AVRDC field, were transplanted to common cabbage. Each planted area, starting from transplanting date, was cordoned off by a 1.2-m-high nylon net fence. At 1, 7, and 13 days after transplanting we placed four sex pheromone traps overnight in each parcel to monitor the presence of DBM adults; none was found. Fourteen days after transplanting, we introduced 100 pairs of DBM in each parcel. A day after DBM introduction, we introduced 1000 *Cotesia* in one parcel, 4000 larvae of *Mallada basalis* in the second, and 1000 *Cotesia* and 4000 Malada in the third parcel. The fourth parcel was maintained as a check.

The natural enemies were introduced again in identical fashion 4, 7, 10, and 13 weeks after transplanting. Starting 1 week after natural enemy release and once a week thereafter, we placed two DBM sex pheromone traps overnight in each parcel and recorded the number of DBM adults trapped. Simultaneously, we observed 50 cabbage plants in each parcel and recorded DBM larvae and pupae on each plant. Monitoring of DBM continued until harvest. We also recorded occurrence of other crucifer pests during the season.

Results

The results of the DBM infestation in the check and treatment plots with natural enemies are summarized in fig. 4. The number of DBM adults trapped in the plot where natural enemies were released were substantially less than in the control plot during the first four observations. The adult population remained low in subsequent observations only in plots where *Cotesia* was released. In *Mallada* plots, the DBM adult population was similar to check plots. *Mallada* is not a specific predator of DBM as *Cotesia* is. Hence, it was probably not able to keep DBM in check. The DBM larvae and pupae population followed the same trend as that of DBM adults. Larval and pupal population increased in plots where natural enemies were released during the last two observations. The increase was substantial in *Mallada* plots but much less in *Cotesia* plots. This is because of the specificity of *Cotesia* towards DBM. At this stage, however, the crop was nearing harvest and the pest damage did not cause any notable loss in quality or quantity of cabbage. The reduction in larval and pupal population in the check plot during the last two observations is due to the natural occurrence of *Cotesia* in that plot.

Other insect pests present include *Crocidolomia binotalis*, which was observed at the beginning of the season, but its infestation tapered off at mid-season. *Spodoptera exigua* and, to a lesser extent, *Spodoptera litura* were present throughout the season. *Mallada basalis* did not seem to have significant influence over these insect pests. Since the crop was grown in four parcels located as far apart as possible, soil fertility may have differed substantially. Therefore, we did not compare yields. In future tests we will increase frequency of natural enemy release early in the season to prevent buildup of other pests. Specific noninsecticidal measures such as sex pheromone and possibly virus for armyworm control will be adopted.

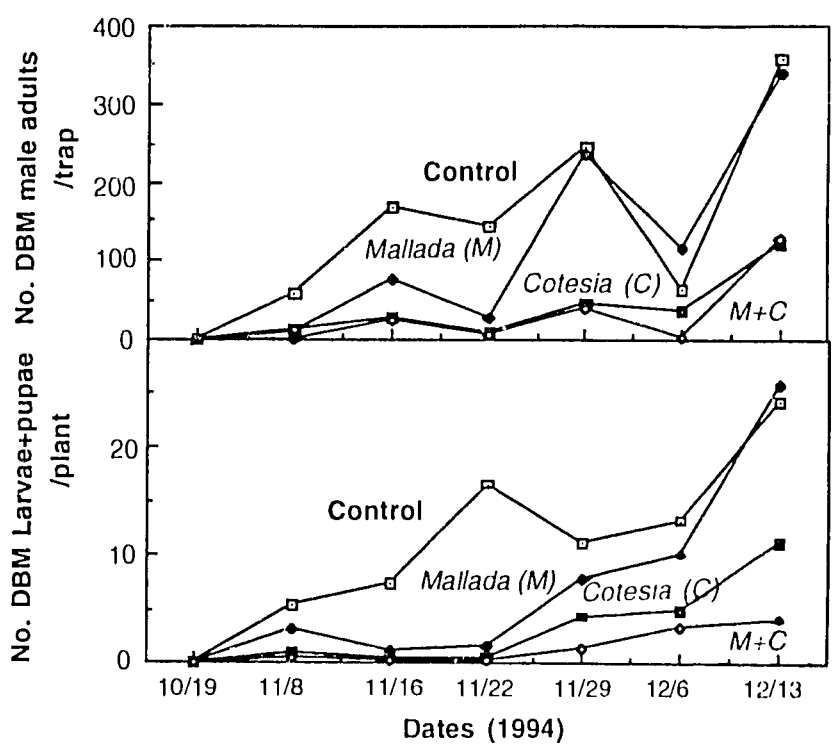


Fig. 4. Population of DBM adults (upper) and larvae and pupae (lower) in the control and plots with *Cotesia* and *Mallada* (Transplanting date: 6 October)

Crucifer Pathology

A DNA-Based Assay to Detect *Xanthomonas campestris* pv. *campestris*

Summary

One clone (pXC233), containing a 2.9-Kb insert of *Xanthomonas campestris* pv. *campestris* (Xcc) DNA, was identified at the Washington State University-Puyallup as having suitable characteristics for use in the development of a polymerase chain reaction (PCR) assay. The DNA sequence of the 2.9-Kb insert has been determined and used to generate oligonucleotide primers for PCR testing. The specificity of primers 4 and 14, 4 and 5, and 5 and 16 were tested using genomic DNAs extracted from 125 strains isolated from crucifer leaves showing black rot symptoms in Taiwan. The pathogenicity of each strain was determined by inoculating cabbage seedlings. One hundred and ten pathogenic Xcc and one nonpathogenic strain were PCR-positive. Twelve pathogenic Xcc and two nonpathogenic strains were PCR-negative.

Introduction

Xanthomonas campestris pv. *campestris* (Xcc), the causal agent of black rot, is perhaps the most serious pathogen of crucifers worldwide. It attacks all cultivated crucifers. After entering through the hydathodes at the leaf margins or through wounds, the bacteria colonize the vascular tissue and move rapidly through the vascular system when temperatures exceed 25°C[1]. Invasion of the flowering stalks leads to infection of the seed. Since Xcc is seedborne, with no reliable seed treatment to eradicate it, it is very important to have pathogen-free seeds for disease control. Thus, a reliable and efficient seed detection method is required.

Current assays for detecting this pathogen involves the isolation and culturing of bacteria from seed washes followed by pathogenicity testing of suspect colonies[2]. These are time-consuming and not very sensitive. A fast, sensitive, and specific assay incorporating polymerase chain reaction was thus developed.

Twenty-one oligonucleotide primers were generated from pXC233, a clone isolated from a genomic DNA library of the neopathotype strain and with sufficient specificity to pathogenic Xcc strains. Three pairs of primers (5 and 16, 4 and 14, and 4 and 5) have shown promise amplifying DNAs from pathogenic strains of Xcc. This study further tested the specificity of these three primer pairs for detecting pathogenic strains of Xcc by comparing the results of pathogenicity tests.

[1] Williams, P.H. 1980. Black rot: A continuing threat to world crucifers. Plant Disease 64(8):736-742.

[2] Schaad, N.W. 1989. Detection of *Xanthomonas campestris* pv. *campestris*. p. 68-75 In: Detection of bacteria in seed and other planting material. Saettler, A.W., Schaad, N.W., and Roth, D.A. (ed.). American Phytopathological Society, St. Paul, MN, USA.

Materials and Methods

Bacterial strains and oligonucleotide primers

A total of 125 strains isolated from cruciferous leaves in Taiwan showing black rot symptoms were used in this study. Oligonucleotide primers no. 4, 5, 14, and 16 were provided by Dr. R. Aline of Washington State University-Puyallup Research and Extension Center.

Genomic DNA isolation

Bacteria were grown overnight in nutrient broth at 30°C, harvested, washed twice, and resuspended in TEN buffer (10 mM Tris, pH8, 1 mM EDTA, 100 mM NaCl). The bacterial suspension was incubated at 56°C for 1 h with proteinase K and SDS at final concentrations of 0.15 mg/ml and 1%, respectively. Phenol and chloroform extractions were performed and the DNA was precipitated by isopropyl alcohol and ammonium acetate. The pellet was washed in ethanol, dried, and dissolved in water.

PCR amplification and gel electrophoresis

PCR amplification of DNA was done in the PTC-100 programmable Thermal Controller (MJ Research, Inc.). Individual PCR samples (25 µl) contained 1x PCR buffer (BRL), 1.5 mM $MgCl_2$ (BRL), 200 µM of each dNTP (Promega), 0.8 µM of each primer, template DNA, and 0.5 unit *Taq* DNA polymerase (BRL). Template DNA was initially denatured in the thermal controller at 95°C for 30 sec. The polymerase was added at 65°C. Each reaction was overlaid with 25 µl of mineral oil. Twenty-five PCR cycles of denaturation at 95°C for 30 sec, primer annealing for 30 sec at 60°C (for primers 5/16), 57°C (for primers 4/14), or 58°C (for primers 4/5), and DNA extension at 72°C for 1 1/2 min were done after the initial denaturation. The samples were cooled to 4°C after a final step of 72°C for 5 min. Gel tracking dye (2.5 µl) was added directly to each reaction tube, and 10 µl of the reaction mixtures were electrophoresed at 50 volts using 1% agarose gel in 0.5x TBE. The gels were stained with ethidium bromide and photographed with a Polaroid MP4 Camera System using 667 Polaroid film.

Pathogenicity tests

Eight- to ten-day-old cabbage (*Brassica oleracea* var. *capitata*) seedlings were inoculated with bacteria by cutting one cotyledon from each plant and applying bacteria to the wound using a cotton swab. Ten plants each were inoculated with each strain. Seedlings were incubated in a growth chamber at 12 h-day photoperiod and 21-28°C (night-day). For each assay, a set of control isolates with known reactions were included. These controls are: a highly and moderately virulent black rot isolate (Xcc); an avirulent isolate (Xc); and an isolate of Xanthomonas leaf spot *X.c.* pv. *armoraciae*, which is closely related to Xcc. Plants were rated on a 0-9 disease severity scale 2 weeks after inoculation: 0, plant healthy; 1, slight vascular darkening at the inoculation site; 3, vascular darkening evident in stem; 5, one or more true leaves with indistinct black rot like lesion; 7, well-developed typical black rot lesion; 9, plant nearly dead or dead. The disease severity index (DSI) was calculated for each bacterial strain as follows:

$$\frac{(\text{No. plants in class } 0 \times 0) + \ldots\ldots + (\text{No. plants in class } 9 \times 9)}{\text{Total number of plants}}$$

Based on the DSI the virulence of bacterial strains can be classified into five categories: 0-1 = avirulent; 1-3 = weakly virulent; 3-6= moderately virulent; 6-8 = virulent; 8-9 = highly virulent.

Results and Discussion

PCR tests

Bacterial genomic DNAs were extracted from 125 strains isolated from cruciferous leaves in Taiwan showing black rot symptoms. The specificity of three pairs of primers (5 and 16, 4 and 14, and 4 and 5) were tested. To summarize, 14 strains showed no amplification product using any of these primer pairs. The other 111 strains were amplified using all pairs of primers.

Pathogenicity tests

To see if the PCR results match the results of traditional pathogenicity tests, 8- to 10-day-old cabbage seedlings were inoculated with bacteria as described in Materials and Methods. The plants were rated and the disease severity index was calculated 2 weeks after inoculation. One hundred and twenty-two strains were pathogenic Xcc and most of them were either highly virulent or virulent. Three strains were nonpathogenic. Two of them (Xcc43 and 103) were yellow and mucoid on 523 media and were able to hydrolyze starch. The other (Xcc108-1) was isolated from a stored culture and was yellow but not mucoid on 523 media. Even though all bacteria were isolated from diseased crucifer leaves, there is still a chance of picking up some epiphytic Xc which look just like Xcc on agar media. Xcc108-1 may be a spontaneous mutant in the stored culture or the stored culture was not pure enough at the beginning. Since Xcc produces a lot of extracellular polysaccharides, it is easy to have mixed culture stored without knowing it. It is necessary to repurify the bacteria several times after isolating from diseased plant tissue.

Correlation between PCR and pathogenicity tests

For most of the strains, the PCR results match the pathogenicity test results. DNAs from 12 pathogenic strains cannot be amplified by any of the three primer sets. This could be due to some deletion or point mutations in the region so that primers cannot anneal to the DNAs. Actually, four of them were found to have a 400-600 bp deletion at the internal BamH1 site and were probably the same strain based on restriction fragment length polymorphism (RFLP) patterns (R. Aline, personal communication). The other strains need to be further analyzed to see if they all have deletion in that region. Ten of these strains were isolated from cabbages in the central mountainous area in Taiwan. The other two strains were isolated from kohlrabi in another central mountainous area. There is a possibility that the first 10 strains were identical or the bacteria in the mountain area were somehow different from those in the lowland area. The nonpathogenic but PCR-positive strain may be a real exception to these primers. The tests need to be repeated to make sure that this strain is really PCR-positive but nonpathogenic. Based on these results, PCR is recommended as a substitute traditional plant pathogenicity assay to detect pathogenic Xcc.

Eggplant Breeding

Observation and Evaluation of Eggplant Cultivars and Germplasm

Summary

A total of 91 eggplant entries, including 2 commercial varieties, 32 landraces, and 57 germplasm accessions, were sown in August 1993 and evaluated in the field. The two commercial lines and some landraces from China and India performed very well; they represented 8 of the top 20 yielders. The yields of the top 20 yielders ranged from 40.7 to 59.9 t/ha with an average of 46.7 t/ha. Among the top 10 yielders, only two varieties had cylindrical fruit.

A large diversity in yield and horticultural characteristics was observed. Cylindrical fruit type and green and purple colors were the predominant shape and colors. The number of branches ranged from 5 to 11 per plant. More than 73% of the entries matured in 73-95 days after transplanting (DAT). The mean marketable yield was 30.7 t/ha. Varieties with good horticultural traits, such as erect plant type, less branches, early maturity, and others, were identified and will be evaluated in future elite variety trials and used in breeding programs.

Introduction

Eggplant (*Solanum melongena*) is widely grown in tropical and subtropical areas. According to the 1993 FAO Production Yearbook, Asia has the largest eggplant production area, which is more than 90% of the world production area, and produced 86% of the world production (data from India not included). To date, AVRDC's eggplant germplasm collections is comprised of 1807 accessions, including 896 accessions of *Solanum melongena* and other 42 *Solanum* species. The observation trial (OT) aimed to: (1) identify promising varieties for future evaluation and testing; (2) define desirable genotypes and postulate for tropical lowland cultivation; and (3) look for genetic materials to be used in the eggplant breeding projects.

Materials and Methods

An observation trial consisting of 91 entries was conducted in 1993-94. The entries included 32 landraces, 2 commercial hybrids, and 57 germplasm accessions from AVRDC. Seeds were sown on 13 August 1993 and seedlings transplanted to the field on 7 September 1993. Single-row planting was applied on each bed (plot). Spacing was 1.5 m between rows and 0.5 m between plants. There were 12 plants in each plot without replications.

Cultural practice was based on the standard procedures adopted by farmers for eggplant production. Chemicals were sprayed when necessary. Fruit were harvested weekly for 8 consecutive weeks from five plants (excluding border plants). Plant height, number of branches, fruit characteristics, and fruit quality were also measured. Quality traits were evaluated by AVRDC's Analytical Laboratory.

Results and Discussion

Diversity of collections. A great diversity in fruit shape, fruit color, fruit size, plant type, and number of branches was observed in this trial (table 1). Forty-nine entries had cylindrical fruit shape and 89 had green or purple fruit color. Among the 91 entries planted, nine related wild species did not fruit. Among the other 82 fruited entries, some landraces and germplasm accessions were not pure and had several fruit shapes or colors.

Table 1. Diversity of color and fruit shape among 82 varieties of eggplants, 1993 OT[a]

Color	Cylinder	Oval	Teardrop	Novelty	Total
Purple	31	8	5	0	44
Green	16	18	9	2	45
White	2	2	0	3	7
Total	49	28	14	5	96[b]

[a] Planting dates: sowing: 13 August 1993; transplanting: 7 September 1993

[b] Total no. of varieties was not 82 because some impure ones had several fruit shapes and colors

Plant types. Plants varied a lot in erectness, plant height, and number of branches. Plant heights ranged from 89 to 154 cm, with a mean of 118 cm (fig. 1). Around one-third of the entries had heights in the range of 110-120 cm. Having less branches is a good trait in eggplant because it contributes to reduced pruning costs. Only three entries had less than six branches per plant (fig. 1). More than half of the total entries produced six to eight branches.

Maturity. Sixty entries matured within 95 DAT (fig. 1). The earliest entry was P.P. Long from Bhutan which took only 73 days; TS 78 matured in 129 days. The nine wild species did not fruit when this trial was ended.

Number of fruit per plant. The distribution of number of fruit per plant is shown in fig. 1. Pant Samrat from India produced almost 72 fruits/plant. It had dark purple and short cylindrical fruit suitable for processing. Macrocarpum produced only 6.2 fruits/plant. A large proportion of the entries had less than 30 fruits/plant.

Fruit weight. Fruit weights of the 82 entries ranged from 15 (Perat from Malaysia) to 317 g (Ching-ho-pao from China). The average fruit weight was 118 g. Among all entries, 18, 23, and 33% had fruit weights in the categories of ≤ 50 g, 50-100 g, and 100-150 g, respectively. Again, there was a negative association between fruit size and number of fruit per plant. The larger the fruit, the less the number of fruit per plant.

Fruit quality. Dry matter (DM), sugar and fiber contents of the 82 entries were also determined. Dry matter ranged from 6.2 to 21.4%, with a mean of 8.8%. Kapal, with a small oval fruit, contained the highest DM, while Teng Pao Chieh, a large oval-fruited landrace, had the lowest DM of 6.2%. Both sugar and fiber contents varied widely. The distribution of sugar and fiber contents ranged from 4.9 to 43.8% and 7.4 to 28.2%, respectively. DM content is positively correlated with fiber content ($r = 0.700$) but negatively correlated with sugar content ($r = -0.750$). A negative correlation between sugar and fiber content was also found to be highly significant ($r = -0.795$).

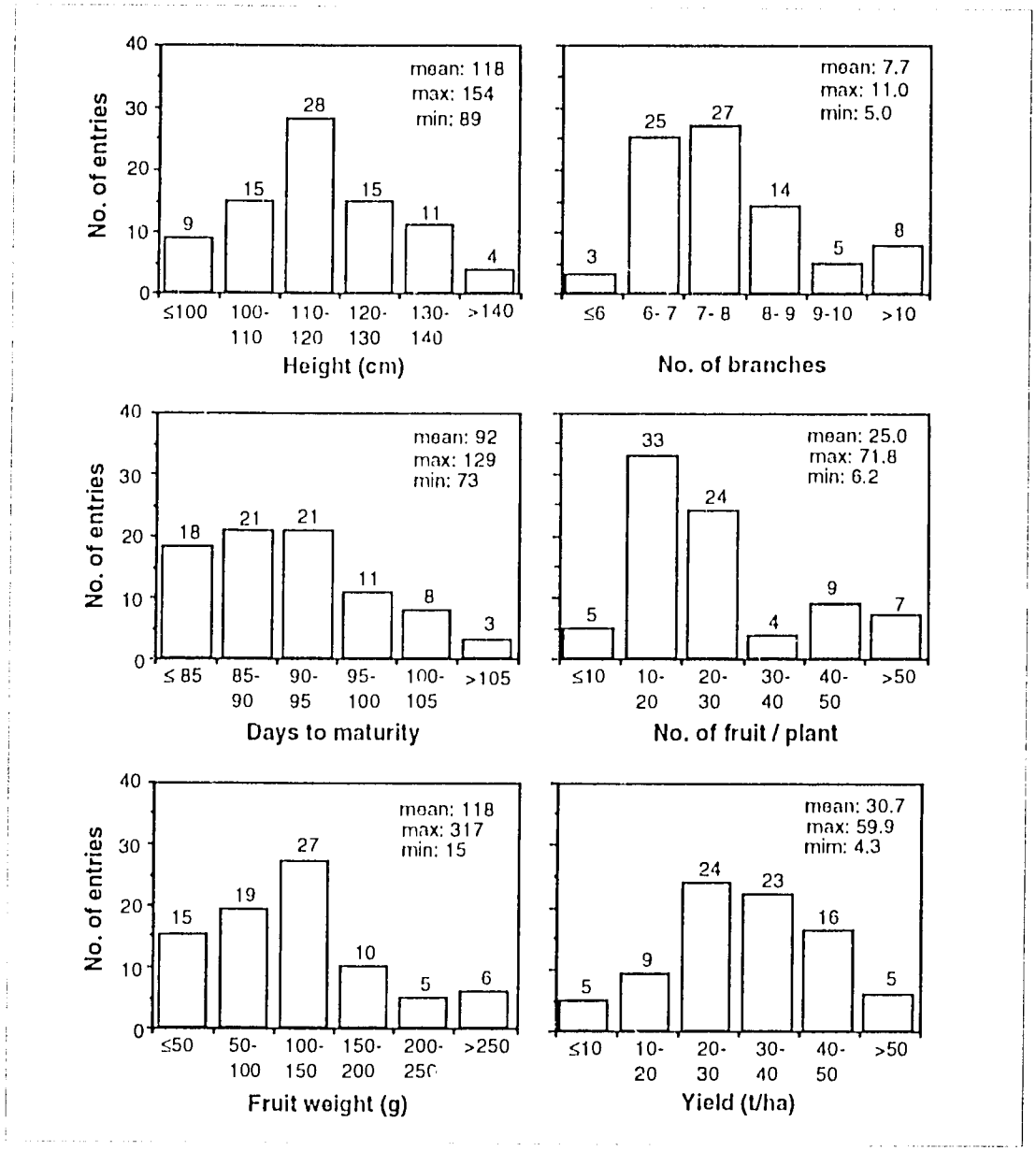


Fig. 1. Distribution of horticultural characteristics among 82 entries of eggplant

Yield. The yield distribution of 82 entries is given in fig. 1. Five entries, i.e., Chu Szu Chieh, Pusa Krant, Teng Pao Chieh, Ching-ho-pao, and Pant Rituraj, produced yields higher than 50 t/ha (table 2). The lowest yield was produced by Macrocarpum, which also had the least number of fruit per plant. Mean yield was 30.7 t/ha; 54% of the 82 entries yielded more than 30 t/ha.

Overall performance. The horticultural characters and yields of the top 20 entries are shown in table 2. These 20 entries had a high average yield of 46.7 t/ha, compared to the total average of 30.7 t/ha (fig. 1). These high-yielding varieties had a mean fruit weight of 163 g and matured 86 DAT. Eight of the top 10 yielders had the teardrop or oval fruit shape. The adaptation and stability of these high-yielding varieties will be further tested in elite variety trials in various seasons.

Table 2. Horticultural characters and yields of top 20 varieties of eggplant, 1993 OT[a]

Variety name	Plant height (cm)	No. of branches	DTF	DTM	Fruit color[b]	Fruit shape[c]	Fruit wt (g)	Fruit size L (cm)	W (cm)	L/W	Fruit no./ plant	Mkt. yield (t/ha)
Chu Szu Chieh	89	7.0	35	86	GP	TD	189	17.1	6.8	2.5	23.8	59.9
Pusa Kranti	117	10.3	35	84	DP	TD	140	11.1	5.5	2.0	30.0	55.9
Teng Pao Chieh	111	6.1	35	77	DP	O	229	9.6	6.8	1.4	17.4	53.2
Ching-ho-pao	106	6.0	41	76	GW	O	317	9.5	8.4	1.1	12.2	51.6
Pant Rituraj	109	9.1	27	82	DP	O	183	7.8	7.7	1.0	20.8	50.7
Fu-chien Long	91	7.5	41	85	DP	C	101	20.8	3.7	5.6	35.8	48.4
TS 79	107	6.8	41	89	W	C	196	16.4	5.9	2.8	18.2	47.5
TS 10	121	6.1	35	92	DP	TD	211	16.6	7.0	2.4	16.4	46.1
Tania F	121	7.8	35	89	DP	O	298	12.5	7.6	1.6	11.6	46.1
TS 110	118	7.7	41	88	DP	TD	154	12.6	6.2	2.0	22.2	45.5
TS 95	111	7.1	41	91	G	TD	219	15.1	6.8	2.2	15.4	45.1
Agora F_1	117	7.0	41	85	DP	TD	251	12.2	6.5	1.9	13.2	44.1
TS 120	111	7.6	41	90	GW	TD	108	10.9	5.8	1.9	30.4	43.9
Pant Samrat	115	9.0	35	86	DP	C	46	12.1	3.0	4.1	71.8	43.6
TS 75	113	6.6	35	94	G	C	129	25.7	3.6	7.1	25.0	42.9
Yao Khaew	116	8.7	49	99	G	C	133	21.8	3.4	6.5	24.2	42.9
Pusa Purple Long	99	10.7	35	77	DRB	C	74	16.6	2.8	5.9	43.8	42.9
P.P. Long	95	10.1	35	73	DRB	C	74	15.7	2.9	5.4	43.0	42.7
Uttara	114	8.8	35	89	LP	C	58	12.9	4.0	3.2	52.4	40.8
Pai Chieh Tzu	118	7.1	37	86	DP	C	150	21.0	5.2	4.0	20.4	40.7
Mean	110	7.9	37	86			163	14.9	5.5	3.2	27.4	46.7

[a] Planting dates: sowing: 13 August 1993; transplanting: 7 September 1993

[b] Fruit color: DP: dark purple; DRB: dark red brown; G: green; GP: green with purple stripes; GW: green with white stripes; LP: light purple; W: white

[c] Fruit shape: C: cylindrical; O: oval; TD: teardrop

The two commercial hybrids and some landraces from China and India performed very well and represented 8 of the top 20 yielders. Two Chinese varieties, Teng Pao Chieh and Ching-ho-pao, exhibited early maturity (76-77 DAT) and also produced high yields of more than 50 t/ha. Some entries with good horticultural traits were identified and are listed in table 3. They will be observed in future trials and used in the breeding project.

Table 3. Promising entries for some horticultural traits from 1993-94 eggplant observation trial

Trait	Genotype
Erect plant type	Yao Khaew, Jan, Cruz, Taso Hung, Duen Ho, TS 124
Fewer branches	TS 117, White Round, Ching-ho-pao, Teng Pao Chieh
Early maturity	P.P. Long, Ching-ho-pao, Teng Pao Chieh, New Hsih, KT 4
High yield potential	Chu Szu Chieh, Pusa Kranti, Teng Pao Chieh, Ching-ho-pao, Pant Rituraj
High no. of fruit/plant	Pant Samrat, Nurki, TS 76, Lai, Terong Keul, Uttara, TS 80
Large fruit	Ching-ho-pao, Tania F_1, New Hsin, Tzu Yuan, Islam Puri, Agora F_1
Small fruit	Perat, TS 78, Bulat Keul, Telunjuk, Terong Kecil, TS 53, TS 80
Processing fruit type	Pant Samrat, KT 4, Nurki, Kopek Putih

Elite Variety Trial

Summary

Promising entries from observational trials were selected and tested in elite variety trials (EVTs) to confirm their adaptation and yield stability. In 1993-94, three EVTs were conducted in spring and summer. Unfortunately, the 1994 summer EVT failed because of typhoons and heavy rain.

The EVT entries were grouped into cylindrical and teardrop fruit types. Significant differences in marketable yields were found in each trial. In the 1993 trial, mean marketable yields were 20.8 and 21.1 t/ha for cylindrical and teardrop fruit, respectively. The yields of the 1994 trial were much higher with averages of 48.8 and 49.4 t/ha for both fruit types, respectively. TS 33 (cylindrical type) performed consistently well in both trials. Marketable yields were highly significantly correlated with number of branches, days to maturity, and number of fruit per plant. On the other hand, the correlation between number of fruit and fruit weight was significantly negative.

Introduction

The objective of EVT is to test the adaptation and yield stability of selected entries from the OT. Selected entries are observed in a replicated experimental design in different cropping seasons, mainly in spring, summer, and autumn. In 1993-94, three EVTs were conducted in spring 1993, and spring and summer 1994. Entries of the spring 1993 trial were selected from the 1992-93 OT, while those of the spring and summer 1994 trials were from stable yielders of the 1993 spring EVT and 1993-94 OT. Unfortunately, the 1994 summer EVT failed because of typhoons.

Materials and Methods

Because of the diversity of eggplant plant type, fruit shape, and fruit color, priorities are set to select entries for EVTs. Purple fruit color is the first priority and fruit shape is the second. Only cylindrical and teardrop fruit types are selected. The selection criteria used are high and stable yield, erect plant type, less branches, disease resistance (bacterial wilt, phomopsis blight, and others), and pest resistance (such as fruit and shoot borers). Local varieties from Taiwan were included as checks.

In the 1993 EVT, 17 cylindrical fruit entries and 22 teardrop entries were sown on 30 March and transplanted on 30 April. For the 1994 EVTs, 17 cylindrical and 17 teardrop fruit type entries were included. The 1994 spring EVT was sown on 4 February and transplanted on 15 March. The 1994 summer trial was sown on 3 May and transplanted on 31 May. The cultural practices and field layout used for the EVTs were the same as those for the OTs except that each entry had four replications.

Results and Discussion

1993 spring EVT. The differences in marketable yields were significant among entries within the trial. For cylindrical fruit type, the highest yield of 38.5 t/ha was given by TS 2, a small fruit type suitable for processing (table 4). Onita F_1, which was selected because of its erectness, had the lowest yield of 8.7 t/ha. The mean yield and fruit weight were 20.8 t/ha and 92.2 g, respectively. The mean yield of the 22 teardrop type entries was 21.2 t/ha, slightly higher than the cylindrical type (table 5). Marketable yields ranged from 6.9 to 41.7 t/ha. Fruit weight ranged from 134 to 321 g with a mean of 245 g which was much higher than that of the cylindrical type. In general, teardrop fruit entries had less number of branches and larger fruit, but less number of fruit per plant than cylindrical fruit types.

Table 4. Horticultural characters and yield of top 5 cylindrical eggplant entries, 1993 and 1994 spring EVT

Trial/Variety	Plant height (cm)	No. of branches	Days to maturity	Fruit Weight (g)	Fruit L (cm)	Fruit W (cm)	Fruit L/W	No. of fruit/ plant	Mkt. yield (t/ha)
1993 spring[a]									
TS 2	97	6.5	93	40	8.7	4.0	2.2	73.0	38.5
TS 33	90	6.8	95	154	18.1	4.8	3.8	17.7	36.2
Uttara	97	7.7	94	45	8.6	4.0	2.1	51.5	30.4
Sufala	77	6.0	86	58	17.0	2.8	6.1	34.7	26.6
Ichiban PU F_1	86	6.5	80	113	17.8	4.2	4.4	14.9	22.5
Mean of 17 var.	83	5.5	84	92	15.3	3.7	4.4	22.7	20.8
LSD (5%)	6	0.7	4	13	2.2	0.7	0.6	7.9	4.1
CV (%)	5	9.0	3	10	9.9	12.6	9.7	24.4	13.9
1994 spring[b]									
Daikokuden F_1	101	4.8	81	134	12.5	4.6	2.7	33.0	58.4
TS 24	116	6.8	88	146	16.4	4.1	4.1	28.3	54.2
TS 33	111	6.2	90	133	16.0	4.4	3.7	30.5	53.4
P.P. Long	94	6.8	80	81	16.8	3.4	5.0	49.4	53.4
Shanshi Naga F_1	93	4.2	81	100	14.2	4.0	3.6	40.2	53.1
Mean of 17 var.	109	5.8	87	132	16.8	3.9	4.5	33.5	48.8
LSD (5%)	8	0.9	3	14	2.0	0.5	0.5	7.8	9.3
CV (%)	5	11.2	3	9	8.3	8.6	8.6	16.3	13.4

[a] Planting dates: sowing 30 March; transplanting 30 April
[b] Planting dates: sowing 4 February; transplanting 15 March

Table 5. Horticultural characters and yield of top 5 teardrop eggplant entries, 1993 and 1994 spring EVT

Trial/Variety	Plant height (cm)	No. of branches	Days to maturity	Fruit Weight (g)	Fruit L (cm)	Fruit W (cm)	Fruit L/W	No. of fruit/ plant	Mkt. yield (t/ha)
1993 spring[a]									
Othello F_1	89	6.1	94	321	10.8	8.2	1.3	9.8	41.7
Oria F_1	125	6.9	95	306	11.6	7.5	1.6	8.4	34.0
Bonesa F_1	110	6.2	90	246	12.0	7.6	1.6	10.0	32.9
Fontana F_1	120	7.1	96	314	11.8	8.2	1.4	8.0	32.4
Queen of Sheba F_1	123	6.6	89	355	12.1	8.0	1.5	6.7	31.5
Mean of 22 var.	105	5.7	86	245	10.9	6.7	1.7	6.7	21.2
LSD (5%)	9	0.8	6	41	1.2	0.7	0.2	2.6	7.4
CV (%)	6	10.4	5	12	8.0	7.8	8.1	27.6	24.7
1994 spring[b]									
Agora F_1	116	5.9	87	285	10.9	7.2	1.5	16.1	61.0
Galine	131	5.4	83	275	8.7	7.1	1.2	16.6	60.3
Tania F_1	120	6.0	86	304	9.9	7.1	1.4	14.7	59.5
Edna F_1	134	5.4	89	257	10.9	7.4	1.5	17.0	56.4
Pusa Kranti	116	6.9	85	160	11.4	5.8	2.0	26.0	55.2
Mean of 17 var.	129	5.7	88	269	9.8	7.0	1.4	14.4	49.4
LSD (5%)	10	0.8	4	34	1.5	0.9	0.1	3.5	8.5
CV (%)	6	10.3	3	9	10.4	9.5	5.8	16.9	12.0

[a] Planting dates: sowing 30 March; transplanting 30 April
[b] Planting dates: sowing 4 February; transplanting 15 March

1994 spring EVT. The highest marketable yields for the cylindrical and teardrop types were 58.4 and 61.0 t/ha, and the lowest yields 38.8 and 31.7 t/ha (tables 4 and 5), respectively. Fruit weights of the cylindrical type ranged from 81 to 146 g; teardrop types ranged from 160 to 317 g. Number of fruit per plant differed largely between the two types. Cylindrical fruit types produced an average of 33.5 fruit, while the teardrop fruit types produced only a mean of 14.4 fruit.

Comparing the entries included in both EVTs, number of fruit per plant and marketable yields in the 1994 EVT were all higher than in the 1993. This was due to better plant growth and crop management in 1994. Among the cylindrical entries, TS 33 performed consistently well in both trials. For the teardrop entries, the yields of Othello F_1, Oria F_1, Fontana F_1, and Bonesia F_1 were stable, but their ranking changed due to the addition of some new entries.

Correlation analysis showed that marketable yields were significantly correlated with number of branches, days to maturity, and number of fruit per plant (table 6). In breeding, less branching is preferred because it contributes to less pruning cost. However, when number of branches decreases, the yield might decrease as well. Number of fruit per plant was found to be negatively correlated to fruit weight (r = -0.729). Increasing the number of fruit per plant but maintaining the fruit size is the key factor to increasing the yield of a given variety.

Table 6. Correlation coefficients and probability between some horticultural characteristics of eggplant[a]

	Plant height	No. of branches	Days to maturity	Fruit weight	No. of fruit/plant	Mkt. yield
Plant height		0.385**[b]	0.581**	0.086	0.094	0.206
No. of branches			0.584**	- 0.065	0.457**	0.742**
Days to maturity				- 0.165	0.541**	0.648**
Fruit weight					- 0.729**	- 0.110
No. of fruit/plant						0.660**

[a] Planting dates: sowing: 30 March 1993; transplanting: 30 April 1993; N = 68

[b] *significant at 5% level; ** significant at 1% level

Effects of Pruning on Yield, Number of Fruit per Plant, and Fruit Weight

Summary

Pruning and defoliation are common cultural practices adopted by eggplant growers in Taiwan to produce straight and uniform colored fruit. These practices are very costly. To study the effects of pruning on yield, number of fruit per plant, and fruit weight, an experiment using four varieties was conducted in a split-plot design in spring 1994. Pruning was done on the lateral shoots below the one just under the first bifurcation on the main stem.

Results of the analysis of variance indicated that the effect of pruning was highly significant for yield and number of fruit per plant. Interaction between pruning and variety for yield was also significant. Yield decreases of 11-25% as affected by pruning were observed among the four varieties. Pruning treatment produced 16-26% less fruit per plant than the control. Considering the time and labor costs involved in pruning, this practice is not recommended for eggplant cultivation under AVRDC conditions.

Introduction

The long cylindrical and glossy purple eggplant type is the only type that Taiwanese consumers prefer. To produce long and straight fruit, they should be borne at least 30 cm aboveground. To enhance uniform fruit color, defoliation must be done periodically to increase the amount of sunlight on the fruit surface, but not strong enough to cause sunscald. Pruning and defoliation are thus commonly practiced by farmers. These practices are very costly because labor is quite expensive in Taiwan. An experiment was conducted to study the effects of pruning on yield, number of fruit, and fruit weight. The feasibility of using this practice in eggplant cultivation at AVRDC was investigated.

Materials and Methods

Four varieties, including two each of the cylindrical and teardrop fruit types, were chosen for the experiment. Each variety has two treatments: pruning and nonpruning (control). The experiment was a split-plot with pruning for the main plot and varieties for the subplot treatments with four replications. Each subtreatment contained two-row plots with 24 plants per plot. Spacing was 1.5 m between rows and 0.5 m between plants. There were two border rows between each main plot. The border row next to the pruning treatment was also pruned.

Seeds were sown on 9 March and seedlings transplanted on 11 April. The first pruning was done on 18 May by removing lateral shoots below the one just under the first bifurcation on the main stem. Periodical removal of lateral shoots was also practiced. A 1.2-m bamboo stick was stuck beside the plant. Along the edges of the pruned beds, sticks were also stuck and nylon ropes were tied on top of the sticks along the edge to support the drooping fruiting stems. Other cultural practices used were based on the standard procedures adopted by farmers for eggplant production. Fruit were harvested weekly for 60 days until the plants were destroyed by typhoons. Yield, number of fruit per plant, and fruit weight were calculated.

Results and Discussion

Analysis of variance for the split-plot experimental design indicated that pruning significantly affected yield and number of fruit per plant. However, pruning had no significant effect on fruit weight (table 7). Pruning reduced yields of the four eggplant varieties by 11-25%. Number of fruit per plant was also reduced significantly by 16-26%. Pruned Othello F_1 produced 9.5 fruits/plant compared to 12.9 for the unpruned. Interaction between pruning and variety for yield was also significant. Pingtung Long was the most affected variety while Epic F_1 was the least affected one. Fruit weight of all four varieties did not differ widely between pruning treatments because experienced workers harvested fruit at the right stage.

Table 7. Yield, number of fruit per plant, and fruit weight of eggplant as affected by pruning[a]

	Yield (t/ha)		No. of fruit/plant		Fruit wt (g)	
Variety	P[b]	UP[b]	P	UP	P	UP
Shanshi Naga F_1	42.8	54.8	31.4	39.7	102	104
Epic F_1	38.9	43.5	12.0	14.3	243	229
Pingtung Long	32.3	42.8	19.0	24.3	128	133
Othello F_1	39.1	49.5	9.5	12.9	310	297
Mean	38.3	47.6	18.0	22.8	196	191
F test[c]	P**		P**		P[ns]	
	V**		V**		V**	
	PxV*		PxV[ns]		PxV[ns]	

[a] Planting dates: sowing: 3 March 1994; transplanting: 11 April 1994

[b] Treatment: P = pruned, with 3 main branches left; UP = unpruned; date of pruning: 18 May 1994

[c] P = pruning, V = variety; *, ** = significant difference at 5%, 1% level, ns = not significant

Pruning slightly enhanced plant and fruit growth. Regardless of the variety, the mean plant height and fruit length were 110 cm and 14.0 cm in the pruned plots compared to 103 cm and 13.8 cm, respectively, in the unpruned plots. Yield is positively correlated to number of branches and number of fruit per plant (information derived from EVTs). Pruning decreased yield and increased labor cost, and thus, it is not recommended for eggplant cultivation at AVRDC.

Crosses and Generation Advance

A total of 122 crosses were made between parents with desirable horticultural traits or with bacterial wilt resistance (BWR) (table 8). Pedigree selections of 10 F_2 populations are being conducted in the field for high yield, earliness, erect plant type, and good horticultural traits. For incorporation of BWR, 10 F_2 populations were screened in the greenhouse for resistance to the disease. The selected resistant plants are being grown in the field for further evaluation and selection. In addition, 15 diallel F_1 crosses were developed to study the genetics of resistance to bacterial wilt.

Table 8. Crosses developed in 1993-94 in the eggplant improvement project

Objectives of crosses	No. of crosses
1. Development of breeding varieties/lines with erect plant type, earliness, and good horticultural traits	37
2. Incorporation of bacterial wilt resistance	85
3. Genetics of resistance to *P. solanacearum*	15

Determination of Natural Outcrossing Rate

Summary

The natural outcrossing rate in eggplant was determined in an experiment using a green-stemmed variety, TS 56B, and a purple-stemmed variety, Slim Jim. Plants of TS 56B were grown in the field surrounded by plants of Slim Jim. Seeds harvested from TS 56B were germinated in the greenhouse and the hypocotyl color of seedlings was examined. The result indicated that the natural outcrossing rate in eggplant at AVRDC fields was 2.7%. On the other hand, only 0.05% outcrossing was detected in plants covered with screen nets.

Introduction

Eggplant is an autogamous species. It is normally self-pollinated. The amount of natural cross-pollination may vary depending on genotype, location, and insect activities. Genetic or environmental modification of stigma position can also affect the degree of cross-pollination. Long-styled and short-styled flowers are commonly found in eggplant. The high rate of crossing poses problems in assuring inbreeding and maintaining genetic purity of varieties in seed production and breeding programs. Furthermore, a successful breeding program may require the determination of rates of crossing for the particular area where it will be conducted. This study, therefore, investigated the percentage of outcrossing in the field by using hypocotyl color as a marker.

Materials and Methods

In eggplant, the gene expressing a purple hypocotyl is dominant. Thus, the purple hypocotyl was used as a marker to determine the rate of outcrossing in the field. To determine the natural outcrossing rate of eggplant, two varieties, TS 56B and Slim Jim, were used in this experiment. TS 56B has green hypocotyls and stems, while Slim Jim has purple ones.

TS 56B and Slim Jim were sown on 19 January. Seedlings were transplanted to the field on 2 March. Spacing used was 1.5 m between rows and plants. Slim Jim was planted in odd rows and TS 56B in even rows. The green-stemmed TS 56B was surrounded by purple-stemmed Slim Jim. When Slim Jim was in full blossom, fruit and flowers of TS 56B plants in one row were removed. Five of the plants were then covered with screen nets. The screen net was 1.5 m long x 1.5 m wide x 1.5 m high and there was only one TS 56B plant in each screen net. Mature fruit was harvested from both open field and net-covered TS 56B plants. Collected seeds from both sources were sown in a greenhouse on 24 August. Two weeks after sowing, the hypocotyl colors of seedlings were examined and the outcrossing rate was calculated.

Results and Discussion

Experimental results showed that under very careful observation, purple hypocotyl was a fairly accurate marker for identifying the outcrossed progenies at the seedling stage. Under open field conditions, a total of 261 out of 9534 seedlings had purple hypocotyl, which indicated that the seedlings were produced from outcrossed seeds of TS 56B. On the other hand, only 2 out of 4328 seedlings were found to be outcrossed under screen-net covered plants. The results showed that the natural outcrossing rate of eggplant in an open field with a spacing of 1.5 m was 2.7% (table 9). When plants were covered with screen nets, the outcrossing rate was only 0.05%. Thus, the use of screen nets is recommended to promote inbreeding and seed production in the field.

Table 9. Outcrossing rate of eggplant when grown in screen nets and in open field at AVRDC, spring 1994

Category	Open field	Screen net
Total no. of seedlings	9534	4328
No. of seedlings with purple hypocotyl	261	2
No. of seedlings with green hypocotyl	9273	4326
Outcrossing rate (%)	2.74	0.05

Identification of Sources of Resistance to Bacterial Wilt

Summary

Six batches of eggplants, including one batch of 19 commercial varieties, two batches of germplasm accessions from AVRDC's GRSU, and three batches of germplasm from India, were screened for resistance to bacterial wilt (BW) in the greenhouse. No resistance was found among the commercial varieties. Among the 76 accessions from GRSU, 24 were classified as resistant and 3 accessions, TS 3, TS 60A (Pale Green), and TS 89, showed no wilt symptoms. Twelve of the 95 Indian germplasm accessions appeared to have a high level of resistance. Eight accessions, Arka Nidhi, Arka Kehav, Arka Neelkantha, BB-1, BB-44, BB-49, EP 49, and Surya, had no symptoms.

A field screening trial for BWR was conducted in late summer. Forty-nine selected accessions which were screened for BWR in the greenhouse were grown in the disease field (prepared by the Plant Pathology Unit). Among the 49 accessions, 8 had high resistance levels with less than 10% disease index (DI). Six varieties, TS 36, Terong Lalap, TS 75, Hijau Besar, Terung Hijau Kecil, and Terong Hijau, exhibited consistent resistance to BW in both greenhouse and field screenings. In contrast, eight resistant accessions showed either moderate resistance or moderate susceptibility in the field. The inconsistent results in resistance screening of some of the accessions will be reexamined in 1995.

Introduction

Bacterial wilt, caused by *Pseudomonas solanacearum*, is an important disease in eggplant production. The disease was identified as one of the most important diseases in our survey of vegetable researchers in Asia. Yield losses of 50% were observed in the U.S., and higher losses of 75-81% and 50-100% were reported in India and Indonesia. The pathogen can survive in the soil in areas with mild to warm climates for years, thus this disease is a serious constraint in the subtropics and tropics. Identifying sources of resistance and breeding for resistance are major focuses of the center's eggplant breeding program.

Materials and Methods

Commercial varieties. In 1993, a total of 47 commercial varieties were evaluated for resistance to BW. The results for 28 varieties had been included in the AVRDC 1993 Progress Report. The other 19 varieties, along with 2 check varieties, were sown on 27 August and inoculated on 29 September 1993.

Germplasm collection from GRSU. In 1994, 76 eggplant accessions from the GRSU collection were screened for resistance to BW. The first 38 accessions were sown on 28 June and inoculated on 28 July. The second 38 accessions were sown on 16 August and inoculated on 16 September. Two other varieties, TS 56B and Bonne, were used as the resistant and the susceptible checks, respectively.

Germplasm collection from India. The screening for resistance of 95 accessions collected from India was done in three batches. The three sowing dates were 26 April, 14 June, and 12 August; and the inoculating dates were 24 May, 13 July, and 13 September, respectively. The two check varieties were also included in each batch.

Experimental design. Seeds were sown in 7.5-cm plastic pots in the greenhouse. The experimental design used was RCBD with three replications. Each replication contained 15 plants (pots).

Inoculation. Inoculation was done by combining root wounding and soil drenching. Roots were wounded by cutting with a knife and then 30 ml of *P. solanacearum* (strain PSS97) inoculum was poured on the soil. The concentration of the inoculum was 10^8 bacterial cells/ml. After inoculation, the media were kept moist and temperatures were maintained at >25°C. Records were taken every other 2 days of the date of symptom appearance until plant death and experiments were terminated 30 days after inoculation (DAI). Disease reading was the same as for 1993, but the grouping of BW reaction was slightly different from last year's. The variety with a DI less than 10% was classified as resistant (R); 10-20%, moderately resistant (MR), 20-40%, moderately susceptible (MS); and more than 40%, susceptible (S).

Field screening trial. A field screening trial for BWR was conducted in late summer. Forty-nine selected accessions from all the four BW reaction groups had been screened for resistance in the greenhouse trials. Seeds were sown on 1 August in the greenhouse and transplanted to the diseased field prepared by the Plant Pathology Unit. However, the typhoons in July-August destroyed the inoculated tomato plants. Therefore, the pathogen distribution was not even, and only a few eggplants showed symptoms. Artificial inoculation was done on 13 October by cutting roots and drenching with 200 ml inoculum/plant. The concentration was 10^8 bacterial cells/ml. Disease reading was the same as for the greenhouse; observations were ended at 77 DAI (the first inoculation, i.e., transplanting).

Results and Discussion

Commercial varieties. There was no resistant variety among the 19 entries screened in 1993. The DI ranged from 68% to 100% with a mean of 90%. The lowest DI of 68% was shown by Patch Bulat, which was also classified as susceptible. However, some varieties have good yield potentials or good horticultural traits and are included in our EVT. Thus, the improvement of their resistance to BW is necessary.

Germplasm collection from GRSU. There were some resistant accessions found in the GRSU germplasm collection. In the first batch, seven accessions were classified as resistant and TS 89 showed no symptoms at all (table 10). In the second screening, as many as 16 accessions had DI equal or less than 10%, and TS 3 and Pale Green (TS 60A) were free from wilt symptoms (table 10). These resistant accessions were mainly collected from Indonesia, Malaysia, and Thailand.

Germplasm collection from India. Among the 95 accessions collected from India, 12 appeared to have a high level of resistance (table 11). Eight accessions showed no wilt symptoms: Arka Nidhi, Arka Kehav, Arka Neelkantha, BB-1, BB-44, BB-49, EP49, and Surya. The reconfirming experiments are ongoing in the growth room.

Field screening trial. Among the 49 entries, eight had high resistance levels with DI less than 10% (including the resistant check TS 56B, table 12). Six entries, TS 36, Terong Lalap, TS 75, Hijau Besar, Terung Hijau Kecil, and Terong Hijau (TS 56B, the resistant check), showed consistent resistance in both greenhouse and field. In contrast, eight resistant entries (from greenhouse screening) were either moderately resistant or moderately susceptible or susceptible in the field.

The inconsistent results might be attributed to factors such as environmental changes between greenhouse and field, growth differences of plants, and genotypic differences within the accession. These differences might have influenced the degree of susceptibility of the accessions. The experiment will be repeated in 1995, and the resistance of the resistant accessions will be confirmed using a soil drenching with root-severing method. In addition, vascular discoloration, ELISA, and selective medium will be employed to characterize resistance.

Table 10. Disease indices and percentage of nonsymptomatic plants of resistant eggplant germplasm collections of GRSU inoculated with *Pseudomonas solanacearum* PSS97[a]

Acc. no.	Variety	Disease Index[b] (%)	Nonsymptomatic plants (%)	Bacterial wilt reaction[c]
First batch[a]				
TS 7	MTE2	7	89	R
TS 55	Terong Lalap	1	98	R
TS 60B	Pale Green	15	82	MR
TS 61	Hijau	12	84	MR
TS 66	Marukan	0	96	R
TS 69	Gelatik	13	87	MR
TS 88	Ihijau	7	93	R
TS 89		0	100	R
TS 91	Hijau Besar	7	93	R
TS 103B	Terung Hijau Kecil	4	96	R
TS 56B	Terong Hijau (R ck)	5	96	R
S 120	Bonne (S ck)	98	0	S
Mean		41.9		
CV		33.2		
LSD (5%)		22.7		
Second batch[a]				
TS 3		0	100	R
TS 12	Manggol	9	77	R
TS 15	Bulat Keul	17	52	MR
TS 36		3	87	R
TS 38	Sarikel var.	7	91	R
TS 43	Dayak	1	96	R
TS 46		1	96	R
TS 47A		1	96	R
TS 56A	Terong Hijau	11	82	MR
TS 59	Terong Putih	10	80	R
TS 60A	Pale Green	0	100	R
TS 74		14	80	MR
TS 75		4	89	R
TS 76		7	84	R
TS 77		7	82	R
TS 79		10	85	R
TS 81		2	91	R
TS 82		12	87	MR
TS 85	Hijau	4	91	R
TS 90		1	96	R
TS 56B	Terong Hijau (R ck)	1	98	R
S 120	Bonne (S ck)	98	0	S
Mean		29.4		
CV		26.2		
LSD (5%)		12.5		

[a] Planting dates: first batch: sowing, 28 June; inoculation, 28 July; second batch; sowing, 16 August; inoculation, 16 September

[b] Disease index = $(\Sigma n_i \times i)/(N \times i_{max})$ where n = no. of plants, i = 0-5, i_{max} = 5, N = total plants tested

[c] R = DI% less than 10%; MR = 10-20%; MS = 20-40%; S = more than 40%

Table 11. Disease indices and percentage of nonsymptomatic plants of resistant eggplant accessions from India inoculated with *Pseudomonas solanacearum* PSS97 for 30 days[a]

Batch no.	Acc. no.	Variety	Disease index[b] (%)	Nonsymptomatic plants (%)
Batch 1	1	Arka Nidhi	0	100
	2	Arka Keshav	0	100
	4	Arka Neelkantha	0	100
	6	BB-1	0	100
	8	BB-49	0	100
	9	EP49	0	100
	15	Green Long	1	99
	21	SM6-6	2	98
Batch 2	141	Surya	0	100
Batch 3	11	BB-44	0	100
	10	BB-13-1	10	90
	7	BB2	9	91

[a] Planting dates: batch 1: sowing, 26 April; inoculating, 24 May; batch 2: sowing, 14 June; inoculating, 13 July; batch 3: sowing, 12 August; inoculating, 13 September

[b] Disease index = $(\Sigma n_i \times i)/(N \times i_{max})$ where n = no. of plants, i = 0-5, i_{max} = 5, N = total plants tested

Table 12. Disease indices and percentage of nonsymptomatic plants of resistant eggplant accessions inoculated with *Pseudomonas solanacearum* PSS97 or showing different reactions at field and greenhouse screening[a]

Acc. no.	Variety	Disease index[b] (%)	Nonsymptomatic plants (%)	Bacterial wilt reaction[c] Field	Greenhouse[d]
S 014	Slim Jim	11	81	MR	R
S 026	M701 F_1	33	56	MS	R
S 030	Senguro No. 2F_1	51	47	S	MR
S 038	Masumi F_1	45	44	S	MR
S 042	Daikokuden F_1	61	33	S	MS
S 116	Ma-su	17	58	MR	S
S 117	Pingtung Long	42	37	S	MR
TS 1		11	61	MR	MS
TS 7	MTE 2	13	78	MR	R
TS 15		12	80	MR	MR
TS 33		12	70	MR	MS
TS 36		2	94	R	R
TS 55	Terong Lalap	4	92	R	R
TS 56A	Terong Hijau	21	72	MS	MR
TS 60A	Pale Green	45	47	S	R
TS 61	Hijau	18	81	MR	MR
TS 63		87	6	S	R
TS 64	Jackpot	16	67	MR	MS
TS 66	Marukan	24	70	MS	R
TS 74	Bulat Keul	4	89	R	MR
TS 75		4	83	R	R
TS 82		2	89	R	MR
TS 87	Glatik	10	86	MR	S
TS 88	Ihijau	28	64	MS	R
TS 89A		10	78	MR	R
TS 89B		27	67	MS	R
TS 91	Hijau Besar	3	97	R	R
TS 103B	Terung Hijau Kecil	5	92	R	R
TS 119		10	78	MR	-
TS 56B	Terong Hijau (R ck)	8	89	R	R
S 120	Bonne (S ck)	94	3	S	S
Mean		40.6			
CV		29.6			
LSD (5%)		19.5			

[a] Planting dates: sowing, 1 August; 1st inoculation, 7 September; 2nd inoculation: 13 October

[b] Disease index = $(\Sigma n_i \times i)/(N \times i_{max})$ where n = no. of plants, i = 0-5, i_{max} = 5, N = total plants tested

[c] R = DI% less than10%; MR = 10-20%; MS = 20-40%; S = more than 40%

[d] From the results of the previous greenhouse screening

Inoculum Concentration, Seedling Age, and Bacterial Wilt Severity

Summary

Experiments were designed and conducted to study the effects of inoculum concentration and seedling age on the bacterial wilt severity of resistant and susceptible eggplant varieties. Susceptibility to BW decreased in resistant varieties as the seedling age increased from 20 to 40 days. In the susceptible Bonne variety, there was no significant decrease in susceptibility with increase in seedling age. Decreasing the inoculum concentration from 10^8 to 10^6 cells/ml did not decrease the disease severity on 20-to 40-day-old seedlings of Bonne. However, it did decrease disease indices of the moderately resistant variety, Pingtung Long, and the resistant variety, TS 56B, when seedlings were 20-30 days old.

Introduction

The technique for BW resistance screening for eggplants at AVRDC was derived from that used for tomato. The inoculum concentration of 10^8 cells/ml is used for tomato and pepper at AVRDC. The response of eggplants to this concentration was fine in the preceding screening trials. However, the effect of inoculum concentration on disease development in eggplant is unknown. On the other hand, resistance to diseases in some crops sometimes increase when the plants grow older. Currently, 30-day-old seedlings are used for screening; the following 30 days are set aside for symptom observation. BW severity in eggplant at different ages also needs to be studied. To investigate the effect of inoculum concentration and seedling age on the BW severity of resistant and susceptible eggplant varieties, an experiment was conducted in summer. The experiment aimed to develop the most effective screening technique for large numbers of plants under greenhouse conditions.

Materials and Methods

Three varieties, TS 56B (a resistant variety), Bonne (a susceptible variety), and Pingtung Long (a moderately resistant variety) were selected for this study. Seeds of the three varieties were sown at 10-day intervals on 3, 13, and 23 June, respectively. Seedlings were inoculated on 13 July when they were 20, 30, and 40 days old. The *P. solanacearum* inoculum was prepared by the Plant Pathology Unit in two concentrations: 10^6 and 10^8 cells/ml. Each variety received six treatments of different combinations, i.e., the three different ages of seedlings were inoculated with two inoculum concentrations. The total of 18 treatments were arranged in RCBD with three replications. Each replication contained 15 plants. The method of inoculation and disease reading were the same as those for the screening trials.

Results and Discussion

The different inoculation methods were not compared. However, soil drenching with root-severing showed fairly good differentiation between resistant and susceptible plants. A decrease in the inoculum concentration from 10^8 to 10^6 cells/ml did not affect disease severity on 20-to 40-day-old seedlings of Bonne and 40-day-old TS 56B and Pingtung Long. However, it did decrease the disease severity in 20-to 30-day-old TS 56B and Pingtung Long.

Susceptibility to BW decreased in both the resistant TS 56B and the moderately resistant Pingtung Long as the seedling age increased from 20 to 40 days (fig. 2). There were no significant differences in the susceptible Bonne in this age range. According to the results, the DI of all three varieties did not significantly differ when seedlings were 20-30 days old. However, mature plant resistance was observed on the resistant and moderately resistant varieties. Inoculation of 30-day-old eggplant seedlings using an inoculum with a concentration of 10^8 cells/ml gave a good estimate of relative levels of resistance. To effectively mass-screen BW resistance, a younger seedling age might be used in future particularly for segregating populations of breeding programs. Another reconfirmation trial is ongoing in the greenhouse.

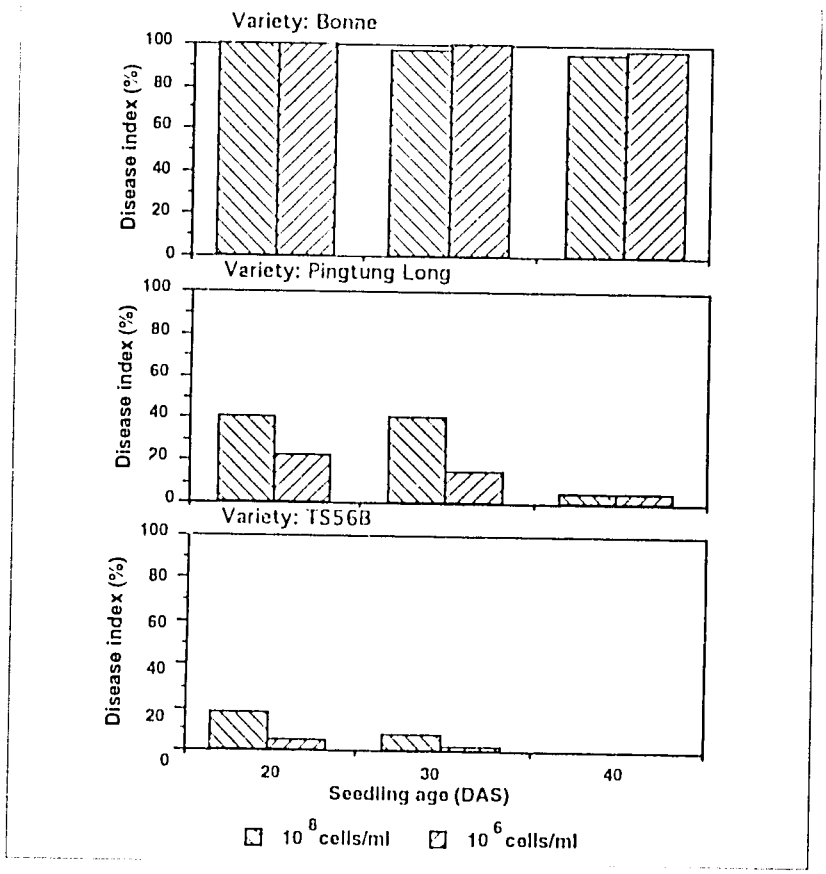


Fig. 2. Effects of seedling age and pathogen concentration on the screening for bacterial wilt resistance in eggplant

Seed Distribution

The Eggplant Breeding Unit distributed 193 seed samples to 24 countries and territories, including Belize, Cambodia, China, Ethiopia, Ghana, India, Jordan, Kenya, Korea, Laos, Liberia, Malawi, Mozambique, Namibia, Nigeria, Philippines, Seychelles, Sigatoka, Sri Lanka, Tanzania, Taiwan, Uganda, Vietnam, and West Africa. So far, no feedback has been received.

Eggplant Entomology

Control of Eggplant Fruit and Shootborer

Summary

Our survey of vegetable researchers in Asia has identified eggplant fruit and shoot borer, *Leucinodes orbonalis* Guenée, as the most destructive pest of eggplant. A pest monitoring study resulted in the discovery of a larval parasite, *Eriborus sinicus* (Holmgren) that attacks *Leucinodes* in the field. We will explore its utility in 1995. We are now developing a simple mass-rearing procedure. A sex pheromone blend which could aid in monitoring the incidence of this pest is being tested in Bangladesh, Philippines, and Taiwan. After we succeed in insect mass rearing, our research on several fronts will be expanded.

Introduction

Since eggplant is a relatively new crop for AVRDC, we conducted a survey in 1993-94 of national program scientists in Asia – where 70% of the world's eggplant is grown – to find out major insect pests. The results of the survey are summarized in table 1. Among the dozen Asian countries surveyed, except for Indonesia and Malaysia, all countries considered the eggplant fruit and shoot borer, *Leucinodes orbonalis* Guenée (Lepidoptera: Pyralidae), as the most destructive pest. Species of *Epilachna* beetles were mentioned as the first or second most important in five countries. Among the other insect pests cited, none was important in more than three countries. In view of the survey results, AVRDC's entomological research thrust for eggplant during the next few years will be confined to the control of the eggplant fruit and shoot borer or *Leucinodes*.

Ongoing research

During 1994 we conducted an exhaustive survey of literature on this pest. So far, we were able to find over 350 citations on this pest. The lion's share of this publication volume comes from India — 293 of 351 publications. Most of the research, thus, is conducted in India where this pest is considered as one of the top three economically important insect pests. Unfortunately, a large portion of the past and current research in Asia, particularly in South Asia, has been devoted to chemical control (table 2). Very scanty information exists on biology, ecology, and biological control. As a result, initially at least, it will be difficult to develop an integrated pest management (IPM) project for this pest. Some of the national program research needs to be redirected to develop alternative control measures to reduce the excessive dependence on chemical insecticides. Countries in South Asia have voted to include *Leucinodes* as a target pest for collaborative research to be funded, hopefully, by the Asian Development Bank, starting in early 1996.

Table 1. Important insect pests[a] of eggplant in Asia

Country	Priority	Pests
Pakistan	1	Eggplant fruit & shoot borer
	2	*Epilachna* beetles
India	1	Eggplant fruit & shoot borer
	2	*Epilachna* beetles
Sri Lanka	1	Eggplant fruit & shoot borer
	2	Green peach aphid
Nepal	1	Eggplant fruit & shoot borer
	2	*Epilachna* beetles
Bangladesh	1	Eggplant fruit & shoot borer
	2	Cotton leafhopper
Thailand	1	Eggplant fruit & shoot borer
	2	*Thrips flavus*
Malaysia	1	*Thrips palmi*
	2	*Epilachna* beetles
Indonesia	1	*Epilachna* beetles
	2	Green peach aphid
Philippines	1	Eggplant fruit & shoot borer
	2	Cotton leafhopper
Cambodia[b]	1	Eggplant fruit & shoot borer
	2	*Thrips palmi*
Laos[b]	1	Eggplant fruit & shoot borer
	2	Fruitfly
Vietnam	1	Eggplant fruit & shoot borer
	2	Green peach aphid

[a] Eggplant fruit & shoot borer = *Leucinodes orbonalis*; *Epilachna* beetles = *E. vigintioctopunctata*, *E. duodecastigma*, *E. indica*; cotton leafhopper = *Amrasca biguttula biguttula*; fruitfly = *Bactrocera latifrons*; green peach aphid = *Myzus persicae*

[b] Information for Cambodia and Laos are based on ACIAR survey

Table 2. Publications on various aspects of *Leucinodes*

Topic	No. papers
Occurrence	15
Biology	19
Ecology	14
Physiology	4
Taxonomy	3
Damage	13
Alternate hosts	9
Mass rearing	4
Sex pheromone	5
Biological control	22
Host-plant resistance	63
Chemical control	153
General	42

Leucinodes occurs in all countries of Southeast Asia including China and Japan, South Asia, Saudi Arabia, and all countries in Africa bordering the Indian Ocean. This pest is also found in Zaire, Ghana, Burundi, Cameroon, Nigeria, Sierra Leone, and Uganda in Africa.

Leucinodes lays eggs on eggplant leaves and neonate larvae bore in tender shoot or fruit if fruits are formed. Larvae feed concealed inside the shoot or fruit. The presence of a larva in the shoot is indicated by fresh frass at the hole it made in that plant part. Later the portion of the shoot distal to the larval entry hole wilts. The presence of the insect in the fruit can be detected by the larval entry hole which is usually plugged by frass. After feeding, full-grown larva pupates in the soil. Because of the concealed nature of larval and pupal stages, this insect is difficult to control by conventional means such as insecticide application. However, due to the lack of suitable practical and effective control measures, farmers spray insecticide to control this pest. Our survey in the Philippines and Taiwan show that farmers spray up to 50 insecticide sprays per cropping season of 5-6 months. This pesticide misuse has resulted in resurgence of other pests such as *Thrips palmi*.

In 1994, we monitored the incidence of this pest at AVRDC by planting eggplant once a month and recording the number of plants damaged once every 2 weeks. We frequently visited the Chinan Branch Station of Kaohsiung DAIS and the National Pingtung Polytechnic College, both located in Pingtung County, to observe the insect damage in the southern part of Taiwan where most of the eggplant is cultivated. This insect occurs practically throughout the year, but its infestation is rather low, with 10-15% plants exhibiting damage symptoms. Insect incidence will increase at AVRDC as the area under eggplant experiments increases with time. During the monitoring exercise at AVRDC, we found a new hymenopterous wasp parasite which was identified as *Eriborus sinicus* (Holmgren). A maximum of 30% of *Leucinodes* larvae were parasitized by this larval parasite in March-April. We will intensify our research on this parasite in 1995.

Two research groups, one in China and the other in Sri Lanka, have identified a sex pheromone of *Leucinodes*. We have purchased this chemical and preliminary trials in the utilization of this pheromone are under way in Bangladesh, Philippines, and at AVRDC. We are seeking collaborators in India, and Pakistan, and Sri Lanka to conduct this research. In all these countries, present infestation of *Leucinodes* is much higher than in Taiwan, hence such trials can be done much more efficiently there.

Top priority is being given to developing a mass-rearing procedure for *Leucinodes* so that we can conduct research on this pest year-round in Taiwan as well as elsewhere in Asia. We found that *Leucinodes* can feed on a blackgram-based artificial diet developed by Indian scientists, but, so far, we have failed to get this pest to lay eggs on artificial surface such as paper, aluminum foil, or cloth to enable us to harvest the eggs easily. Once we succeed in mass rearing, we will initiate research on sources of resistance to this pest. Reports from India reveal certain wild *Solanum* species to be highly resistant to this pest. We will reconfirm this observation before deciding upon the feasibility of using these materials for resistance breeding.

Mungbean Breeding

The mungbean breeding program was transferred to the AVRDC Asian Regional Center in June 1993. The mungbean breeding programs of ARC and its host institute, Kasetsart University, have been combined to maximize the use of resources. The goal of the program is to develop high-yielding lines with resistance to major diseases and insects, early and uniform maturity, insensitivity to photoperiod and temperature, resistance to shattering, and improved seed quality. Major focuses in 1994 were management of mungbean yellow mosaic virus (MYMV), management of bruchid storage insect, and restriction fragment length polymorphism (RFLP) mapping of mungbean and its application in breeding.

Yield Trials

The 19th and 20th International Mungbean Nursery (IMN) trials, one Advance Yield Trial, two different Intermediate Yield Trials, six different Preliminary Yield Trials, and three different Observation Yield Trials were conducted in 1994 dry, early wet, and late wet seasons at ARC. VC 3541B of the 19th IMN and VC 6075B of the 20th IMN outyielded the check VC 1973A and showed good agronomic traits.

Mungbean Yellow Mosaic Virus

MYMV is the most serious constraint in mungbean production in South Asia. Since the International MYMV Expert Consultation Workshop was organized by AVRDC in July 1991 in Bangkok, collaboration among AVRDC scientists and national program partners has been very active. The first International MYMV Nursery Trials have been conducted in all South Asian countries. For all entries, MYMV ratings in New Delhi, India, and Faisalabad, Pakistan, were similar, indicating no difference in race between the two countries. In Bangladesh, however, only four Pakistani lines (NM 51, NM 92, NM 94, and NM 20-21) were highly resistant. This is probably because of high pressure of disease or different races.

A conventional genetic study indicated that the resistance in NM 92 was controlled by a single major recessive gene. RFLP linkage analysis located the MYMV resistance gene near the bruchid resistance gene. Since MYMV is not present in Taiwan or in Thailand, a shuttle breeding program has been established in cooperation with the Nuclear Institute of Agriculture and Biology (NIAB) in Pakistan. The MYMV-resistant lines (NM 51, NM 92, and NW 94) were developed from crosses between AVRDC breeding lines and a local MYMV-resistant line from Pakistan. They are more resistant to MYMV than their resistance source, indicating the presence of the minor/modifying gene(s). These lines were crossed again with the AVRDC lines to improve the agronomic traits further. Some lines selected through shuttle breeding showed high MYMV resistance and even higher yield and 1000-seed weight than the parents. They are very promising not only in South Asia but also in other regions.

Bruchid Resistance

Bruchid is a very serious insect pest of mungbean and other legumes during storage. A wild mungbean accession, *Vigna radiata* ssp. *sublobata* (TC 1966), and two mungbean accessions (V 2709 and V 2802) are highly resistant to bruchid, *Callosobruchus chinensis* (L.). Genetic studies showed that bruchid resistance in each resistant line is controlled by a single dominant gene, and that these three resistant genes were nonallelic. Bruchid-resistant lines produced by conventional backcross eight times show promise, and the resistant lines with only two generations of RFLP-based backcross performed similarly as the recurrent parent.

RFLP Mapping and Application in Breeding

The University of Minnesota has developed a mungbean RFLP map with 220 markers. The total length covered by this linkage map is 1776 centimorgans (cM), and the average distance between markers is 8 cM. Some of the most important RFLP loci have been converted into PCR-based markers because RFLP markers require the use of radioactive nucleotides.

RFLP-based backcross breeding has been done in collaboration with the University of Minnesota using TC 1966 as the donor parent for bruchid resistance and VC 3890A as recurrent parent. The resistance genes for both bruchids and MYMV were found to be located on linkage group 9 near RFLP markers R26/Bng110/Bng64. Because these loci are closely located but derived from different mungbean accessions, DNA markers can be used to select specifically for those lines that have the two resistance genes joined together on the same chromosome homologue.

International Cooperation

Some 691 seed packets were distributed to 25 cooperators in 15 countries in 1994. As of 1994, 54 mungbean cultivars from AVRDC have been released by the national programs in 19 countries and are extensively grown in Thailand, China, Indonesia, and the Philippines. Recently, MYMV-resistant lines have been developed using AVRDC lines as parents. The planting area devoted to mungbean with high MYMV resistance, high yield, and other good agronomic traits, is increasing very rapidly in South Asia. A mungbean session emphasizing MYMV was organized by AVRDC during the International Symposium on Pulses Research in April 1994 in New Delhi, India.

Pepper Breeding

Genetic Resources, Enhancement, and Varietal Development

Seed Production, Observation and Evaluation Trials of New and Advanced Germplasm

Summary

Sixteen AVRDC hot pepper Szechuan purelines were inbred for two more generations for seed production and further studies to test for inbreeding depression. Three evaluation trials were conducted in 1993-94. Twenty sweet pepper F_6 lines, one parent check, and three commercial hybrids were evaluated in the 1993 hot-rainy season. Average fresh weight marketable yield of the six best lines was 19.8 t/ha, whereas the mean of all entries was 16.1 t/ha. Nine of the lines were resistant to bacterial wilt, as confirmed by partners in Sri Lanka. Five hot pepper entries were evaluated in a split-split plot design with three replications to test the effect of shade intervals and shade intensity on yield and quality traits. The significant findings from one experiment were, in general, that both fruit color value and pungency increased under shaded conditions, whereas, dry matter, lipids and yield (fruit number) significantly decreased under shade. Genotype by shade intensity interactions were significant for all the quality traits, fruit number, yield and plant height. The significant output suggested that quality trait evaluations under shaded conditions led to inflated or deflated estimates of genotype performance compared to nonshaded production, an important consideration for germplasm evaluation. The line Szechuan 921206 appeared to be relatively shade tolerant. Another evaluation trial was conducted in two seasons to test the interaction of five hot pepper genotypes with three methods of seedling establishment. Based on this preliminary work, direct sowing of hot pepper may be an effective management practice to reduce labor and cost of transplant management without consequences on yield selection.

Introduction

The objective of this subproject is to maintain seed of breeding lines and to identify superior genotypes for advanced testing or breeding via observation or evaluation trials. New testing methods are explored under evaluation trials.

Seed Production

Materials and Methods

Sixteen hot pepper Szechuan purelines were inbred for two more generations under screenhouse conditions. Five plants per line were sown and offtypes, if any, were rogued. Seed was bulked per line. Seed production under field-plot cage was conducted for INTHOPE 4.

Results and Discussion

Most of the Szechuan lines are now inbred to the OP_1S_7 (OP=open-pollinated; S=self pollination). These lines will be used for further inbreeding and future studies to test for inbreeding depression. For the distribution of the INTHOPE 4 hot pepper sets, seed production was achieved for 14 entries.

Evaluation Trials

Experiment 1

Materials and Methods

Twenty sweet pepper F_6 lines (of tapered shape), one parent check (CA 8, waxy tapered, rugose, resistant to bacterial wilt), and three commercial hybrids (Gypsy, Chung Chiao No. 5, and Mito Lee) were evaluated in the 1993 hot-rainy season. The experimental design was a randomized complete block with three replications. Plant populations were 66,667 plants/ha. The breeding lines and check were derived from Sri Lankan sweet peppers carrying resistance to bacterial wilt (screened in Sri Lanka and Taiwan) and tolerance to CVMV and CMV (screened in Taiwan).

Results and Discussion

Average fresh weight marketable yield of the six best sweet pepper lines was 19.8 t/ha, whereas the mean of all entries was 16.1 t/ha with an average fruit weight of 19.3 g. The average fresh weight marketable yield of the three hybrids was 22.0 t/ha, and their average individual fruit weight was 34 g. The average crop duration to the first harvest was 106 days from sowing. The mean sum of marketable fruits per plant was 13. Average fruit length, width, and wall thickness significantly decreased between the first and the last harvest (a 7-week period) by 4.7, 0.8, and 0.03 cm, respectively. Details of this work are reported in the 1994 training report of visiting scientist Dr. Le Thi Anh Hong from Vietnam. Analyses of variance and mean comparisons are on file with the Pepper Breeding Unit. Four of the lines turned out to be pungent types so they are targeted instead for hot pepper improvement. Three other lines were dropped because of poor performance or nonuniformity. Resistance to bacterial wilt was confirmed under controlled screening conditions in Sri Lanka in 1994 in cooperation with the Horticultural Crops Research and Development Institute (HCRDI) and Field Crops Research and Development Institute (FCRDI). Nine of the lines were most outstanding for resistance to bacterial wilt.

Experiment 2

Materials and Methods

Five hot pepper entries were evaluated in a split-split plot design with three replications to test the effect of shade intervals and shade intensity on yield and quality traits. The two shade intervals were shade at transplanting and at flowering, respectively. The three shading factors were 0, 24, and 42% shade. The experiment was conducted in two seasons.

Results and Discussion

The significant findings from the first experiment were that, in general, both fruit color value and pungency increased under shaded conditions, whereas, dry matter, lipids, and yield (fruit number) significantly decreased under shade. Genotype by shade intensity interactions were significant for all the quality traits, fruit number, yield, and plant height. The significant output from the first phase of the work was that quality trait evaluations under shaded conditions led to inflated or deflated estimates of genotype performance compared to standard nonshaded production. This has bearing on germplasm management, quality evaluation procedures, and performance under protected cultivation or mixed cropping. At present, AVRDC conducts quality trait analyses on fruit samples grown under cages with ±24% shade. On a positive note, the pungency and color composition of hot pepper could significantly be improved when grown in intercropping schemes or protected cultivation that keeps the pepper under slight shade. A shade threshold must still be determined to avert a negative correlation with fruit yield. The AVRDC line Szechuan 921206 appeared to be relatively shade tolerant. The experiment was repeated in fall 1994. Details of the study may be found in the training report of Mr. Yayeh Zewdie, AVRDC research fellow in pepper breeding from Nazareth Research Station, Ethiopia.

Experiment 3

Materials and Methods

An evaluation trial was conducted in two seasons to test the genotype by environment interaction (g x e) of five hot pepper genotypes with three methods of seedling establishment. The experimental design was a randomized complete block design in a 5 x 3 factorial arrangement. The three methods of seedling establishment were: (1) direct sowing, (2) containerized transplants, and (3) bareroot transplants. The purpose of the experiment was to find a way to improve selection efficiency and population management for the pepper breeding program. In tropical hot pepper production areas, any one of these methods is already common in commercial practice depending on resource and labor availability. Regardless of the best method for commercial crop productivity, an absence of g x e interactions under test conditions would suggest that plant breeders could employ any of the seedling methods during selection processes without adversely affecting selection.

Results and Discussion

The significant output of the first season experiment was that the g x e was not significant for fruit yield, whereas, it was for days to flowering. Therefore, based on this preliminary work, containerized transplants would not need to be used for effective selection for yield in hot pepper. Direct sowing may be a cost- and labor-efficient method of establishing breeding plots. Details of the study may be found in the training report of Mr. Yayeh Zewdie.

Hybridization and Selection of New Genotypes with Combined Disease Resistance, Quality and Regional Adaptation

Summary

Second backcrosses were completed with CVMV-resistant BC_1 plants to 18 recurrent parents of tropical hot pepper varieties and five recurrent parents of sweet pepper. From June 1993 through December 1994, three generations were completed to advance selections from F_2:F_3 lines to F_5:F_6 seed in a hot pepper pedigree selection activity. The selection criteria used were (1) survival after flooding, (2) plant vigor, (3) field resistance to virus, (4) fruit-set, (5) field tolerance to anthracnose, and (6) field tolerance to powdery mildew. A testcross analysis with a subset of these lines indicated that three of 48 testcrosses had the best flooding survival; nine had good vigor and vegetative development, and two were more resistant to bacterial spot. Two generations of selection were conducted from summer 1993 through spring 1994 on two sweet pepper (SP1 and SP2) and one hot pepper (HP1) populations. Total C_1 selections from SP1, SP2, and HP1 were 32.4, 26.7, and 64.4%, respectively, of all the plants inoculated for independent disease screening. Two cycles of a honeycomb mass selection scheme were carried out in 1993-94. From the first cycle of selection, 38 plants (5.26% selection pressure) were selected based on total dry yield (g/plant). The average performance of the selected plants over the base population was 48 fruits/plant, 49.8 g/plant, 0.2 cm, and 0.2 cm for total fruits per plant, total dry weight yield, fruit length, and fruit width, respectively. Sweet pepper was advanced from F_1:F_2 lines and 62 new crosses were made in the 1993-94 cool season. During summer 1994, 526 of the F_2 lines were screened for flood and heat tolerance. Despite severe flooding stress, many lines performed better than commercial checks or new sweet pepper accessions. Twenty-one lines were advanced to F_5:F_6 seed. Hot pepper was advanced to 337 F_1:F_2 lines and 23 new crosses were made in the 1993-94 cool season.

Experiment 1

Introduction

The objective of this activity is to incorporate resistance to CVMV in known hot and sweet pepper varieties or landraces. The breeding method employed after the first screening and selection of F_2 resistant plants is now continuous backcrossing with screening and selection for resistance. One-hundred and one different crosses involving nine sources of resistance were initially used in this breeding scheme. Six of the resistance sources were related by pedigree, but were not necessarily carrying the same genes for resistance.

Materials and Methods

A second backcross was made involving 18 recurrent parents of tropical hot pepper varieties and five recurrent parents of sweet pepper. Of the 18 hot pepper parents, 11 represent market types for AVNET countries, 4 for SAVERNET, and 3 from Mexico.

Results and Discussion

Backcrosses were completed and preparations are under way to proceed with sowing and screening to develop the BC_2.

Experiment 2

Introduction

The objectives of this activity were: (1) to develop hot pepper breeding lines with field resistance/tolerance to CVMV, CMV, anthracnose, and powdery mildew via pedigree selection. The F_3 selection process began with 925 F_2:F_3 lines among 62 single-cross combinations that were targeted to incorporate CVMV and CMV resistance into various Asian hot pepper market types; and (2) to test for combining ability of some of the F_3:F_4 lines in an early-generation testcross with a virus-susceptible Ethiopian variety named Mareko Fana that has good color value.

Materials and Methods

From June 1993 through December 1994, three generations were completed to advance selections from F_3:F_4 lines to F_5:F_6 seed. Selection among F_4 lines was based on an augmented randomized complete block design with four check lines in each of 10 blocks. Selection among F_5 lines was based on an incomplete block-partial lattice design (6 x 6) for larger fruited types (≥ 4.5 - 7.8 g), and an augmented randomized complete block (with two checks randomized in each of nine blocks) for smaller fruited types (<4.5 - 0.8 g). Final F_5 selections were done on single plants only and self-pollination was assured by nylon bags that covered individual branches. The selection criteria used were (1) survival (after heavy winds and rain), (2) plant vigor, (3) field resistance to virus (CVMV, CMV), (4) fruit-set, (5 field tolerance to anthracnose and, (6) field tolerance to powdery mildew.

Results and Discussion

Ten of the best F_5 lines were derived from four single crosses involving five parents. The testcross analysis indicated that three of 48 testcrosses had the best flooding survival; nine had good vigor and vegetative development; and two were more resistant to bacterial spot. In 1995, the 10 selections of the 34 larger fruited lines will be tested in advanced yield trials [(F_6 for main summer; F_7 in fall (maybe at ARC-Thailand)] along with any other F_6 derived from the parents that had the best combining ability. Final selections will be entered in the INTHOPE 7 set in 1996. Testcross F_2 seed will be bulked among the best crosses and entered into a mass selection program under Ethiopian and Taiwan conditions.

Experiment 3

Introduction

The objective of this activity is to develop germplasm pools of hot and sweet pepper as useful sources of disease resistance and heat tolerance in various horticultural backgrounds. The breeding method used is mass selection for disease resistance with progeny tests for heat tolerance.

Materials and Methods

Two generations of selection were conducted from summer 1993 through spring 1994 on two sweet pepper (SP1 and SP2) and one hot pepper (HP1) populations. $Cycle_0$ (C_0) was screened by inoculation for CVMV resistance in two of the populations before and after transplanting, and subsequently, all populations were selected for heat tolerance under summer field conditions. $Cycle_1$ was screened under controlled conditions with independent screening against five disease reactions (bacterial spot-*Bs2* or *Bs3* resistance, bacterial spot-quantitative resistance, bacterial wilt, cucumber mosaic, and phytophthora root rot).

Results and Discussion

Total selections from SP1, SP2, and HP1 were 32.4, 26.7, and 64.4%, respectively, of all the plants inoculated. The probabilities of selecting a plant with all types of resistance were 0.03, 0.09, and 8.58% for SP1, SP2, and HP1, respectively (table 1). The probabilities of recovering plants with resistance to ≤3 pathogens were much higher. Seed was bulked from the resistant plants to form C_2 populations, a slight departure from original plans to maintain the selections by individual plants for progeny testing. The C_2 are now at the S_1 level of inbreeding (F_2=S_0) without consideration of natural outcrossing. The following guidelines are suggested for managing the populations in 1995. The HP1 can be distributed to other pepper breeders as it is for deriving inbred lines. At AVRDC it should go through one round of 'multiple' disease screening on individual plants, and then progeny tests for heat tolerance should be conducted from seed derived from individual resistant plants. The next phase would be preliminary yield trials and quality trait analysis to group the selections into market types. The sweet pepper populations need to be improved for fruit size. It is suggested that they be screened for two disease resistance combinations, e.g., chili veinal mosaic virus (CVMV) or cucumber mosaic virus (CMV) plus bacterial spot, CVMV(or CMV) plus bacterial wilt, and CVMV plus phytophthora blight. Resistant plants should be crossed to large fruited sweet pepper types. Seeds from these crosses become new C_0 populations or may be advanced by pedigree breeding.

Table 1. Cycle one mass selection controlled disease screening results in hot and sweet pepper, 1994[a]

Disease/ population[b]	SP1	SP2	HP1
	% resistant plants		
Bacterial spot (*Bs2* or *Bs3*)	2.5	17.2	na
Bacterial spot (Qt.)	14.8	25.8	29.6
Bacterial wilt	30.5	16.1	53.4
CMV (tolerance)	53.8	28.8	82.0
Phytophthora root rot	41.0	45.1	66.1
Probability % for all in one plant	0.025	0.093	8.57

[a] Cycle$_0$ was selected partly for CVMV resistance after inoculation and heat tolerance under summer field conditions, 1993

[b] SP = sweet pepper, HP = hot pepper

Experiment 4

Introduction

The objective of this activity is to improve yield in an Ethiopian hot pepper variety through a honeycomb mass selection technique.

Materials and Methods

Two cycles of a honeycomb mass selection scheme were carried out in 1993-95.

Results and Discussion

From the first cycle of selection, 38 plants (5.26% selection pressure) were selected based on total dry yield (g/plant). The average performance of the selected plants over the base population was 48 fruit/plant, 49.8 g/plant, 0.2 cm, and 0.2 cm for total fruit per plant, total dry weight yield, fruit length, and fruit width, respectively. The second cycle of selection is still under way in the field. Details of

the study may be found in the training report of Mr. Yayeh Zewdie. C_2 seed will be sent to Ethiopia for continuous selection and variety development.

Experiment 5

Introduction

The objective of this activity is to advance breeding lines through pedigree selection and to continue crossing new genotypes to improve heat/flood tolerance, disease resistance, fruit size, and yield potential.

Materials and Methods

Sweet pepper. Sweet pepper was advanced from F_1:F_2 lines and 62 new crosses were made in the 1993-94 cool season. During summer1994, 526 of the F_2 lines were screened for flood and heat tolerance. Relatively small populations (≤100 plants) were used per line but the priority was to detect the best families for further selection.

Hot Pepper. Hot pepper was advanced to 337 F_1:F_2 lines and 23 new crosses were made in the 1993-94 cool season.

Results and Discussion

Despite severe flooding stress, many sweet pepper lines performed better than commercial checks or new sweet pepper accessions. Sixteen (3%) lines had the best fruit-set, but overall, 126 lines (24%) were selected as first priority and another 41 lines (8%) as second priority for further selection. Twenty-one lines were advanced to F_2:F_3 seed. For 1995 the following guidelines are suggested. For sweet pepper, remnant F_2 seed as well as the select group of F_3 lines of the first priority lines will be sown at larger population sizes for selection during the 1995 spring sowing. The new crosses were advanced to S_0:S_1 lines in a 1994 fall planting, along with 155 crosses that were made in 1993. For hot pepper, the 337 F_1:F_2 lines, 23 new crosses as well as 466 crosses that were made in 1993, will be sown in February 1995 for selection of the F_2 or generation advance of the F_1.

Collection, Evaluation, Multiplication and Distribution of INTHOPE Entries

Summary

The International Hot Pepper Trial Network-set 4 (INTHOPE 4) was evaluated for field performance and quality traits at AVRDC in summer 1993 and fall-winter 1993-94. Disease screening was completed against the usual 10 pathogens (see Pepper Pathology). Of the 26 entries evaluated, 16 were chosen for distribution based on their overall performance. A multilocation analysis has been conducted on the INTHOPE 1 feedback to date.

Introduction

The objective of INTHOPE is to introduce adapted hot pepper landraces and elite germplasm from different countries into appropriate production regions and to compile a database for hot pepper production and disease screening results over many geographic regions. At AVRDC, one new INTHOPE set is evaluated per year comprising two growing seasons.

Materials and Methods

The INTHOPE 4 was evaluated for field performance and quality traits at AVRDC in summer 1993 and fall-winter 1993-94. The experimental designs were randomized complete blocks with three replications. The summer planting involved two experiments, one transplanted on 1.0-m beds (T1) and the other on 1.5-m beds (T2) with population densities of 44,444 and 29,630 plants/ha, respectively. The fall trial was on 1.5-m beds at 29,630 plants/ha (T3). Disease screening was completed against the usual 10 pathogens (see Pepper Pathology).

Results and Discussion

Of the 26 entries evaluated, 16 were chosen for outside distribution based on their overall performance. Summaries of the experiment means are presented in tables 2 and 3. Fall season yields were significantly less than for the summer season, partly because there were no typhoons in summer 1993. In the fall trial, however, virus and fruitworm incidence was high, as well as some loss due to rodent damage. Means of entries are presented in table 4 for the summer experiments combined (mostly nonsignificant g x e) and the fall experiment. The highest yielding entries were LC-Serdang, PBC 581, and Matikas, whereas, Banglen, PBC 592, LV 1592, and Pant C-1 (check) had the highest fruit number for summer trials. Quality trait analyses are summarized in table 5. The mean color value significantly dropped by 20 units (x100) in the fall season trial. Otherwise there was little seasonal effect on quality traits. The specifics of all the analyses of variance and mean comparisons are on file with the Pepper Breeding Unit.

Table 2. Comparisons of INTHOPE 4 field performance in two seasons, AVRDC

Season 1993-94	Marketable fruit/plant	Total fruit/ plant	Marketable yield (t/ha)[c]	Total yield (t/ha)	Harvest index (%)
Summer[a]	146.7	180.3	8.7	10.2	36.0
Fall/winter[b]	55.5	67.0	4.4	5.1	46.1
t-value, 27 df	6.30**	7.26**	6.13**	7.07**	4.28**

[a] No typhoons in summer 1993; crop duration: 209 days after sowing (DAS)
[b] Disease pressure severe, virus, blight?; crop duration: 209 DAS
[c] Fresh weight; ** significant at P = 1%, two-tailed paired t-test

Table 3. Comparisons of INTHOPE 4 field performance under different bed spacing, AVRDC summer 1993 (no typhoons)

Bed spacing	Marketable fruit/plant	Total fruit/ plant	Marketable yield (t/ha)[c]	Total yield (t/ha)	Fruit weight (g)	Harvest index (%)
1.0 m[a]	95.2	117.1	9.2	10.8	2.84	30.5
1.5 m[b]	146.7	180.3	8.7	10.2	2.71	36.0
t-value, 27 df	5.7**	20.1**	2.16*	1.90[ns]	2.60*	5.7**

[a] Crop duration: 208 (DAS)
[b] Crop duration: 209 DAS
[c] Fresh weight; *, ** significant at P = 5% and 1%, respectively, two-tailed paired t-test; ns= not significant

Table 4. INTHOPE 4 summary of yield components and fruit characteristics, AVRDC, Shanhua, 1993-94

Name	Origin/Source	Season[1]	FN[2]	Yield components CN %	t/ha fresh[3]	CW %	t/ha dry[3]	HI	Fruit characteristics[4] IFW (g)	FL (cm)	WD (cm)	FL/WD	TH (cm)	EX (μ)
Athi slim	Kenya/Kenya Seeds	summer	39	28.6	1.5	18.8	0.5	8.4	0.9	4.9	0.7	7.6	0.1	62
Athi slim	Kenya/Kenya Seeds	fall	3	16.7	0.1	10.2	0.04	2.6	1.3	4.6	1.0	4.9	0.1	62
B.L. 39	Sri Lanka/RARC-MI	summer	203	14.7	12.9	10.0	3.00	55.5	1.8	7.3	0.8	8.9	0.1	63
B.L. 39	Sri Lanka/RARC-MI	fall	35	41.1	2.4	31.7	0.6	61.2	2.3	11.5	0.8	13.6	0.1	73
Bangchang	Thailand/ARC	summer	53	23.2	9.3	15.1	1.4	36.0	5.1	8.2	1.2	6.6	0.2	48
Bangchang	Thailand/ARC	fall	30	27.2	7.1	20.2	1.2	58.4	8.0	10.1	1.6	6.5	0.2	62
Banglen	Thailand/AVNET	summer	315	8.2	10.7	8.1	3.0	34.5	1.0	5.2	0.7	7.3	0.1	48
Banglen	Thailand/AVNET	fall	136	5.6	3.8	4.5	1.2	41.9	0.9	5.1	0.8	6.4	0.1	64
BG-1	India/MARDI	summer	219	4.6	11.0	3.5	2.7	34.2	1.5	5.6	0.8	7.6	0.1	52
BG-1	India/MARDI	fall	148	7.5	7.1	5.7	1.7	44.5	1.6	5.5	0.9	6.3	0.1	72
Cayenne Cajun 1	USA/LSU	summer	20	29.2	4.9	24.3	0.8	22.4	6.5	8.4	1.2	7.1	0.2	37
Cayenne Cajun 1	USA/LSU	fall	4	14.7	0.7	8.8	0.1	13.9	5.9	12.5	1.5	7.8	0.2	69
Chilli NP46A	India/United Seeds	summer	121	29.4	6.7	23.5	1.7	29.7	1.5	8.2	0.8	10.3	0.1	83
Chilli NP46A	India/United Seeds	fall	29	49.1	1.6	38.4	0.5	42.1	1.7	7.0	0.9	8.0	0.1	58
Chillies Giant	Sri Lanka/AVRDC	summer	199	15.5	13.4	11.5	3.4	48.1	1.9	7.4	0.9	9.1	0.1	51
Chillies Giant	Sri Lanka/AVRDC	fall	69	26.5	4.8	16.3	1.3	59.6	2.4	7.9	1.0	8.3	0.1	72
CNPH703	India/CNPH-Brazil	summer	45	25.9	1.2	20.0	0.3	7.0	0.8	5.7	0.6	9.7	0.1	48
CNPH703	India/CNPH-Brazil	fall	10	48.3	0.4	36.2	0.1	14.6	1.4	6.3	0.9	7.3	0.1	58
Kalmicho	Korea/KPNU	summer	66	26.6	7.6	16.5	1.8	45.9	3.2	6.5	1.2	5.5	0.1	73
Kalmicho	Korea/KPNU	fall	21	46.8	3.7	29.8	0.8	66.2	6.0	9.2	1.5	6.1	0.2	72
Korea 11	Korea/HES	summer	52	27.6	7.7	23.0	1.4	34.1	4.3	7.3	1.4	5.3	0.2	53
Korea 11	Korea/HES	fall	24	28.2	5.1	18.7	1.0	64.3	7.6	9.6	1.5	6.6	0.2	82
Korea 13	Korea/HES	summer	66	29.2	9.6	20.4	1.8	42.6	4.1	7.6	1.3	6.3	0.4	61
Korea 13	Korea/HES	fall	34	21.0	6.2	12.4	1.2	66.7	6.0	9.2	1.4	7.0	0.2	60
Korea 4	Korea/HES	summer	70	20.9	8.0	17.9	1.6	29.5	3.2	6.9	1.2	6.2	0.2	67
Korea 4	Korea/HES	fall	41	23.6	5.2	11.5	1.2	64.4	4.3	9.3	1.4	6.6	0.2	65
LC-Serdang	Malaysia/AVNET	summer	78	16.5	18.3	13.5	2.9	38.7	6.6	8.4	1.6	5.4	0.2	53
LC-Serdang	Malaysia/AVNET	fall	31	23.9	7.4	16.0	1.2	54.9	7.9	9.4	1.7	5.3	0.2	57
Lv. 1592	Indonesia/AVNET	summer	223	12.6	10.0	8.5	2.6	37.3	1.3	6.4	0.8	8.0	0.1	51

Table 4. continued.

Name	Origin/Source	Season[a]	FN[b]	Yield components CN %	t/ha fresh[c]	CW %	t/ha dry[c]	HI	Fruit characteristics[c] IFW (g)	FL (cm)	WD (cm)	FL/WD	TH (cm)	EX (μ)
Lv. 1592	Indonesia/AVNET	fall	65	31.9	3.3	21.9	0.8	39.7	1.8	6.5	0.9	7.6	0.1	83
Mareko Fana	Ethiopia	summer	29	19.6	3.9	11.7	0.7	23.3	3.7	6.3	1.5	4.2	0.2	58
Mareko Fana	Ethiopia	fall	13	15.4	2.8	9.8	0.6	45.6	6.5	7.2	1.8	4.1	0.2	39
Matikas	Philippines/AVNET	summer	93	34.4	13.4	24.0	2.2	43.3	4.0	10.3	1.2	8.6	0.2	62
Matikas	Philippines/AVNET	fall	23	30.2	3.0	18.0	0.5	47.4	4.3	9.0	1.2	7.3	0.2	72
Pant C-1	India/IARI	summer	261	28.1	12.5	19.3	3.3	47.9	1.3	5.8	0.8	7.3	0.1	58
Pant C-1	India/IARI	fall	135	12.6	7.0	7.9	2.0	58.3	1.8	5.3	0.9	5.7	0.1	63
PBC 455	India/AVRDC	summer	63	19.3	3.5	14.7	0.8	15.0	1.6	5.8	0.8	7.9	0.1	64
PBC 455	India/AVRDC	fall	49	16.9	2.4	15.0	0.7	41.9	1.8	7.1	0.9	7.8	0.1	69
PBC 539	AVRDC	summer	154	33.7	8.4	26.1	1.8	42.9	1.5	7.2	0.7	10.4	0.1	49
PBC 539	AVRDC	fall	184	10.1	10.7	7.2	2.6	67.4	2.0	7.8	0.9	8.8	0.1	54
PBC 581	Malaysia/AVRDC	summer	79	12.8	15.8	10.6	2.6	33.3	5.6	10.3	1.3	8.0	0.2	49
PBC 581	Malaysia/AVRDC	fall	29	11.7	6.5	6.7	1.0	45.7	7.6	11.1	1.6	7.0	0.2	72
PBC 582	Malaysia/AVRDC	summer	81	18.0	2.4	15.2	0.6	11.8	0.8	4.5	0.4	10.3	0.1	52
PBC 582	Malaysia/AVRDC	fall	37	13.8	1.1	9.3	0.3	10.8	1.1	5.2	0.6	8.4	0.1	55
PBC 592	Thailand/AVRDC	summer	258	9.3	13.9	7.9	3.7	49.7	1.6	6.1	0.9	6.8	0.1	48
PBC 592	Thailand/AVRDC	fall	97	7.7	5.2	5.1	1.4	44.4	1.8	6.1	0.9	6.7	0.1	54
PBC 622	AVRDC	summer	203	12.3	11.9	11.1	2.8	45.0	1.7	6.1	0.9	7.1	0.1	49
PBC 622	AVRDC	fall	127	7.2	8.0	4.6	1.8	51.1	2.1	6.3	0.9	6.7	0.1	69
PBC 632	Bangladesh/AVRDC	summer	93	30.6	5.9	23.6	1.4	23.7	1.8	5.6	1.1	5.3	0.1	56
PBC 632	Bangladesh/AVRDC	fall	58	19.5	3.7	13.5	0.9	36.6	2.1	5.2	1.1	4.8	0.1	79
Prapadaeng	Thailand/AVNET	summer	208	6.8	11.4	5.1	3.0	33.6	1.5	6.1	0.8	8.5	0.1	46
Prapadaeng	Thailand/AVNET	fall	74	11.2	4.0	8.2	1.0	28.2	1.8	5.8	0.8	6.9	0.1	65
Szechuan 8	Taiwan/AVRDC	summer	64	23.8	7.0	18.7	1.3	27.3	3.0	8.5	0.7	13.2	0.1	48
Szechuan 8	Taiwan/AVRDC	fall	32	15.6	5.8	9.1	1.0	59.5	6.3	11.2	1.3	8.6	0.2	60
Tumpang	Indonesia/LEHRI	summer	35	26.6	7.8	20.4	1.4	29.7	6.2	8.1	0.9	9.1	0.1	58
Tumpang	Indonesia/LEHRI	fall	17	25.6	5.6	17.5	1.0	59.4	11.4	13.3	1.6	8.7	0.2	86
Mean		summer	121	21.0	8.9	15.8	2.0	33.2	2.8	6.9	1.0	7.8	0.1	56
Mean		fall	56	21.8	4.4	14.8	1.0	46.1	3.9	8.0	1.16	7.1	0.13	66

[a] Means of three replications of two experiments for summer trial; means of three replications for fall trial (or < 3 for a few entries)

[b] Marketable

[c] Mean of 10 marketable subsamples; FN=fruit number per plant, CN=cull fruit number, CW=cull fruit weight, HI=harvest index, IFW=individual fruit weight, FL=fruit length, WD=fruit width, TH=fruit wall thickness, EX=exocarp thickness

Table 5. INTHOPE 4 summary of quality traits as determined by near infrared reflectance spectroscopy[a], AVRDC, 1993-94

Name	N[b]	Dry matter (%)	Capsaicin (mg/g DM)	Sugars (%)	Lipid (%)	Color value (*100)[c]
Athi slim	6	21.0	2.69	10.0	13.3	145.11
B.L. 39	9	23.0	1.76	14.0	11.8	151.74
Bangchang	9	13.1	2.49	20.1	9.1	150.21
Banglen	9	26.2	2.87	13.1	16.4	145.01
BG-1	9	23.9	3.93	9.1	16.7	119.04
Cayenne Cajun 1	3	13.9	1.16	27.2	7.3	145.00
Chilli NP46A	9	21.5	2.58	10.1	12.2	119.76
Chillies Giant	9	22.6	2.85	11.8	13.5	140.30
CNPH703	6	26.4	1.41	11.1	13.8	129.01
Kalmicho	9	21.0	1.79	22.2	12.3	153.98
Korea 11	9	14.5	3.15	19.2	10.6	151.24
Korea 13	9	16.9	2.15	19.8	11.2	155.60
Korea 4	6	21.0	2.78	18.1	10.8	153.24
LC-Serdang	9	14.0	2.14	19.6	9.8	148.56
Lv. 1592	9	21.1	2.62	12.0	12.1	143.16
Mareko Fana	9	15.2	2.28	15.5	9.4	185.41
Matikas	9	13.7	1.71	18.5	9.5	143.37
Pant C-1	9	23.9	1.55	10.5	14.1	125.40
PBC 455	9	21.7	3.05	10.0	15.1	129.41
PBC 539	9	19.3	1.88	12.9	11.7	118.60
PBC 581	9	14.7	2.35	20.2	10.2	147.59
PBC 582	3	23.1	2.39	11.0	12.1	141.84
PBC 592	9	22.9	2.17	15.2	13.9	124.87
PBC 622	9	21.8	1.34	17.9	13.2	139.22
PBC 632	9	21.3	3.01	11.7	14.8	155.31
Prapadaeng	9	22.9	3.08	13.7	14.8	137.42
Szechuan 8	9	15.0	2.33	18.8	9.3	154.94
Tumpang	1	16.6	3.60	22.9	11.0	147.89
MEAN	212	19.8	2.37	15.3	12.3	142.68
SD		4.8	0.96	4.65	2.45	23.20

[a] NIRS assays were conducted by AVRDC's Analytical Laboratory

[b] Summer experiments (2) and fall experiment (1) combined means, three replications per experiment unless N<9; 'Tumpang' not included in grand mean

[c] Note: Hungarian paprika standard color value = 20,274

An updated summary of INTHOPE evaluation, distribution and feedback is presented in table 6. A multilocation analysis has been conducted on the INTHOPE 1 feedback to date. We are awaiting the return of more data sets to supplement the analysis. A summary bulletin for INTHOPE 1-4 is expected to be completed by 1995. The INTHOPE 5 set is presently being evaluated and will be distributed in 1995. The INTHOPE 6 entries will be evaluated as usual in summer and fall 1995. It might be timely to develop an evaluation questionnaire to send to all INTHOPE participants to understand if the network needs to shift focus in any way to better serve the participants.

Table 6. Present status of the International Hot Pepper Trial Network

Sets	Entries evaluated[a]/ distributed	Disease screening[b]	Recipients	Test sites	Countries	Recipient feedback
I (1990-91)	43/30	A, BS, BW,	67	94	28+	22
II (1991-92)	39 / 15	A, BW, PB, F, V, VW	50	72	24+	10
III (1992-93)	30 / 16	A, BS, BW, PB, V, VW	40	74	18+	6
IV (1993-94)	28 / 16	A, BS, BW PB, V, VW	46	73	23+	3

[a] 2 or 3 seasons, horticultural traits, field performance, chemical traits (NIRS)

[b] Anthracnose (A), bacterial spot (BS), bacterial wilt (BW), Pnytophthora blight (PB), [Fusarium wilt (F)], Viruses (V) = CMV, CVMV, PVY, TMV, ToMV, PMMV, [TEV, PeMV, TSWV], [Verticillium wilt (VW)]; [items in brackets indicate outside cooperators]

[c] Some additional country distribution through regional offices

Note: feedback column represents a normal delay between seed distribution, implementation of experiments and data compilation from outside cooperators

Strategic and Supporting Studies

Genetics of Resistance to Pepper Diseases

Summary

Preliminary results indicated that resistance to CVMV was inherited by dominance and interactive gene effects at two loci. The resistance to PVMV was controlled by double recessive alleles at two loci. Cultivars, Perennial HDV and PSP-11 appeared to have the same genes for resistance to CVMV and pepper veinal mottle virus (PVMV). There was no evidence of linkage of the resistance genes for CVMV with those for PVMV. Of 32 F_3 lines derived from each of the crosses Cheongryong / Perennial HDV and Cheongryong / PSP-11, five and six lines, respectively, were nearly fixed for resistance to three viruses, CVMV, PVMV, and potato virus Y (PVY).

Introduction

A study was conducted to understand the inheritance of resistance to pepper viruses CVMV, PVMV, and PVY from two sources of resistance, Perennial HDV and PSP-11. Both these accessions originated from India and are being used in the pepper breeding program.

Materials and Methods

Populations developed among three accessions, a susceptible hot pepper variety Cheongryong (S) from Korea and two sources of resistance to CVMV, PVMV and PVY, Perennial HDV (R1) and PSP-11 (R2) indirectly of Indian origin, were independently screened for resistance to CVMV, PVMV, and PVY. The population sizes for the S, R, F_1, BC-R, F_2, and F_3 were approximately 12, 12, 12, 40, 80,

and 32 (with 32 plants/line), respectively, for each virus-cross combination tested. The BC-S crosses were unsuccessful at the time, but were repeated with success in 1994 and the study is to be repeated in 1995 with CVMV and PVMV only. Remnant seed of the F_2 (S x R1) was sown to test the segregation from multiple-virus inoculation in comparison with single-virus inoculation. Inoculum was prepared and inoculations were conducted by the Virology Unit, in batches based on plant phenology at the three to four-true leaf stage. Double antibody sandwich-enzyme linked immunosorbant assay (DAS-ELISA) was conducted approximately 2 weeks after the last inoculation of each batch, also by the Virology Unit. Visual symptoms were recorded 1 month after the last inoculation of each batch. DAS-ELISA and/or inoculation (after pruning) was repeated in a few cases where symptom expression did not concord with the ELISA reaction. Plants inoculated with CVMV or PVY were raised under glasshouse conditions with controlled temperature, whereas, plants inoculated with PVMV were raised in a quarantine-design screenhouse under natural ambient conditions.

Results and Discussion

Preliminary results indicated that resistance to CVMV was inherited by dominance and interactive gene effects at two loci (15R:1S digenic F_2 ratio). The resistance to PVMV was controlled by double recessive alleles at two loci (15S:1R digenic F_2 ratio). Perennial HDV and PSP-11 appeared to have the same genes for resistance to CVMV, as well as the other set of genes for resistance to PVMV. There was no evidence of linkage of the resistance genes for CVMV with those for PVMV. The results with respect to PVY were inconclusive because the seed lot of the putative susceptible parent resulted in ELISA-negative reactions after inoculation. Of 32 F_3 lines derived from each of the crosses Cheongryong / Perennial HDV and Cheongryong / PSP-11, five and six lines, respectively, were nearly fixed for resistance to three viruses, CVMV, PVMV, and PVY. Segregation patterns based on DAS-ELISA of F_2 populations that were inoculated with one or the other virus did not match segregation patterns from a mixed inoculation of three viruses (CVMV, PVMV, PVY). In fact, no F_2 plants were ELISA-positive for CVMV or had strong CMV symptoms, despite ELISA-positive reactions for infectivity from mixed inoculation on alternate hosts. These findings indicate that independent screening and selection must be carried out to develop varieties with resistance to more than one of these potyviruses. For 1995, the epistatic gene effects will be confirmed by one more set of experiments with larger F_2 and all backcross populations. Field selection should be conducted for heat tolerance and fruit size on the 11 F_3 lines that had combined CVMV, PVMV, and PVY resistance.

Genetics and Adoption of Unique Traits for Pepper Breeding

Summary

Three activities were conducted to adopt unique traits for tropical hot pepper improvement. The three traits of interest were (1) shortened internodes to increase the harvest index of 10 recurrent parents, (2) cleistogamy [closed flower *(cf)* gene] to assure self-pollination before flower bloom in 14 recurrent parents, and (3) fasciculate (clustered) internodes to improve uniform ripening and labor efficiency at harvest of three recurrent parents.

Introduction

The objective of this activity is to adopt traits such as fasciculate growth, multiple flowers, deciduous fruit, cleistogamy, and male sterility in tropical pepper breeding lines.

Materials and Methods

Three major activities were conducted to adopt unique traits for tropical hot pepper improvement. All of the work was done at the interest of two training scholars (one research intern and one research fellow) who joined the pepper programs from 1993 to 1994. The three traits of interest were (1) shortened internodes to increase the harvest index of 10 recurrent parents (target country: Indonesia), (2) cleistogamy [closed flower *(cf)* gene] to assure self-pollination before flower bloom of 14 recurrent parents [target countries: Indonesia, Ethiopia (two recurrent parents) and Sri Lanka], and (3) fasciculate (clustered) internodes to improve uniform ripening and labor efficiency at harvest of three recurrent parents (target country: Indonesia).

Results and Discussion

Details of the activities may be found in the training reports of Ms. Yenni Kusandriani, research intern from the Lembang Horticultural Research Institute (LEHRI), Indonesia, and Mr. Yayeh Zewdie, AVRDC research fellow from Nazareth Research Station, Ethiopia.

Seed Distribution

Besides the INTHOPE distribution of test sets, the Pepper Breeding Unit distributed 50 seed shipments to 22 countries in 1994.

Pepper Pathology

Greenhouse Screening Method for Resistance to Bacterial Spot

Summary

A dipping method with surfactant (Silwet L-77) was developed for greenhouse screening for resistance to bacterial spot in pepper. When 0.075% of L-77 (v/v) was added to the bacterial suspension of *Xanthomonas campestris* pv. *vesicatoria* (Xcv) for 6 h at 30°C, no obvious reduction in bacterial population was observed. Pepper seedlings at the three true-leaf stage were kept under shade before inoculation to avoid stomata closure. Seedlings were dipped into the Xcv suspension (10^8 cfu/ml) with L-77 for 30 sec. Inoculated plants were kept in a 28°C growth room with natural light and high humidity was maintained by overhead sprinkling irrigation. When 0.05% or 0.1% of L-77 was added to the bacterial suspension, the infected leaf area in the susceptible cultivars ranged from 25 to 50% (rating of 5), which were significantly higher than 3% (rating of 1) in the resistant cultivars. No damage was observed on pepper leaves with these concentrations of L-77. The dipping method with 0.05% of L-77 was used to screen two sets of hot peppers International Hot Pepper Trial Network (INTHOPE) 4 and 5. The resistant and susceptible controls can be clearly differentiated with a rating of 1.8 and 5.2, respectively. Six accessions from INTHOPE 4 and three from INTHOPE 5 were identified as resistant.

Introduction

Bacterial spot caused by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye is one of the major constraints of pepper production in the warm and humid tropical and subtropical regions. Planting resistant cultivars is the best approach for disease control. Both qualitative and quantitative resistance sources have been used in the resistance breeding program on bacterial spot. Presently, atomization of bacterial suspension is used as a greenhouse screening method for quantitative resistance. However, this method requires a large volume of inoculum, and the selection results are not correlated with field screening results significantly. Thus, it is necessary to develop a more efficient and reliable greenhouse screening method. Silwet L-77 (OSi Specialties, Inc. USA), a nonionic organosilicone surfactant, can reduce the surface tension of water on leaves and has been reported to enhance the foliar infection of pathogenic bacteria through stomata or hydathodes[1]. Delivery of bacteria directly into natural openings on leaf surface would protect bacteria from environmental stress such as radiation and desiccation. This study was conducted to test the potential for using this surfactant to facilitate infection of peppers with the bacterial spot pathogen and develop a greenhouse screening method for quantitative resistance. Materials from INTHOPE 4 and 5 were screened for resistance by this method.

[1] Zidack, N.K., Backman, P.A., and Shaw, J.J. 1992. Promotion of bacterial infection of leaves by an organosilicone surfactant: implications for biological weed control. Biological Control 2: 111-117.

Materials and Methods

Effect of different concentrations of Silwet L-77 on the survival of Xcv

The bacterial suspension of XcvT28, a strain of race 1 of pepper group of *Xanthomonas campestris* pv. *vesicatoria* (Xcv), with a concentration of 10^8 cfu/ml (A_{600}=0.3) was prepared. Different amounts of L-77 (0, 5, 10, and 15 µl) were added to 20 ml of Xcv suspension separately, and each suspension was mixed well and kept at 30°C. The final concentrations of L-77 in each treatment are 0, 0.025, 0.05, and 0.075%. Each treatment has three replicates. Population of bacteria was measured at 0, 2, 4, and 6 h after incubation by dilution plating method on 523 medium.

Effect of different concentrations of Silwet L-77 on disease severity and leaf damage

Seedlings of Early Calwonder (ECW), ECW-10R, ECW-20R, and ECW-30R at three to four fully expanded leaf stage were kept under shade before inoculation to avoid the closure of stomata. Treatments consisted of four concentrations of Silwet L-77 (0, 0.025, 0.05, and 0.1%, v/v) in distilled water with or without XcvT28 at a concentration of 10^8 cfu/ml. The experiment was based on randomized complete block design (RCBD) with two blocks and six replicates. Seedlings were dipped in suspensions of each treatment for 30 sec and kept in a 28°C growth room with natural light. High humidity was maintained in the growth room by overhead sprinkling irrigation at a frequency of 30 sec every 2 h. Disease severity or damage on the leaves was rated based on the Barratt and Horsfall scores 12 days after inoculation.

Screening of resistance to bacterial spot in hot pepper

A total of 29 accessions of INTHOPE 4 and 20 of INTHOPE 5 were screened for resistance to bacterial spot by dipping in Silwet L-77. Seedlings at four fully expanded leaf stage were kept under shade overnight and inoculation was conducted in the early morning. The experiment was based on RCBD with three blocks and eight replicates. Seedlings were dipped in the XvT28 suspension of 10^8 cfu/ml with 0.05% (v/v) L-77 for 30 sec. Inoculated plants were kept in a 28°C growth room with the same condition mentioned earlier. Disease severity was rated based on the Barratt and Horsfall scores 7 days after inoculation.

Results and Discussion

Effect of different concentrations of Silwet L-77 on the survival of Xcv

Bacterial population was maintained at around 10^8 cfu/ml for 6 h at 30°C in all treatments. No obvious reduction in bacterial population was observed when as high as 0.075% of L-77 was added to the Xcv suspension. Thus, inoculation density can be maintained at the same level throughout the inoculation period.

Effect of different concentrations of Silwet L-77 on disease severity and leaf damage

About 4 days after inoculation, circular, raised, and water-soaked lesions appeared on the lower leaf surfaces. Lesions became necrotic 7 days after inoculation. Leaves with a high percentage of infested area dried and fell off. No symptom was observed on new growth areas. Disease severity increased in both resistant and susceptible pepper lines when L-77 was added to the bacterial suspensions (table 1). Resistant and susceptible lines can only be differentiated when 0.05% or 0.1% of L-77 was added to the inoculum. The means of infected leaf area in the susceptible lines were 5.1 in ECW and 5.4 in ECW-10R; the mean in the resistant lines were 1.1 for both ECW-20R and ECW-30R. There were no increases in disease severity when more than 0.05% of L-77 was added to the inoculum. No visual damage was observed on leaves with L-77.

Table 1. Effect of Silwet L-77 on disease severity of bacterial spot in peppers caused by XcvT28, a strain of race 1 of pepper group of *Xanthomonas campestris* pv. *vesicatoria*

Concentration of L-77 (% in v/v)	ECW	ECW-10R	ECW-20R	ECW-30R[a]
0	1.4[b]a[c] A[d]	1.4 aA	0.7 aA	0.7 aA
0.025	1.9 aA	2.4 bA	0.7 aA	0.9 abA
0.05	5.1 bB	5.4 cB	1.1 bA	1.1 abA
0.1	5.2 bB	5.5 cB	1.3 bA	1.3 bA

[a] ECW: Early Calwonder; ECW-10R, -20R, and -30R are near-isogenic lines of ECW and each contains resistance genes of *Bs1*, *Bs2*, and *Bs3*, respectively

[b] Means of Barratt and Horsfall scores of plants in two replications

[c] Grouping by LSD of treatment in same line at 5% level

[d] Grouping by LSD of line in a fixed treatment at 5% level

Screening of resistance to bacterial spot in hot pepper

Using the dipping method, the resistant control PBC 137 and susceptible PBC 416 can be clearly differentiated with the rating of 1.8 and 5.2 in INTHOPE 4, and 1.5 and 4.9 in INTHOPE 5 (tables 2 and 3), respectively. The tested lines can be divided into resistant, moderately resistant, moderately susceptible, and susceptible based on the means of ratings of 2.0, 3.0, and 4.5. Six resistant accessions were selected from INTHOPE 4: PBC 643, 455, 581, 632, 456, and 483. Three resistant accessions were selected from INTHOPE 5: PBC 743, 142A, and VC 44a.

We, therefore, concluded that the dipping method had the following advantages over atomization for resistance screening: (1) less inoculum required; (2) lesions with more uniform size can be generated; (3) difference between resistant and susceptible control is more consistent.

Table 2. Results of greenhouse screening of INTHOPE 4 by dipping in XcvT28, a strain of race 1 of pepper group of *Xanthomonas campestris* pv. *vesicatoria*

Code no.	Name	Mean	Resistance level
PBC 643	Athi slim	1.3[a]	R[b]
PBC 455		1.5	R
PBC 581		1.8	R
PBC 632		1.8	R
PBC 456	Chilli NP 46A	1.8	R
PBC 137	CNPH 703 (R ck)	1.8	R
PBC 483	B.L. 39	2.0	R
PBC 582		2.0	MR
PBC 600	LC-Serdang	2.2	MR
PBC 464	Mareko Fana	2.3	MR
PBC 142A	Pant C-1	2.4	MR
PBC 612	Banlen	2.6	MR
PBC 550A	Lv. 1592	2.7	MR
PBC 539		2.7	MR
PBC 731	Korea 13	2.9	MR
PBC 450	Cajun 1	3.0	MS
PBC 592		3.1	MS
PBC 074	Szechuan 8	3.1	MS
PBC 613	Prapadaeng	3.4	MS
PBC 522	BG-1	3.7	MS
PBC 621	Kalmicho	3.9	MS
PBC 580	Chillie Giant	4.1	MS
PBC 534	Tumpang	4.5	MS
PBC 615	Maticas	4.6	S
PBC 830	Bangchang	4.7	S
PBC 622		4.9	S
PBC 730	Korea 11	4.9	S
PBC 729	Korea 4	4.9	S
PBC 416	YJ 81032 (S ck)	5.2	S
LSD		0.63	

[a] Means of Barratt and Horsfall scores of plants in three replications

[b] R: resistant; MR: moderately resistant; MS: moderately susceptible; and S: susceptible

Table 3. Results of greenhouse screening of INTHOPE 5 by dipping in XcvT28, a strain of race 1 pepper group of *Xanthomonas campestris* pv. *vesicatoria*

Code No.	Name	Mean	Resistance level
PBC 137	CNPH 703 (R ck)	1.5[a]	R[b]
PBC 743	Chinda 2	1.7	R
PBC 142A	Pant C-1	1.8	R
VC 44a	PI 163201	1.9	R
PBC 715	Jwalla	2.1	MR
PBC 074	Szechuan 8	2.5	MR
PBC 583	Ruhunu Miris	2.6	MR
PBC 619	PI 244670	2.6	MR
PBC 636	Galkunda Miris 01146	2.8	MR
PBC 075	Szechuan 921207	3.1	MS
PBC 549	Lv. 2722	3.3	MS
VC 19a	Paul Smith's Serrano 1534	3.4	MS
PBC 717	Sheetal-51	3.8	MS
PBC 711	Dedo de moca	3.9	MS
PBC 584		4.0	MS
PBC 450	Cayenne Cajun 1	4.0	MS
PBC 593		4.3	MS
PBC 559	Tabasco L-167	4.6	S
PBC 416	YJ 18032 (S ck)	4.9	S
PBC 602		5.4	S
PBC 065	Szechuan 921203	5.6	S
LSD		1.0	

[a] Means of Barratt and Horsfall scores in three replications

[b] R: resistant; MR: moderately resistant; MS: moderately susceptible; and S: susceptible

Screening for Pepper Virus Resistance

Summary

From the results of several years of screening we were able to identify many sources of resistance to the major pepper viruses, i.e., PVY, tomato mosaic virus (ToMV), tobacco mosaic virus (TMV), and CVMV. However, only very few good sources of resistance to CMV, and only two lines with pepper mild mottle virus (PMMV) resistance were found so far. This year, therefore, we concentrated on CMV resistance, with emphasis on improvement of the screening method and confirmation of resistance previously identified; routine screening of selected lines for the major viruses was ongoing.

Twenty-two PBC lines, 10 new VC accessions collected by the Virology Unit, 198 GRSU accessions, and various other lines were screened for resistance to all or some of the major viruses. Screening methods were those previously adopted, except for CMV where two plant stages (cotyledon and five-leaf stage, decapitated above the third leaf) were used for the inoculation. Another 26 lines found resistant or moderately resistant to CMV in previous screenings were rescreened to confirm resistance.

One PBC line (PBC 522) was identified to be highly resistant to CMV at the cotyledon stage and moderately resistant at the three-leaf stage; six other lines were resistant at the three-leaf stage but only moderately susceptible at the cotyledon stage. VC 211a was resistant to both CVMV and PVY, while VC 216 was found to have a high percentage of CMV-resistant plants by both inoculation methods.

Among the accessions screened, C 004923 was resistant to CMV at the three-leaf stage, but moderately susceptible at the cotyledon stage. C 00265 was resistant to CVMV and C 00303 to PVY, TMV, and ToMV. Fifty-nine GRSU accessions, which were mainly *C. chinense* and *C. frutescens* types, were resistant to both TMV and ToMV. Resistance to CVMV and PVY was also found in some accessions received from Louisiana State University (LSU) through Dr. L.L. Black.

The resistance to CMV was confirmed by the two inoculation methods in five of the 26 lines rescreened: PBC 569-5 (c), VC 16 a-5-4 (c), VC 16 a-2-4 (c), VC 16 a-1-4 (3), and PBC 549-3 (3). In nine other lines, resistance at the three-leaf stage and moderate resistance levels at the cotyledon stage was confirmed. Resistant plants will be selfed, and seed will be collected to increase and stabilize the resistance level by both inoculation methods.

By using two inoculation methods, we are able to distinguish general resistance that is often expressed in older plants from other types of resistance, such as the one which is expressed at the early growth stages. Only if both types of resistance are combined will durable resistance be ensured. Quantitative ELISA tests are not necessary for routine screening. However, they may be useful to confirm resistance.

Introduction

Routine screening methods have been developed for all major pepper viruses, CMV, CVMV, PVY, PVMV, and the tobamoviruses, and routine screening of PBC lines, INTHOPE entries, and accessions has been an ongoing activity. Many different sources of resistance to PVY and two tobamoviruses – ToMV and TMV – and some for CVMV have been identified. We have therefore, slowed down screening for those viruses. However, since only very few sources of CMV resistance/field tolerance are available, we have emphasized CMV resistance in 1994, with two major objectives: to confirm the previous resistance and to improve the screening methodology.

Materials and Methods

Routine resistance screening

The following lines were screened by methods described previously:

PBC lines. Twenty-two lines were screened for CMV resistance using two inoculation methods, i.e., the cotyledon and the three-leaf stages. One line was screened for PMMV resistance.

VC lines. Ten new accessions, received from INRA, Montfavet (France), were screened for resistance to CVMV, CMV, and PMMV. Several of these accessions have been reported to possess CMV resistance.

AVRDC accessions. One hundred and ninety-eight lines were screened for resistance to CVMV, PMMV, and CMV using the two inoculation methods described in previous reports.

Sixty-six lines were screened for resistance to PVY, ToMV, and TMV.

Others. One line (Lucio LE) received from Petoseed Co. was screened for resistance to CMV, CVMV, and PMMV. Sixteen lines received from LSU were screened for resistance to CMV, CVMV, PVY, PMMV and pepper veinal mottle virus (PVMV).

Confirmation of CMV resistance and testing of two different inoculation methods

Several lines, which in previous screenings showed a high percentage (48-100%) of resistant plants, were rescreened by two quantitative inoculation and rating methods. Seeds were collected from resistant plants. Twenty-four plants/line were inoculated each at the cotyledon and at the five-leaf stage with CMV isolate P-522, originally isolated from peppers in Taiwan. Inoculum was prepared by grinding in a Waring blender one part CMV- infected *Nicotiana glutinosa* leaves in four parts (w/v) 0.01 Na phosphate buffer pH 7.0. The homogenate was filtered through four layers of cheesecloth. With a micropipet, 200 µl of inoculum was layered on the cotyledon or on the third leaf of a plant at the five-leaf stage decapitated above the fourth leaf. Prior to inoculation the leaves were dusted with carborundum. The inoculum was gently distributed across the leaf with the aid of a glass rod.

DAS-ELISA tests were conducted at 2 (E1) and 4 (E2) weeks after inoculation. From plants inoculated at the cotyledon stage, two 1-cm-diam leaf disks were removed from the fourth leaf for E1 and two 1-cm leaf disks from the seventh leaf for E2. From plants inoculated on the third leaf, one leaf disk each was removed from the first and second leaf of the axillary shoots growing from the third and fourth leaf axis (E1), and one leaf disk each from the third and fourth leaf of the same axillary shoots (E2). The two leaf disks were ground together in 400 µl extraction buffer pH 7.4 (phosphate buffered saline with 20 g/l of PVP-40, and 0.5% Tween-20) and used for the ELISA tests.

Twenty-six selections of 21 pepper lines were tested, and the line VC 10a was used as a susceptible check.

Results and Discussion

Routine resistance screening

PBC lines. One line (PBC 522) was found resistant at the cotyledon stage and had only 19% infected plants when inoculated on the third leaf. Six lines (PBC 142A, 455, 456, 483, 612, and 632) showed no symptoms, and no virus was detected when the third leaf was inoculated with CMV. The same lines had 42, 42, 54, 39, 67, and 54% infected plants, respectively when inoculated at the cotyledon stage.

Five lines (PBC 074, 534, 592, 613, 621) had less than 10% infected plants when CMV was inoculated on the third leaf, but when the same lines were inoculated on the cotyledon, infection ranged from 21 to 62%.

Two lines, PBC 345 and 535, were susceptible when both inoculation methods were used. The remaining eight lines had more than 20% infected plants when both inoculation methods were used.

All of the lines will be rescreened by both inoculation methods using seed of resistant plants to confirm resistance.

VC lines. All the lines were susceptible to PMMV. One line (VC 211a) was resistant to CVMV and PVY. Line VC 216 was found to have a high percentage of CMV-resistant plants by both inoculation methods, i.e., 75% when inoculated on the third leaf and 83% on the cotyledon.

AVRDC accessions. One accession (C 00265) was resistant to CVMV and another (C 00303) to PVY, TMV, and ToMV. Fifty-nine accessions which were comprised mainly of *C. chinense* and *C. frutescens* were resistant to both TMV and ToMV: C 0027e, 303, 735, 777, 780, 872, 874, 886, 893, 915, 942, 1379, 1435, 1553, 1556, 1622, 1706, 4402, 4511-A, 4511-B, 4546, 4674, 4677, 4678, 4679, 4680, 4682, 4684, 4685, 4686, 4687, 4688, 4690, 4691, 4697, 4698, 4699, 4700, 4701, 4702, 4703, 4704, 4707, 4708, 4709, 4710, 4712, 4713, 4715, 4840, 4875, 4876, 4878, 4879, 4880, 4882, 4883, 4884, and 4885.

Only one line (C 4923) was found resistant to CMV (no infection, ELISA-negative) when inoculated on the third leaf. When inoculated at the cotyledon, this line only had 36% resistant plants.

Others. The cultivar Lucio LE was resistant to CVMV but susceptible to CMV and PMMV. All lines from LSU except two (P 3, P 26) were resistant to PVY, and nine lines were resistant to CVMV (P 3, P/C 24, P 28, P 34, P 40, PBC 473 PeMV-Tex no. 1, PBC 473-Nic, PBC 473-TEV-C1, PBC 535-CMV DR93-22).

Two lines (PBC 473 PeMV-Tex no. 1 and PBC 473 PeMV-Nic) had no CMV infection when inoculated on the third leaf and two other lines, P 3 and PBC 535 CMV DR93-22, showed less than 10% infection when inoculated at the five-leaf stage. These two latter lines also had high percentages (38 and 64%) of resistant plants when inoculated at the cotyledon stage.

Confirmation of CMV resistance

Symptoms were generally more severe and the percentage of CMV infection higher in plants inoculated at the cotyledon stage than in plants inoculated at the five-leaf stage.

Six lines, PBC 621 (Kalmicho), PBC 142A (Pant C-7), C 00480-1 (Kardee Kiew), PBC 534 (Tumpang), VC 224 (Kheenoo Kbon-2), and VC 223 (Chau Mau Kau) exhibited high levels of resistance in percentage of resistant plants when plants were older, i.e., at the five-leaf stage at the time of inoculation, but only low levels of resistance when inoculated at the cotyledon stage.

This was most pronounced in line PBC 142A, which had only 25% resistant plants when inoculated at the cotyledon stage, but an average of 70% resistant plants when inoculated at the five-leaf stage.

One line, C 01915, was 100% susceptible when inoculated at the cotyledon stage, but when inoculated at the five-leaf stage had a high level (83%) of resistance. Two of the lines, VC 222-5 (LP-1) and VC 160-1 (*C. frutescens* PSP-11), showed low levels of resistance (32-33% resistant plants) when inoculated at the cotyledon stage and moderate levels of resistance (50-58% resistant plants) when inoculated at the five-leaf stage.

Two lines, PBC 550A(5) and PBC 483-1(c), had high levels of resistance (96 and 98%, respectively) when inoculated at the five-leaf stage and high levels (87%) of resistance at the cotyledon stage.

Seven lines/selections showed no symptoms and gave a negative ELISA reaction when inoculated on the third leaf: VC 41a-3-1(3), PBC 521-2(c), VC 41a-2-5(3), VC 40a-4-1(c), PBC 370-2(c), PBC 522(c), and PBC 146-1 (3). When inoculated at the cotyledon stage, these lines also had high percentages of resistant plants (96, 96, 92, 92, 92, 83, and 78%, respectively) (table 4).

However, six lines/selections were found highly resistant by both inoculation methods, i.e., none of the plants showed symptoms or gave a positive ELISA reaction: PBC 569-5(c), VC 16a-5-1(c), VC 16a-2-4(c), VC 16a-1-4(3), VC41a-3-1, and PBC 549-3(3) (table 5).

This quantitative screening showed that for plants inoculated at the cotyledon stage one ELISA reading at 2 weeks after inoculation was sufficient. Although the percentage of infected plants was generally the same for both ELISA tests, the overall average ELISA values were slightly higher in the second ELISA test, indicating that the virus had moved into the upper leaves rapidly and was multiplying there.

In decapitated five-leaf stage plants inoculated at the three-leaf stage, there was no significant difference in the percentage of infected plants and the ELISA values whether the ratings were done on the third- or fourth leaf axillary shoot. However, the average ELISA value in the second ELISA tests (conducted 4 weeks after inoculation) was again significantly higher than the first ELISA test.

Table 4. Reactions (% resistant plants) of selected pepper lines previously found resistant or moderately resistant to CMV in a second screening using seed of resistant plants and two different inoculation methods

Line/ Selection	Description	1st screening[a] Inocul. method[b]	% resistant plants	2nd screening[c] cotyledon E1	E2	3rd leaf E1(3)	E2(3)	E1(4)	E2(4)
PBC 522	BG-1	c	100	83	83	100	100	100	100
PBC 370-2	not known	c	96	92	92	100	100	100	100
VC 41a-3-1	HDA 295	3	95	96	100	100	100	100	100
PBC 621	Kalmicho	c	92	79	71	96	92	96	92
PBC 142A	Pant C-1	c	87	25	25	87	75	100	67
VC 41a 2-5	HDA 295	3	87	92	92	100	100	100	100
PBC 549-3	Fudi Jiao	3	85	100	100	100	100	100	100
VC 216-5	Rama	c	83	67	67	87	79	87	83
VC 223-1-4	Chau Mau Kau	3	83	87	87	96	92	92	92
VC 16a-1-4	Perennial HDV	3	79	100	100	100	100	100	100
PBC 550A	Lv 1592	3	79	87	87	100	100	96	96
PBC 521-2	Tiwari	c	78	100	96	100	100	100	100
VC 16a-2-4	Perennial HDV	c	75	100	100	100	100	100	100
VC 222-5	LP-1	3	74	37	27	58	58	62	58
VC 40a-4-1	HDA 273	c	71	92	92	100	100	100	100
C 00480-1	Kardee Kiew	c	70	46	46	87	83	87	83
PBC 146-1	Ludhiana Long	3	70	78	78	100	100	100	100
VC 41a 3-1	HDA 295	c	69	96	96	100	100	100	100
C 01915A	not known	3	67	0	0	83	83	83	79
VC 16a-5-1	Perennial HDV	c	67	100	100	100	100	100	100
PBC 483-1	BL 39	c	65	87	87	96	96	96	96
PBC 534-3	Tumpang	c	65	74	74	100	100	96	96
VC 160-1	*C. frutescens* PSP-11	3	62	33	33	50	50	54	46
VC 224-2	Kheenoo Ubon	3	60	38	38	92	87	87	83
PBC 569-5	not known	c	60	100	100	100	100	100	100
VC 223 4-2	Chau Mau Kau	c	48	58	58	100	96	100	96
VC 10a (S ck)	Yolo Wonder HDV	c	0	0	0	0	0	0	0
Avg.				75	74	94	92	97	91

[a] First screening was done in 1992/93 by two inoculation methods. Rating was by a nonquantitative ELISA test, conducted 4 weeks after inoculation

[b] c = inoculation of the cotyledon; 3 = inoculation of the third leaf of five-leaf stage plants decapitated above the fourth leaf

[c] Second screening was in 1994 by two inoculation methods (cotyledon and third leaf). Rating was by two quantitative ELISA tests conducted 2 weeks (E1) and 4 weeks (E2) after inoculation

E1(3) and E1(4) were conducted on leaf disks one each from the first and second leaf of the new axillary shoots emerging from the third and fourth leaf axillary bud, respectively

E2(3) and E2(4) were conducted on leaf disks, one each from the third and fourth leaf of the new axillary shoots emerging from the third and fourth leaf axillary bud, respectively

Table 5. Average ELISA values of selected pepper lines previously found resistant or moderately resistant to CMV, in a second screening, using seed of resistant plants[a]

Line/Selection (initial inocul. method)		Description	Cotyledon E1	Cotyledon E2	3rd leaf E1(3)	3rd leaf E2(3)	3rd leaf E1(4)	3rd leaf E2(4)
PBC 522	(c)	BG-1	0.031	0.081	0.000	0.025	0.007	0.000
PBC 370-2	(c)	not known	0.075	0.025	0.000	0.000	0.000	0.000
VC 41a-3-1	(3)	HDA 295	0.072	0.000	0.043	0.000	0.035	0.000
PBC 621	(c)	Kalmicho	0.153	0.298	0.000	0.066	0.000	0.049
PBC 142A	(c)	Pant C-1	0.952	0.760	0.053	0.242	0.040	0.330
VC41a-2-5	(3)	HDA 295	0.036	0.061	0.000	0.000	0.000	0.000
PBC 549-3[b]	**(3)**	**Fudi Jiao**	**0.003**	**0.001**	**0.000**	**0.000**	**0.000**	**0.000**
VC 216-5	(c)	Rama	0.353	0.279	0.049	0.140	0.026	0.183
VC223-1-4	(3)	Chau mau Kau	0.056	0.079	0.000	0.071	0.000	0.082
VC 16a-1-4	(3)	Perennial HDV	0.016	0.000	0.000	0.000	0.000	**0.000**
PBC 550A	(3)	Lv 1592	0.069	0.000	0.008	0.000	0.081	0.097
PBC 521-2	(c)	Tiwari	0.000	0.000	0.000	0.000	0.000	**0.000**
VC 16a-2-4	**(c)**	**Perennial HDV**	**0.000**	**0.000**	**0.000**	**0.000**	**0.014**	**0.000**
VC 222-5	(3)	LP-1	0.144	0.445	0.162	0.348	0.159	0.360
VC 40a-4-1	(c)	HDA 273	0.019	0.066	0.000	0.000	0.000	0.000
C 00480-1	(c)	Kardee Kiew	0.434	0.727	0.047	0.100	0.059	0.147
PBC 146-1	(3)	Ludhiana Long	0.258	0.198	0.000	0.000	0.000	0.000
VC41a-3-1	(c)	HDA 295	0.000	0.007	0.000	0.000	0.000	**0.000**
C 01915A	(3)	not known	0.232	0.873	0.007	0.159	0.044	0.126
VC 16a-5-1	**(c)**	**Perennial HDV**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
PBC 483-1	(c)	BL 39	0.086	0.050	0.024	0.040	0.026	0.061
PBC 534-3	(c)	Tumpang	0.254	0.186	0.001	0.000	0.029	0.006
VC 160-1	(3)	*C. frutescens* PSP-11	0.596	0.448	0.461	0.453	0.534	0.518
VC 224-2	(3)	Kheenoo Ubon-2	0.297	0.335	0.050	0.088	0.000	0.127
PBC 569-5	**(c)**	**not known**	**0.000**	**0.000**	**0.000**	**0.000**	**0.016**	**0.000**
VC 223-4-2	(c)	Chau Mau Kau 4-2	0.175	0.401	0.000	0.039	0.000	0.032
VC 10a inoc. (S ck)		Yolo Wonder HDV	0.451	0.803	1.038	1.388	0.964	1.667
VC 10a non-inoc. (H ck)		Yolo Wonder HDV	0.024	0.026	0.036	0.025	0.036	0.025
Avg.[c]			0.166	0.205	0.035	0.068	0.041	0.081

[a] Inoculations and ELISA tests; see footnotes on table 4

[b] Bold indicates that the line was 100% resistant by both inoculation methods and after the first and second ELISA tests

[c] Average values (without check) adjusted for healthy check values

Previously, French researchers had noted that in certain lines inoculated on the third leaf, virus was only detectable in axillary shoot averaging from the third leaf axis, but not in the fourth leaf axillary shoot. This was attributed to a resistance mechanism that prevented the virus from moving laterally from one side of the plant to the other side and then up. We did not observe this type of resistance mechanism in any of the plants tested.

The results indicate that quantitative ELISA tests are necessary when large scale screening for resistance to CMV in peppers is conducted. They are useful, however, to effectively confirm the absence of virus in resistant plants. However, the two screening methods, cotyledon and third leaf should be maintained in routine screenings, because plants that show resistance when inoculated at the three-leaf stage may not necessarily be resistant at the cotyledon stage. To breed for stable resistance, it is better to select lines with confirmed resistance at both young and old growth stages.

Conclusion

Additional sources of resistance to CMV, CVMV, ToMV/TMV, and PVY have been identified. For CMV routine resistance screening both young plants (cotyledon stage) and older plants (five-leaf stage, decapitated above the fourth leaf) should be inoculated to distinguish between a resistance mechanism that is apparently operating in older plants and other resistance mechanisms that protect young seedlings from infection with CMV. Quantitative ELISA tests are not necessary for routine screening, but should be used to confirm resistance in plants where a resistance mechanism based on reduced virus multiplication is expected.

Detection, Characterization, and Production of Monoclonal Antibodies for Pepper Virus

Summary

We have prepared highly specific polyclonal antisera to most of the pepper viruses, except TMV and ToMV, which polyclonal antisera cannot distinguish. Therefore, two monoclonal antibodies (MABs) were prepared with high specificity to ToMV and TMV. These MABs only reacted with homologous antigen, but not with heterologous tobamoviruses present singly or in mixture in plant sap. A detailed ELISA protocol has been prepared.

Tobamoviruses are very important because of their seed-transmitted nature. Most commercial pepper lines possess resistance to ToMV and TMV but not to PMMV, which is present worldwide and is known to cause considerable damage to pepper fruit. Therefore, four seed treatments (HCl, heat, NaOH, and Na_3PO_4) were investigated for their effectiveness in eliminating seedborne PMMV. One hot and one sweet pepper cultivar (Passion and Yolo Wonder, respectively) were mechanically inoculated at the three-leaf stage. The presence of PMMV on and in the seed was measured by ELISA, which was supplemented by a bioassay where seed washings as well as ground up seed were inoculated on *Nicotiana glutinosa*. Tobacco reacts with PMMV with local lesions.

ELISA tests indicated that virus was still present in all samples following the treatments. However, bioassays indicated that the HCl and heat treatments had eliminated PMMV from both seed coat and endosperm in hot pepper. In sweet pepper, only the HCl treatment had eliminated PMMV from both seed coat and endosperm. Germination was reduced by 10% in hot pepper by HCl, while sweet pepper was not affected. Although ELISA tests are useful to detect seedborne viruses, they do not give any indication of virus infectivity, which can only be tested by bioassays.

Introduction

Specific diagnostics are important for resistance screening and surveys to determine the distribution and importance of viruses. Tomato mosaic virus and tobacco mosaic virus , both members of the tobamovirus group, commonly infect tomato and peppers. Since the two viruses are very closely related, it has not been possible to distinguish them by polyclonal antisera in either indirect or DAS-ELISA. Similarly, DNA probes which were developed last year were not able to differentiate the two viruses in nucleic hybridization tests. The production of monoclonal antibodies was, therefore, attempted since they are known to be highly specific.

In 1992 and 1993, several seed treatments were tested for their efficiency in eliminating seedborne pepper mild mottle virus (PMMV). Only the heat treatment was found effective in eliminating virus from the seed coat, but not from the endosperm. The presence of virus on and in the seed after the

treatments was measured by ELISA. However, this did not give any indication of the infectivity of the virus. Therefore, the same seed treatments were repeated and the presence of virus was measured by ELISA as well as an infectivity test.

Materials and Methods

Monoclonal antibodies for the identification of ToMV and TMV

Virus purification. ToMV (isolate 1511) was multiplied in *Nicotiana tabacum* Samsun and TMV (isolate 1508) in *N. sylvestris*. The viruses were purified using the method slightly modified from that described by Garcia-Luque et al. (1990)[2] for pepper mild mottle virus, another tobamovirus. In this method, PEG and Triton X precipitation was followed by two cycles of differential centrifugation at 10,000 rpm.

Immunization and hybridoma production. Four- to eight-week-old BALB/c mice were used for immunization. The mice received two injections: the first into the tail vein with 50 µl (50 µg) of semipurified virus; the second, 1 week later, directly into the spleen with 80 µg virus. The fusion was done 3 days after the second injection. The fusions and culture of hybridoma lines were done using the method employed by Suh (1993)[3]. Seventeen to eighteen days after the fusion, 100 µl of culture supernatant was collected from hybridoma growing wells and tested by indirect ELISA for antibody production using extracts of healthy and virus-infected plant tissue for coating the plates. Antibody producing hybridomas were then tested by a second indirect ELISA against leaf extracts (100 µl/well) of pure isolates of TMV, ToMV, and PMMV.

Hybridomas which reacted with either TMV or ToMV but not with PMMV were cloned by the limiting dilution method. A third ELISA test was conducted on these hybridomas using the method described for the second ELISA test. Antibody isotypes were determined with the mouse-monoclonal antibody isotyping kit (Sigma ISO) following the supplier's instructions.

Antibody specificity test. To test the specificity of the selected monoclonal antibodies, an indirect ELISA test was conducted using a dilution series of virus-infected leaf extracts (TMV, ToMV, and PMMV) at 1:1, 1:10, 1:20, 1:50, 1:100, 1:200, 1:400, and 1:800 for coating the plates. The MAB-producing culture supernates were used at 1:10 (TMV) and 1:500 (ToMV) dilutions. For comparison, ToMV polyclonal antiserum at (100 µl of 1:1000 ToMV IgG) was used in DAS-ELISA.

The efficiency of the MABs for detecting ToMV or TMV in double-infected plants was tested in the susceptible tomato line L 4840. Plants were artificially inoculated at the two-leaf stage with a mixture of TMV and ToMV. Indirect ELISA tests were performed 2 weeks after inoculation. ELISA plates were coated with 100 µl virus-infected plant extracts (1:5 w/v) and challenged with TMV MAB culture fluid at 1:1000 and ToMV MAB culture fluid at 1:500.

PMMV seed transmission

Seed treatments were performed on seed collected from hot (Passion) and sweet pepper (Yolo Wonder) mechanically inoculated with PMMV at the two-leaf stage. Four seed treatments (76°C dry heat, HCl 5.3%/4 h, NaOH 2%/10 min, Na_3PO_4 10%/2 h) described in the AVRDC 1992 Progress Report were tested.

[2] Garcia-Luque, C., Sera, M.T., Alouso, E., Widce, B. Ferrero M.L., and Diaz-Ruiz J.R. 1990: Characterization of a Spanish strain of pepper mild mottle virus (PMMV-S) and if relationship to other tobamovirus es. J. thylopathoe. 129: 1-8.

[3] Suh, S.K. 1993. Production of monoclonal antibodies against turnip mosaic virus and their application to strain identification and resistance genetics in Chinese cabbage. Ph.D. thesis, Dept. of Horticulture, Seoul National University, 98 p.

For each treatment, batches of 30 seeds were used with five replications. ELISA and infectivity tests (by inoculation of *N. glutinosa*) were conducted on each seed batch. Infectivity was evidenced by the appearance of local lesions on the inoculated leaves of *N. glutinosa*.

The presence of virus on the surface of the seed coats was measured by shaking the seeds for 2 days in 3 ml extraction buffer. The buffer was then used for the ELISA and infectivity tests. The seed batches were then rinsed five times in tap water and ground in 3 ml sodium phosphate buffer (pH 7.0). This homogenate was used to measure the presence and infectivity of virus in the seed endosperm. A germination test on moist filter paper was conducted on separate seed batches which had undergone the same seed treatments.

Results and Discussion

Monoclonal antibodies against ToMV and TMV

Virus yield was 85 mg virus per 100 g infected leaves, using an extinction coefficient of $E_{0.1\%,1cm}$ at 260 nm = 3.18.

A total of nine fusions were made with ToMV and 10 fusions with TMV. After cloning 14% ToMV antibody-producing hybridoma lines and only 0.5% TMV lines were obtained (table 6). The monoclonal antibodies for TMV 14 and ToMV 3 were selected for further testing.

Table 6. Percent antibody producing hybridoma lines in the three ELISA tests (E1, E2, E3) following the fusions

Virus	Total no. of fusions	Antibody producing-hybridoma lines (%) E1[a]	E2	E3
ToMV	9	4[b]	21	14
TMV	10	9.2	2.6	0.5

[a] E1: culture supernate was challenged with homologous virus in infected plant sap and healthy plant extract; E2: culture supernate was challenged with healthy plant sap and ToMV-, TMV-, and PMMV-infected plant sap; E3: same as E2, culture supernate after cloning from single hybridoma lines

[b] Average percent positives per fusion

Isotype tests showed that MAB TMV-14 belongs to the group IgG3 and MAB ToMV-3 to the group IgG2a.

Specificity tests, in which the two MABs and ToMV polyclonal antiserum were challenged with three tobamoviruses, TMV, ToMV, and PMV, in crude plant sap, showed that the monoclonal antibodies were much more specific than the polyclonal antiserum.

The TMV MAB (1:10) did not react with the heterologous antigens, even at dilutions as low as 0.5×10^{-1} antigen. The ToMV MAB (1:500) showed background reactions. At 10^{-1} and 0.5×10^{-1} antigen dilutions, the heterologous and homologous antigens could no longer be differentiated (fig. 1). In contrast to this, the ToMV polyclonal antiserum could not differentiate TMV at any of the antigen dilutions used because of strong cross reactivity. It was, however able to distinguish PMMV.

Therefore, for screening tests or surveys where ToMV and TMV are to be detected, specific monoclonal antibodies have to be used: TMV MAB-14 at 1:10 and plant sap dilutions ranging from 8×10^{-2} to 10^{-1} and ToMV 3 MAB at 1:500 and plant sap dilutions ranging from 8×10^{-2} to 2×10^{-1}.

The two MABs are also useful to detect homologous virus in plants that are infected with more than one virus (table 7), although the ELISA values observed were about 50% lower in the double-infected plants. However, these values were still significantly higher than the ELISA values of the healthy plant sap.

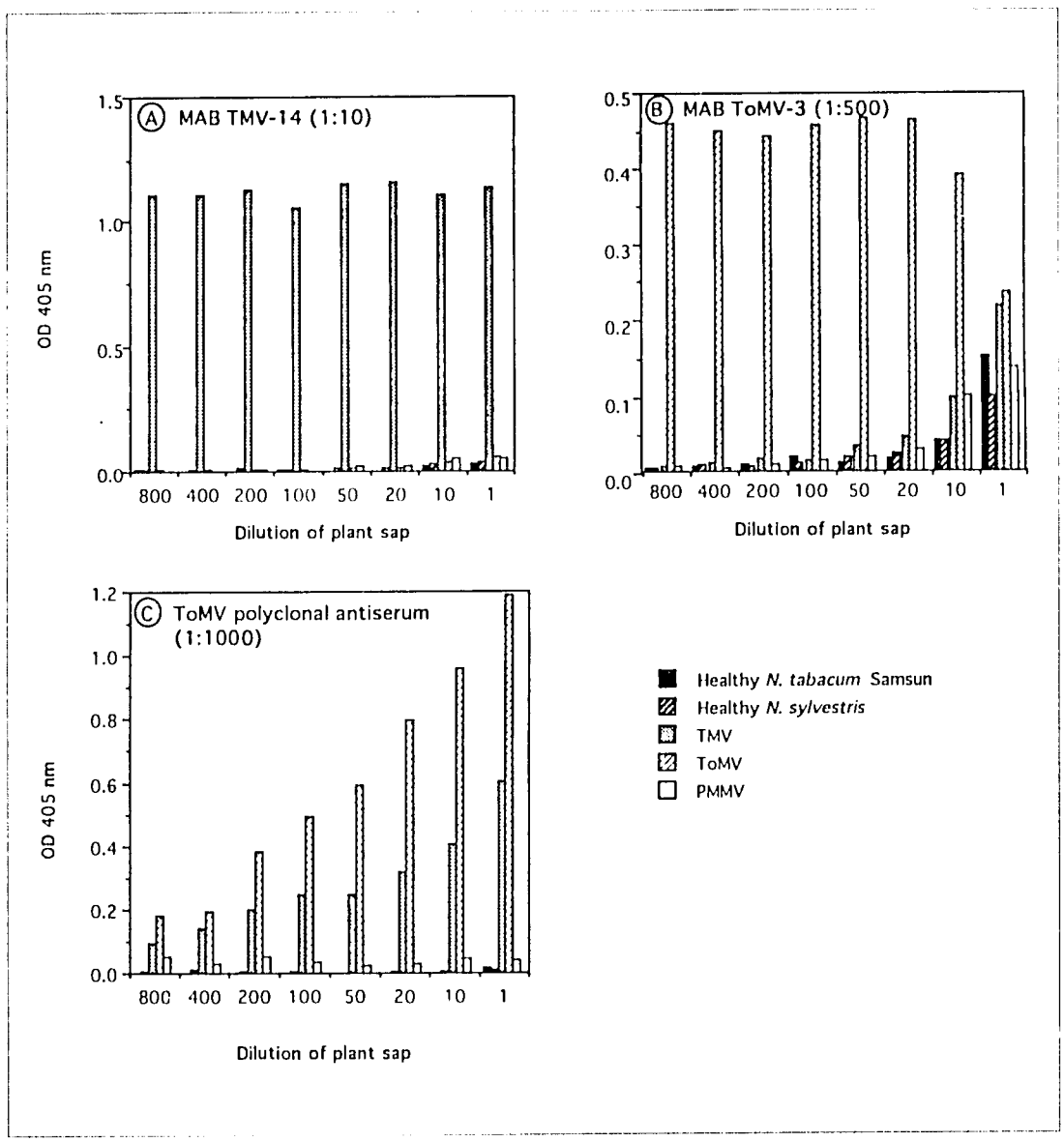


Fig. 1. ELISA OD values of MAB TMV-14 at 1:10 (a), MAB ToMV-3 at 1:500, (b) and ToMV polyclonal antiserum (c), challenged with three tobamoviruses in plant sap at various dilutions

Table 7. Average ELISA OD 405 nm values of TMV and ToMV MABs challenged with homologous and heterologous antigen and a mixture of both

Antigen[a]	MAB TMV-14	MAB ToMV-3
TMV	0.5777 a[b]	0.0279 c
ToMV	0.0307 c	0.7873 a
TMV + ToMV	0.2692 b	0.3957 b
Healthy plant sap	0.0095 c	0.0095 c

[a] In crude plant sap, diluted 1:5
[b] Mean separation within columns at the 5% level (DMRT)

PMMV seed transmission

The results of the seed treatments on germination and on PMMV presence and infectivity are shown in table 8. Germination was affected by the different seed treatments for virus elimination, and hot pepper reacted differently from sweet pepper to the treatments. In hot pepper, the HCl and NaOH treatments reduced germination by 8 and 7%, respectively. In sweet pepper, three seed treatments (heat, Na_3PO_4, and NaOH) reduced germination by 25, 30, and 77%, respectively. The ELISA test only indicated whether virus was present on the seedcoats and on the endosperm, but not whether the virus present was still infectious. Thus, while ELISA detected virus in all seed samples after all the treatments, infectivity tests showed that the virus was not infectious in the seedcoats of hot pepper seeds treated with HCl, heat, and Na_3PO_4 and of sweet pepper seeds treated with HCl and heat. However, only the HCl and heat treatments eliminated PMMV from the endosperm of hot pepper, while only the HCl treatment eliminated PMMV from the endosperm of sweet pepper.

Table 8. Effect of seed treatments on gerrnination and virus presence and infectivity in PMMV-contaminated pepper seed

Seed treatment	Hot pepper					Sweet pepper				
	Germination (%)	Seedcoat		Endosperm		Germination (%)	Seedcoat		Endosperm	
		ELISA positive[a] (%)	Infectivity[b] (%)	ELISA positive (%)	Infectivity (%)		ELISA positive (%)	Infectivity (%)	ELISA positive (%)	Infectivity (%)
HCl 5.3% (4 h)	89 b	100	0	100	0	911 a	100	0	100	0
Heat 73 C (3 days)	96 a	100	0	100	0	71 b	100	0	100	20
Na_3PO_4 10% (2 h)	92 ab	100	0	100	60	66 b	100	40	100	80
NaOH 2% (10 min)	90 b	100	40	100	60	22 c	100	80	100	100
ck (no treatment)	97 a	100	100	100	100	95 a	100	100	100	100

[a] % of seed batches showing positive ELISA reaction/seed batches tested (5);
Positive ELISA reaction: OD 405 nm absorbance values of 10 times those of the untreated check
[b] Positive infectivity reaction: appearance of local lesions on inoculated leaves of *N. glutinosa*
[c] Values are averages of batches of 30 seeds each and five replications.
Mean separation within columns at 5% level (DMRT)

Conclusions

Monoclonal antibodies were shown to be more useful than polyclonal antisera or nucleic acid probes to differentiate tobacco mosaic from tomato mosaic virus. They are useful in detecting these viruses in singly infected plants as well as in plants infected with a mixture of viruses.

To eliminate infectious PMMV from hot pepper seedcoats as well as endosperm while not adversely affecting germination, a heat treatment of 76°C/3 days is recommended for hot pepper, while for sweet pepper a treatment of HCl 5.3%/4 h is recommended.

Evaluation of Pepper Accessions for Their Anthracnose Reactions

Summary

Pepper accessions in the fourth International Hot Pepper Trial Network (INTHOPE 4) were evaluated for their reactions to anthracnose based on the percentage of fruit that developed symptoms when grown in the field with natural inoculum. The percentage of fruit developing anthracnose symptoms ranged from 1 to 23% among the 28 pepper accessions tested. Percent infection averaged less than 3% in two separate field experiments for six entries (PBC 455, PBC 582, PBC 592, PBC 612, PBC 613, and PBC 643) suggesting that they possess resistance to anthracnose. The predominant pathogen was *Colletotrichum gloeosporioides*.

Introduction

Anthracnose fruit rot is one of the most important diseases of pepper; it frequently causes severe yield loss when pepper is grown during the rainy season. *Colletotrichum capsici* (Syd.) E.J. Butler & Bisby and *C. gloeosporioides* (Penz.) Penz. & Sacc. in Penz. are generally reported as the two major causal agents of anthracnose. *C. gloeosporioides* is predominant in Taiwan. Resistant pepper accessions have been reported, but most are not suitable types for cultivation. The objective of this study was to evaluate pepper entries included in INTHOPE 4 for their reactions to anthracnose under field conditions.

Materials and Methods

INTHOPE 4 included 28 pepper entries. Each accession was planted in RCBD with three replications in two separate fields (PP93SU02, transplanted 21 June 1993; PP93SU03 transplanted 22 June 1993). Each treatment replication consisted of four 4.5-m-long double rows. Fruit evaluations for anthracnose symptoms were made on the 4th (22 September), 7th (12-13 October), and 10th (2-3 November) harvest from each field. The percentage of fruit showing symptoms at harvest was determined visually and confirmed microscopically by observing the total fruit at each harvest.

In addition, a 20-fruit sample of asymptomatic fruit was taken from each treatment replication to determine the percentage of fruit that developed symptoms during postharvest. The fruits were washed in tap water, immersed in 1% sodium hypochlorite for 2 min, rinsed in distilled water, and incubated at room temperature for 2 weeks on a wire screen in an enclosed plastic bag. The percentage of fruit from each accession that developed anthracnose symptoms from latent infections was determined by visual observation and confirmed by microscopic examination of the spores. The pre- and postharvest percentages of symptomatic fruit were combined to determine the percentage of infected fruit for each accession at each harvest. Finally, the percentages of infected fruit at three harvest periods from two fields were averaged to derive the mean incidence of anthracnose infection for each pepper accession. Data from the two fields were treated as a combined experiment, analyzed by ANOVA, and the means separated by LSD.

Results and Discussion

Although both *C. capsici* and *C. gloeosporioides* were associated with lesions in this study, *C. gloeosporioides* was the causal agent in more than 95% of the lesions. Responses of individual pepper accessions to anthracnose were similar in separate field plantings; therefore, data from the two fields were highly correlated ($r = 0.83$). The mean incidence of fruit from both fields that developed anthracnose symptoms ranged from about 1 to 23% among the 28 pepper entries (table 9). Entries with

the lowest (≤ 2.8%) mean incidence of infected fruit and presumably the most resistant to anthracnose were PBC 582, PBC 612, PBC 643, PBC 613, PBC 592, and PBC 455. Entries with the highest (≥ 14.8%) mean incidence included PBC 731, PBC 142, PBC 456, PBC 539, and PBC 615.

Anthracnose pressure in these fields was relatively low compared to previous years. Summer and fall 1993 were very dry with rainfall far below normal. Even though disease pressure was not great, distinct differences were observed among the responses of the pepper entries which permit assessment of their relative field reactions to anthracnose caused by *C. gloeosporioides*.

Field reactions based on relative incidence of symptomatic fruit appear to be a reproducible method of evaluating pepper accessions for their response to anthracnose. However, we are not certain about all the factors that contribute to the different reactions among the accessions. In particular we are unable, at present, to distinguish between observed differences that may be due to escape based on fruit size, number, or location on the plant and inherent resistance to infection and/or disease development that may occur in the fruit itself.

Table 9. Incidence of anthracnose-infected fruits among the pepper entries in the INTHOPE 4 field trials grown AVRDC with natural inoculum at AVRDC

		Symptomatic fruits (%)[a]		
Entry	Name/Origin	Field 02	Field 03	Mean[b]
PBC 731	Korea 13	23.0	22.8	22.9
PBC 142	Pant C-1	10.8	26.8	18.8
PBC 456	Chilli NP46A	19.3	15.9	17.6
PBC 539	(AVRDC)	13.2	18.5	15.9
PBC 615	Matikas	14.4	15.2	14.8
PBC 729	Korea 4	11.9	12.0	12.0
PBC 830	Bangchang	12.4	11.1	11.8
PBC 730	Korea 11	9.9	11.2	10.6
PBC 600	LC-Serdang	8.0	12.2	10.1
PBC 483	B. L. 39	9.8	9.6	9.7
PBC 581	(Malaysia)	8.2	8.6	8.4
PBC 580	Chillies Giant	3.6	8.2	5.9
PBC 137	CNPH 703	5.0	6.7	5.9
PBC 464	Mareko Fana	6.3	5.1	5.7
PBC 621	Kalmicho	3.8	7.3	5.6
PBC 450	Cay. Cajun1	5.4	4.8	5.1
PBC 534	Tumpang	3.9	5.6	4.8
PBC 632	Bangladesh	4.8	3.8	4.3
PBC 622	(AVRDC)	6.7	1.8	4.3
PBC 074	Szechuan 8	3.8	3.8	3.8
PBC 550	LV. 1592	3.0	3.4	3.2
PBC 522	BG-1	0.8	5.3	3.1
PBC 455	(India)	3.0	2.6	2.8
PBC 592	(Thailand)	2.3	2.7	2.5
PBC 613	Prapadaeng	2.6	1.9	2.3
PBC 643	Athi Slim	1.4	2.0	1.7
PBC 612	Banglen	1.7	1.3	1.5
PBC 582	(Malaysia)	0	1.3	0.7

[a] Combined pre- and postharvest percentage; based on means of three replications in each field; correlation coefficient between fields r = 0.83

[b] LSD = 5.4 at 5%

We initiated in 1994 and will continue into 1995 studies on laboratory inoculation and disease evaluation techniques that hopefully will allow us to assess the degree of resistance or susceptibility to anthracnose among pepper fruit of various sizes and shapes. Current laboratory methods do not appear to be reliable for this purpose.

Evaluation of Pepper Accessions for Phytophthora Blight Reactions

Summary

Twenty-four pepper entries in INTHOPE 4 were evaluated in the greenhouse at the seedling stage for their reactions to *Phytophthora capsici*, the causal agent of phytophthora blight. The disease reaction of each accession was determined based on the percentage of surviving plants 12 days after inoculation (300 ml of a 10^4 zoospore/ml suspension applied to the soil surface of each planter flat at 30 days after sowing). Only one entry, PBC 534 (Tumpang from LEHRI/Indonesia), was found to express a useful level of resistance with 68% of the plants surviving. Survival among the other accessions ranged from 0 to 21%.

Introduction

Phytophthora blight of pepper caused by *Phytophthora capsici* Leonian causes severe losses in pepper crops in both tropical and temperate climates. The disease occurs in both rainfed and irrigated dry land pepper crops. The fungus is soilborne and causes root rot, stem blight, foliar blight, and fruit rot in pepper. This study was conducted to evaluate pepper entries in INTHOPE 4 for their reactions to *P. capsici* in greenhouse inoculation studies.

Materials and Methods

Seeds of the pepper lines to be tested were sown in a steam-sterilized soil mix with four lines per soil flat (32 x 48 cm) and thinned after emergence to 20 plants per line. Each line was replicated four times with each replication planted in a different flat and arranged in a randomized complete block design on a greenhouse bench. In addition to the 24 INTHOPE 4 lines being tested, highly resistant (PI 201234) and highly susceptible (Blue Star) pepper lines were included as controls.

To prepare the inoculum, isolate Pc-4 of *P. capsici* was grown on V-8 juice agar in petri plates for 1 week. Each plate colony along with the agar medium was cut into four equal sections and each section moved aseptically to a sterile petri plate and diced into approximately 0.5-1 cm^2 pieces. Sterile distilled water was added to cover the agar blocks; the plates were incubated at room temperature for 1-2 h, and the water decanted. Sufficient sterile distilled water was then added to each plate to bring the water level even with the upper surface of the agar blocks and the plates were incubated under continuous light at 28°C for 24 h for sporangial development. The plates were then transferred to an incubator at 4°C for 1 h to trigger zoospore formation and returned to the 28°C incubator for 1 h for zoospore release. Water containing zoospores was decanted from the plates and diluted to 10^4 zoospores/ml.

Plants were inoculated by drenching the soil surface of each flat with 300 ml of the 10^4 zoospores/ml suspension 30 days after seeds were sown. The disease reaction of each entry was evaluated based on the percentage of surviving plants 12 DAI. Disease reaction categories were based on the system

described in the AVRDC 1989 Progress Report: highly resistant = 80-100% survival; moderately resistant = 50-79% survival; moderately susceptible = 30-49% survival; and highly susceptible = 0-29% survival.

Results and Discussion

Disease reactions among the 24 entries ranged from 0 to 67.5% survival 12 DAI (table 10). None of the susceptible check, Blue Star, survived while 100% of the highly resistant check, PI 201234 survived. Only one accession, PBC 534 (Tumpang from LEHRI/Indonesia), was found to possess a moderate level of resistance with 67.5% survival. Survival of all other accessions ranged from 0 to 21% placing them in the highly susceptible category. This study is a portion of a larger effort at AVRDC to evaluate pepper accessions for their reactions to phytophthora blight, to identify additional sources of resistance, and to develop phytophthora-resistant pepper lines and cultivars.

Table 10. Percentage survival of pepper plants among entries in INTHOPE 4 at 12 days after inoculation with *Phytophthora capsici*

Entry	Name/Origin	Survival (%)[a]
PBC 534	Tumpang	67.5[b]
PBC 592	(Thailand)	21.3
PBC 830	Bangchang	17.5
PBC 612	Banglen	16.3
PBC 731	Korea 13	15.0
PBC 581	(Malaysia)	13.8
PBC 550	Lv. 1592	11.3
PBC 729	Korea 4	8.8
PBC 613	Prapadaeng	7.5
PBC 580	Chillies Giant	3.8
PBC 730	Korea 11	3.8
PBC 455	(India)	2.5
PBC 539	(AVRDC)	2.5
PBC 522	BG-1	2.5
PBC 622	(AVRDC)	2.5
PBC 632	Bangladesh	2.5
PBC 456	Chilli NP46A	2.5
PBC 582	(Malaysia)	1.3
PBC 464	Mareko Fana	1.3
PBC 483	B. L. 39	0.0
PBC 600	LC-Serdang	0.0
PBC 615	Matikas	0.0
PBC 621	Kalmicho	0.0
PBC 643	Athi Slim	0.0
C 01176	PI 201234 (R ck)[c]	100.0
Commercial cv.	Blue Star (S ck)[d]	0.0
LSD (P = 1%)		11.4

[a] Values based on 20 plants in each of four replications
[b] Moderately resistant (range 50-70% survival)
[c] Highly resistant check
[d] Highly susceptible check

Pepper Physiology

Identification of RAPD Markers Linked to PVMV and CVMV Resistance Gene(s) in Pepper

Summary

Bulk segregation method in conjunction with random amplified polymorphic DNA (RAPD) analysis was employed in this study on rapid identification of genetic markers which are associated with either pepper veinal mottle virus (PVMV) or chili veinal mottle virus (CVMV) resistance in pepper. Two pairs of bulked DNA samples were generated from F_3 segregating populations originated from two separated single crosses between a susceptible pepper breeding line (S, PBC 186) and a resistant pepper breeding line (i.e., R1, VC 16a; and R2, VC 160). PBC 186 is susceptible to both PVMV and CVMV, whereas, VC 16a and VC 160 are resistant to both PVMV and CVMV. A total of 250 primers were employed in screening through all bulks, and more than 1400 loci were detected. Several potential markers were found to be associated with either CVMV or PVMV resistance; primer UBC 71 produced reproducible band in PVMV-susceptible bulk but not in resistant bulk. Thirty-eight of the tested primers (~15%) detected band variation between the two F_3 families derived from R1xS and R2xS, which revealed high genetic diversity between VC 16a and VC 160.

Introduction

PVMV and CVMV cause virus diseases and result in severe losses in pepper production. Thus, the introduction of resistance genes into cultivated lines is important in breeding for resistant lines. PVMV resistance is determined by double recessive alleles at two loci, whereas CMV resistance is determined by dominant and interactive gene effects at two other loci. Definitive confirmation of these resistance mechanisms have not yet been done. The newly developed bulk segregant analysis procedure by RAPD provides a shortcut which leads to rapid identification of genetic markers linked to important genes. The objective of this study was to identify genetic markers linked to PVMV/CVMV resistance genes and thus facilitate resistance breeding.

Materials and Methods

Plant material

Two F_3 families were developed from two separated single crosses betwen a susceptible pepper breeding line (S, PBC 186) and a resistant pepper breeding line (i.e., R1, VC 16a; and R2, VC 160). PBC 186 is susceptible to both PVMV and CVMV, whereas VC 16a and VC 160 are resistant to both PVMV and CVMV. F_3 segregating analysis for resistance to PVMV and CVMV, respectively, was done by ELISA in cooperation with the Virology Unit.

PVMV-resistant/susceptible bulked DNA

Fifty-two F_3 progenies derived from the three strongest PVMV-resistant F_2 lines (100, 97, and 88% of the F_3 progenies from each line were resistant to PVMV) originated from R1xS were bulked to generate the resistant bulked DNA. Fifty-two F_3 progenies derived from 16 susceptible F_2 lines (putative homozygous dominant) originated from the same cross were bulked to generate the susceptible bulked DNA. Another pair of bulked DNA originated from R2xS were generated by the same strategy. DNA of the selected lines was extracted separately as previously reported and bulked in equal proportion.

CVMV-resistant/susceptible bulked DNA

F_2 individuals were tested for CVMV resistance susceptibility. Ten resistant and 20 susceptible individuals were bulked separately to form two bulks of DNA for RAPD analysis.

RAPD analysis

RAPD reactions were prepared according to standard protocols and amplified using modified thermocycling conditions. DNA amplications were performed in 25 µl volume containng 50 mM KCl, 10 mM Tris-HCl (pH 8.3 at 25°C), 3 mM MgCl2, 0.1 mM dNTPs, approximately 0.2 µM primer, 0.66 unit *Taq* polymerase (Perkin Elmer Cetus) and 50 ng of pepper template bulked DNA. Two hundred fifty primers which were selected from preliminary screening of 140 primers from Operon kits A, I, M, T, U, V, and W (20 primers per kit) and 5000 primers from UBC set 1, 2, 3, 6, and 7 were tested. Reaction mixtures were overlayed with 1 drop of mineral oil. Amplifications were performed in a Perkin Elmer Cetus DNA Thermal Cycler model 480 or Thermolyne Temperature Tronic Thermal Cycler for 3 cycles (1 min at 94°C, 1 min at 40°C, and 2 min at 72°C), followed by 40 cycles (30 sec at 94°C, 30 sec at 40°C, 1 min at 72°C) and further extension at 72°C for 10 min. Polymerase chain reaction (PCR) products were analyzed by electrophoresis in 1.4% agarose gels run at 100V in TBE buffer for approximately 3 h. Gels were stained in ethidium bromide solution and photographed under UV light.

Results and Discussion

A total of 250 primers were tested against DNA bulks which were resistant/susceptible to either PVMV or CVMV, and more than 1400 reproducible loci were scored (5-6 loci per primer on average). Several potential loci associated with PVMV or CVMV resistance were identified.

Primer UBC71 (^{5}'GAGGGCGAGG^{3}') detected polymorphisms between PVMV-resistant/susceptible DNA bulks. A reproducible 0.78 kb PCR product was yielded when PVMV-susceptible bulked DNA and the DNA of the susceptible parent were used as template. Since PVMV resistance is controlled by double recessive alleles at two loci and the F_3 segregation ratio was 15:1 (susceptible: resistant), there was no major dominant allele in the resistant bulked DNA. Theoretically, bulk segregant analysis in conjunction with RAPD, is able to discriminate this dominant allele from the recessive one. However, whether this 0.78 kb PCR product is tightly linked to a dominant allele or not is still being investigated. Primer OPW 15 (^{5}'ACACCGGAAC^{3}') identified variation in band intensity between PVMV-resistant and susceptible bulks from both R1xS and R2xS crosses (i.e., brighter in resistant bulks). According to Michelmore et al. (1991)[1], bulk segregant analysis is sensitive to any markers generated within 15 cM from both sides to the target locus (30% recombination window), and the variation between bulks should be detectable at least as bands of unequal intensity. Primer OPW15 might detect a marker loosely linked to the recessive allele.

[1] Michelmore, R.W., Paran, I., and Kesseli, R.V. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl. Acad. Sci. 88:9828-9832.

CMV resistance is determined by dominant and interactive gene effects at two loci. Primer UBC600 (5'GAAGAACCGC3') detected polymorphism between CVMV-resistant and susceptible bulks. A 2.0 kb PCR product was observed only when CVMV-resistant bulked DNA and DNA of resistant parents were used as template. Based on the bulking strategy, there was no dominant allele in CVMV-susceptible bulks; thus potentially, this 2.0 kb PCR product might link to the dominant allele which determines CVMV resistance. Further confirmation needs to be done in the future.

Thirty-eight of the tested primers (-15%) detected band variation between the two F_2 families derived from R1xS and R2xS, which revealed high genetic diversity between VC 16a and VC 160.

Transformation of Truncated CMV RNA-2 Replicase Gene in Pepper

Summary

Sweet pepper C 00157D and hot pepper Szechuan were transformed with a truncated CMV RNA-2 replicase gene via *Agrobacterium*-mediated transformation. The truncated CMV RNA-2 replicate gene ligated with CaMV 35A promoter and NPT II gene (kanamycin resistant gene) provided by Dr. M. Zaitlin (Cornell University) was transferred to *Agrobacterium tumefaciens* LBA 4404 by freeze-thaw method. Excised cotyledons from 15-day-old seedlings were infected with the transformed *A. tumefaciens* and shoots were induced within 3 weeks after inoculation. About 70% and 15% of the calli of sweet pepper C 00157D and hot pepper Szechuan, respectively, developed shoots on the selection medium containing 50 mg/l kanamycin. The shoots were transferred to modified MS medium supplemented with 0.1 mg/l NAA and 25 mg/l kanamycin for rooting. Several regenerated plants were obtained. The dot blot analysis revealed that the regenerated shoots contained the introduced truncated CMV RNA-2 replicase gene. Further confirmation with Southern and Northern blot analysis, and CMV testing are still in progress.

Introduction

Cucumber mosaic virus causes large production losses in *Capsicum* in tropical Asia. In Taiwan, CMV infection decreases pepper yields by 80% or more. Since there is no efficient way to prevent the infection, the availability of CMV-resistant cultivars becomes a priority in the pepper breeding program. Anderson et al. (1992)[2] transferred a truncated CMV RNA-2 replicase gene into tobacco plants. The truncated CMV RNA-2 replicase gene was cloned from the *Fny* strain of CMV and modified by a deletion of a 94-base-pair region spanning nucleotides 1857-1950. The deletion caused a shift in the open reading frame and resulted in a truncated translation product about 75% of the full length protein. A high degree of resistance to systemic CMV disease was observed in the R_0 and R_1 lines of the transgenic tobacco plants. Some researchers concluded that resistance to CMV might be due to suppression of replication and/or to inhibition of systemic movement of CMV. In this study, we plan to transfer this CMV RNA-2 replicase gene to sweet pepper C 00157D and hot pepper Szechuan via *Agrobacterium*-mediated transformation system to obtain CMV-resistant transgenic plants in pepper.

[2] Anderson, J.M., Palukaitis, P., and Zaitlin, M. 1992. A defective replicase gene induces resistance to cucumber mosaic virus in transgenic tobacco plants. Proc. Natl. Acad. Sci. USA. 89:8759-8763.

Materials and Methods

Preparation of *Agrobacterium tumefaciens*

Plasmid pCMV N/B-23, containing an insertion of the modified *Fny*-CMV RNA-2 replicase gene (from Dr. M. Zaitlin of Cornell University), was transferred to *Agrobacterium tumefaciens* LBA 4404 strain by freeze-thaw method. The transformed *A. tumefaciens* was selected by a supplement of 50 mg/l kanamycin and 25 mg/l streptomycin to the medium.

Plant material and cotyledon disk transformation

Seeds of sweet pepepr C 00157D and hot pepper Szechuan were germinated on 1/2 MS medium. The cotyledons of 15-day-old seedlings were stripped and precultured on MS3BA medium (MS + 3 mg/l BA) for 5 days. The strips were immersed in *A. tumefaciens* solution at a concentration of 4×10^8 cells/ml. The infected strips of sweet pepper C 00157D and hot pepper Szechuan were then cultured on MS3BA and 10BI media (10BI medium: MS + 10 mg/l BA + 1 mg/l IAA), respectively for a 2-day co-cultivation, and then transferred to MS3BA and 10BI selection media with a supplement of 50 mg/l kanamycin and 500 mg/l carbenicillin. After 3 weeks, shoots formed from the developing calli were transferred to B4 media (MS medium with supplement of 1 mg/l myo-inositol, 20 mg/l adenine sulfate, 200 mg/l casein hydrolysate, 4 mg/l BA, 10 mg/l GA_3 and 15 mM silver thiosulfate) with 50 mg/l kanamycin and 200 mg/l carbenicillin and cultured under 3000 lux illumination for shoot elongation. The regenerated shoots were excised and cultured on OMSSN medium (MS + 0.1 mg/l NAA) with 25 mg/l karamycin under 3000 lux illumination for rooting.

Dot blot analysis

Total DNA was extracted from the regenerated shoots. Eight milligrams of DNA was dotted onto Hybond-N membrane and hybridized with DIG-dUTP labeled 3 kb DNA fragment of pCMV N/B-23.

Results and Discussion

Regeneration is a key to the success of transformation in higher plants. In this study, we found that regeneration rate was dependent on age of the seedlings and was species-dependent. The number of regenerated shoots was significantly reduced when the explants were excised from older seedlings (more than 15 days old). Infected explants of sweet pepper C 00157D produced more multiple shoots; infected explants of hot pepper Szechuan had lower shoot regeneration rate, and most of the explants became necrotic within 3 weeks after *A. tumefaciens* inoculation. Explants from different parts of the cotyledon had different shoot-forming potential. Trimmed cotyledons with petioles were more effective in shoot formation, because most multiple shoots developed around the cut end of petioles.

About 70 and 15% of the infected explants of sweet pepper C 00157D and hot pepper Szechuan, respectively, developed shoots on B4 selection medium containing 50 mg/l kanamycin. The dot blot analysis confirmed that these regenerated shoots contain the introduced truncated CMV RNA-2 replicase gene. Thereafter, these regenerated shoots were transferred to OMSSN medium with a supplement of 25 mg/l kanamycin and 0.1 mg/l NAA for root formation. So far, we have obtained one regenerated plant of sweet pepper C 00157D and two regenerated plants of hot pepper Szechuan. These three regenerated plants will be transferred to the soil for further growth. Further confirmation by Southern and Northern blot analyses and CMV testing are still in progress.

Soybean Breeding

Genetic Resources Enhancement and Varietal Development in Soybean

Breeding Rust-tolerant Soybean Lines

Summary

Soybean rust caused by *Phakopsora pachyrhizi* results in yield losses of up to 95%. Extensive research at AVRDC and elsewhere has shown that single-gene resistance is not dependable due to multiple virulent genes in the pathogen. Soybean rust tolerance and partial resistance appear to be the solution to the soybean rust problem. Breeding has made considerable progress in making selections for rust tolerance. Utilizing the advanced yield trial data from spring and autumn, a rust tolerance index (RTI) similar to a stress tolerance index (STI) was developed. Data from 4 years of research showed that the STI is able to effectively discriminate the high-yielding, rust-tolerant selections in environments with and without rust.

Introduction

The yield loss due to soybean rust caused by *Phakopsora pachyrhizi* has been reported to be 5-95%. Four independent single dominant genes conferring resistance to four specific races of the soybean rust pathogen have been identified (Hartwig 1986[1]). However, they were ineffective against other virulent races of the pathogen. Although rate reducing resistance has been identified, the difficulties associated with the identification and quantification made us change our strategy and select genotypes tolerant to *P. pachyrhizi* instead. Tolerance to *P. pachyrhizi* infection was defined as the yielding ability of soybean genotypes grown under severe rust stress relative to those grown under no or minimum rust stress. The objective of this study is to select genotypes with high yield potential and genes for partial resistance and tolerance to soybean rust. The aim is to minimize the use of fungicides, resulting in reduced production cost and yield increase. There is also a need to develop a reliable selection criteria for identifying both high yield and rust tolerance.

[1] Hartwig, E.E. 1986. Inheritance of a fourth major gene conferring resistance to soybean rust. Crop Science 26(6): 1135-1136.

In 1992, a stress tolerance index was found to be a better predictor of soybean rust tolerance and high yield potential. STI is estimated as: $(Yp \times Ys)/(Yp)^2$ where Yp = the yield under no-rust environment or potential yield; Ys = yield under environment with rust; and Yp = mean yield potential. The rust intensity (SI) was determined using one minus the ratio between the mean yield in a rust environment and mean potential yield: 1-(Ys/Yp).

Materials and Methods

Two advanced yield trials (AYT 1 and 2) were conducted in autumn 1993. Eleven breeding lines and three check varieties were planted on 7 September (AYT 1) and 8 September (AYT 2), respectively.

A split-plot design with fungicide and no fungicide as main plots and genotypes as subplots with four replications was used. Entries were planted in 6-m-long, six-row plots. The harvest plot size was 5 x 2 m (four middle rows).

Fungicide-treated plots were allowed to have only natural disease infection (if any). The plots without fungicide were inoculated with soybean rust pathogen inoculum prepared by the Plant Pathology Unit. Data on percent soybean rust infection, grain yield, and 100-seed weight were collected and subjected to pooled analysis of variance to determine the source of variation for various components. In analyzing the data the genotypes, treatments, and seasons were all fixed while the replication was considered random.

The rust tolerance index (AVRDC 1991)[2] was calculated as follows:

$$RTI = \frac{\text{Yield (Ys) or 100-seed weight (100 SWs) w/o fungicide}}{\text{Yield (Yp) or 100-seed weight (100 SWp) with fungicide}} \times 100$$

$$= \frac{\text{Ys or 100SWs}}{\text{Yp or 100SWp}}$$

A combined analysis of 1993 spring and autumn planting was done and an estimate of component of variance was also determined. The stress tolerance index, as reported in AVRDC (1994)[3] and Fernandez (1993)[4], was also computed.

Results and Discussion

When fungicide was not used the maximum rust severity was 71 %, which is similar to the 1991 and 1992 seasons. In AYT 1, SRE-D-14A had the best RTI of 96 for yield while SRE-B-15C and GC 82146-43-9-9 had an RTI of 94 and 90, respectively. However, based on STI, SRE-D-14B, GC 00002-100, and SRE-D-14A were superior in both stress and no-stress environment (table 1). In AYT 2, again based on STI, GC 84040-16-1, GC 86017-170-1N, GC 86049-35-2-1-1-8-1N, and GC 84051-32-1 appear to perform well under stress and no-stress conditions (table 1).

[2] AVRDC, 1991. AVRDC 1990 Progress Report. AVRDC, Shanhua, Tainan, Taiwan.

[3] AVRDC. 1994. AVRDC 1993 Progress Report. AVRDC, Shanhua, Tainan, Taiwan.

[4] Fernandez, G.C.J. 1993. Effective selection criteria for assessing stress tolerance. In: Kuo, C.G. (ed.) Adaptation of Food Crops to Temperature and Water Stress: Proceedings of an International Symposium. Taiwan. 13-18 August 1992. p. 257-270, AVRDC, Tainan, Taiwan.

Table 1. High yielding lines selected from AYT, autumn 1993

Entry	RTI[a]		Yield (t/ha)		STI[b]		100-seed wt. (g)		Rust lesion (%)	
	Yield	100-seed wt	W fungicide	W/O fungicide	Yield	100-seed wt	W fungicide	W/O fungicide	W fungicide	W/O fungicide
AYT 1										
SRE-D-14B	82	87	3.2	2.6	1.3	1.4	25.8	22.4	16	49
GC 00002-100	68	81	3.1	2 1	1.0	0.9	21.4	17.4	15	51
SRE-D-14A	96	83	3.1	3.0	1.4	1.4	26.1	21.6	13	45
SRE-B-15C	94	89	2.5	2.4	0.9	0.8	20.0	17.7	16	52
GC 82146-43-9-9	90	89	2.4	2.2	0.8	0.9	20.5	18.2	19	45
AGS 302 (ck)	89	85	2.8	2.5	1.1	1.1	22.7	19.4	21	58
AGS 129 (ck)	89	91	2.8	2.5	1.1	0.7	18.0	16.4	22	43
Mean of 14 entries			2.6	2.2			20.4	17.8	20.2	50.2
CV			10.6	9.2			5.5	3.6	31.8	13.6
LSD (5%)			0.4	0.3			1.6	0.9	9.2	9.8
AYT 2										
GC 84040-16-1	70	77	3.5	2.5	0.98	0.7	19.8	15.2	12	60
GC 86017-170-1N	77	80	3.4	2.6	1.0	1.0	23.6	18.8	10	47
GC 86049-35-2-1-1-8-1N	87	88	3.1	2.7	0.9	1.0	22.6	20.0	15	55
GC 84051-32-1	87	90	3.0	2.6	0.9	0.4	13.1	11.8	9	43
SRE-D-14B (ck)	80	86	3.0	2.4	0.8	1.4	27.2	23.5	10	50
AGS 129 (ck)	85	90	2.8	2.4	0.8	0.7	18.6	16.7	10	47
Mean of 14 entries			3.0	2.6			21.0	18.7	11.0	48.8
CV			7.8	5.5			2.8	3.4	36.4	13.9
LSD (5%)			0.3	0.2			0.8	0.9	5.8	9.7

[a] RTI = rust tolerance index
[b] STI = stress tolerance index

Combined analysis of AYT 1 and AYT 2 data from spring and autumn showed that the differences between genotypes (G), seasons (S), and treatments (T) were highly significant for both grain yield and 100-seed weight, which is an important yield component influenced by soybean rust. Among the first order interactions, G x S, V x T, and S x T for yield and 100-seed weight were highly significant.

The results of soybean rust screening trials in the past 4 years suggest that $\acute{O}^2F$ was the major component of variance for yield in spring and autumn, with the exception of 1991 in which $\acute{O}^2S$ was the major component of variance.

Conclusion

Four years of screening for soybean rust tolerance suggest that the use and non-use of fungicide effectively discriminates the tolerant lines. The stress tolerance index method is able to identify those lines with high yield potential under both rust stress and no-rust stress situations. Occasionally, seasonal differences may play a role in the intensity of soybean rust incidence, which may influence the stress tolerance index.

Breeding Appropriate Vegetable Soybean

Summary

Vegetable soybean has great potential to enhance nutritional health in the developing countries of Asia, Africa, and Latin America. Many Asian, African, and American countries are currently using grain soybeans as vegetable soybeans. Therefore, AVRDC's vegetable soybean program is specifically directed to develop varieties adapted for the tropics and subtropics with high yield potential, disease resistance, and good consumer qualities. A total of 1589 crossed seeds from 41 hybridizations were obtained. Selections from the crosses were entered into advanced and intermediate yield trials at AVRDC and at the Tainan District Agricultural Improvement Station (DAIS) in spring and autumn. New breeding lines with standard pod yield of up to 10.5 t/ha have been identified in the 1993 trials. In the 1994 trials, the standard pod yield was 10.1 t/ha for selected entries at AVRDC. In Tainan DAIS, the highest yield obtained was 13.8 t/ha in 88 days. The quality characteristics differed between genotypes.

About 25% of the total biomass are pods. About 75% of the biomass and the N in it is recycled into the soil.

Introduction

Vegetable soybean is harvested after the R6 and before R7 growth stage while the pod is still green and the seeds have developed to fill 80-90% of the pod. It has great potential to enhance the nutritional value of most cereal-based diets, particularly in developing Asian and African countries. The green beans can be shelled, cooked alone or with meat and other vegetables. They have a slightly sweet taste compared to grain soybean. In the past, vegetable soybeans adapted to the tropics were unavailable. Therefore, many countries used grain soybeans as vegetable soybeans.

AVRDC's vegetable soybean program is designed specifically for the tropics and subtropics. The spin-off from the program could be used for temperate environments as well. The major breeding objectives in developing tropical and subtropical vegetable soybean are higher yield potential, disease resistance, and good consumer qualities. The horticultural quality characteristics include pod and seed color, appearance, flavor, texture, taste, size of pod and seed, and the number of seeds per pod.

High yield and quality characters are some of the criteria used for making new cross combinations. Selections from F_2 to F_5 generations are used to cross with better quality vegetable soybean to incorporate quality characteristics. Large-pod soybeans from the tropics are used as one parent to retain the tropical adaptation, while temperate vegetable soybeans are used as the other parent. Bacterial pustule, downy mildew, and soybean rust resistance/tolerance are the other objectives in crossing.

Crosses were made between large-seeded tropically adapted selections and vegetable soybeans adapted to temperate latitudes. Selected pedigrees were backcrossed to vegetable soybeans to recover the quality traits for tropical adaptation. AGS 292 (Kaohsiung No. 1) has been found to be adapted to the tropics since it has already been back-released in Thailand as KPS 292. Similarly, AGS 190 has been noted to be adapted to the tropics; it is promising in Sri Lanka and the Philippines. Additional crosses and backcrosses have been made to combine yield and quality traits.

Vegetable soybean breeding activities include greenhouse and field observation of new accessions, generation advance, pedigree evaluation, quality assessment in the laboratory, and yield trials. Yield trials were conducted both at AVRDC and at Tainan DAIS. The results of crosses and yield trials are presented here.

Materials and Methods

A number of pedigrees from F_2 to F_7 are used in a backcrossing program to improve the quality traits and select for high yield and tropical adaptation.

In 1993, six trials were conducted in spring, summer, and autumn, four at AVRDC and two at Tainan DAIS. In spring 1994, five trials were conducted, one at Tainan DAIS and four at AVRDC (table 2). All trials were conducted using RCBD. AYT VS had four replications while IYT VS had two. AYT VS trials were planted in four-row plots of 5 x 2 m, and the harvest plot size was 5 x 1 m (the middle two rows). IYT VS was planted in four-row plots of 3 x 2 m. AVRDC's suggested cultural practices were followed at AVRDC. At Tainan DAIS, vegetable soybean was planted following rice with land preparation. Observations were made on days to R1 and R2 growth stages and 100-seed weight at R6 growth stage; graded pods (two-seeded unblemished pods), weight, and total plant weight were taken for five randomly chosen plants for each variety from each replication. Number and weight of one-, two-, and three-seeded pods, and pod length and width of two- and three-seeded pods were noted. Number and weight of damaged pods, as well as harvest index for graded pod and total pod, were also calculated.

Table 2. Vegetable soybean yield trials at Tainan DAIS and AVRDC, 1993 and 1994

Experiment	No. of entries and (checks)	Date of planting
1993		
IYT VS (Tainan DAIS)	42 (3)	12 Feb
AYT VS (Tainan DAIS)	14 (3)	5 July
IYT VS (AVRDC)	17 (3)	13 Sept
VS-OT/New Acc. (AVRDC)	56 (4)	13 Sept
1994		
AYT VS (AVRDC)	17 (3)	2 Mar
AYT VS (Tainan DAIS)	13 (2)	4 Mar
IYT VS (AVRDC)	47 (5)	17 Feb
IYT VS/COA (AVRDC)	48 (4)	25 Feb

Results and Discussion

A total of 1589 crossed seeds from 41 cross combinations were obtained. Eight cross combinations were made specifically as part of the ROC Council of Agriculture's special project. All the other crosses were made for adaptation to the tropics and to combine resistance/tolerance to downy mildew, bacterial pustule, and soybean rust.

Yield trials, 1993. Sixty new vegetable soybean accessions were evaluated for yield in spring and autumn. The results clearly indicated that the standard pod yield was influenced more by seasons than by genotypic differences: $\hat{O}^2S$ = 86.88% (P = .01%) (table 3). Six of the new accessions gave more than 5 t/ha graded pod yield and one had the highest 100-seed weight of 79 g (table 4). The quality characteristics of these entries are not presented here. These selected entries will be used in the breeding program.

The advanced yield trial 1 conducted during autumn gave a maximum biomass yield of 11.4 t/ha and a graded pod yield of 7.2 t/ha in 77 days. Seven entries gave a yield of 6 t/ha or more, which was significantly higher than that of the check variety KS No. 1 (table 5).

Table 3. Estimates of the components of variance for standard pod yield of VS-OT/new accession vegetable soybean, 1993

Component of variance	SP + AU estimate
$\sigma^2 G$	3.92 % (0.0001)
$\sigma^2 S$	86.88 % (0.0001)
$\sigma^2 SG$	3.88 % (0.0001)
σ^2	5.32 %

Table 4. Promising selections from VS-OT/new accession vegetable soybean at AVRDC, autumn 1993

Entry	Yield (t/ha) Graded pod wt	Total pod wt	Harvest index (%)	Graded pod harvest index (%)	Days to matur-ity	Pod length/ 2 seeds (cm)	Pod width/ 2 seeds (cm)	100-seed wt (g)	No.of graded pod/ 500 g
Neu Ta Pien No. 1	6.8	12.9	49.6	26.2	80	7.3	1.8	74.2	141
Neu Ta Pien No. 2	5.9	10.9	52.0	28.1	80	6.1	1.5	71.7	189
Tsuzunoko Daizu	5.6	10.5	45.6	24.3	72	5.2	1.4	65.3	210
White Lion	5.6	10.0	45.4	25.4	72	4.7	1.3	67.4	214
Shoya	5.5	10.2	44.4	23.9	72	4.5	1.3	67.9	211
Hakucho	5.1	8.9	49.4	28.3	72	4.7	1.3	63.8	240
Hiden Edamame	3.8	7.4	43.9	22.7	77	5.6	1.6	79.0	173
KS No. 2 (ck)	5.1	9.2	43.7	24.3	77	4.7	1.3	53.7	255
Ryokkoh (ck)	4.8	9.2	40.1	21.0	75	5.2	1.5	63.1	223
KS No. 3 (ck)	4.7	9.0	42.9	22.3	80	5.1	1.4	59.1	223
AGS 292 (ck)	4.0	8.2	48.2	23.5	72	5.2	1.3	65.1	193
Mean of 60 entries	4.6	8.7	47.3	24.9	70.7	4.8	1.3	61.9	234.5
CV	16.2	13.2	6.9	13.0	1.1	4.6	4.4	6.4	6.4
LSD (5%)	1.49	2.28	6.51	6.47	1.58	0.43	0.11	7.97	29.85

Table 5. Promising selections from AYT 1 vegetable soybean at AVRDC, autumn 1993

Entry	Yield (t/ha) Graded pod wt	Total pod wt	Harvest index (%)	Graded pod harvest index (%)	Days to matur-ity	Pod length/ 2 seeds (cm)	Pod width/ 2 seeds (cm)	100-seed wt (g)	No.of graded pod/ 500 g
GC 87012-10-B-4-2	7.2	11.4	49.7	31.6	77	5.0	1.3	64.1	202
GC 87012-10-B-6	6.7	10.6	51.6	32.7	74	5.5	1.3	67.5	186
GC 87012-10-B-4	6.7	11.3	48.6	28.9	74	5.0	1.3	65.2	202
GC 87009-71-1-8	6.2	11.1	46.3	26.0	77	5.2	1.3	61.5	196
GC 87020-20S-B-5	6.1	10.2	48.7	29.1	71	5.3	1.3	73.7	172
GC 87021-10-B-1	6.1	10.5	48.9	28.3	74	5.6	1.4	68.3	163
GC 87021-10-B-1-1	6.0	10.5	49.4	28.5	74	5.4	1.4	70.5	164
KS No. 2 (ck)	5.9	10.4	44.5	25.1	74	4.9	1.3	60.4	227
KS No. 3 (ck)	5.2	9.6	48.0	25.8	78	5.1	1.4	64.6	199
KS No. 1 (ck)	5.2	9.7	51.0	26.8	70	5.4	1.4	72.2	186
Means of 20 entries	5.7	10.3	49.2	27.0	73.1	5.3	1.3	68.4	190.7
CV	10.5	6.8	2.7	8.5	1.5	3.2	3.0	4.8	2.9
LSD (5%)	0.84	0.99	1.88	3.24	1.58	0.24	0.06	4.63	7.85

Yield trials, 1994. In AYT 1 at AVRDC during spring the graded pod yield was not significantly different from that of the check, KS No. 1. Three entries had significantly large pod size reflected by the lesser number of graded pod per 500 g (table 6). The quality characteristics of these selections are not presented in this report.

Table 6. Promising selections from AYT 1 vegetable soybean at AVRDC, spring 1994

Entry	Yield (t/ha) Graded pod wt	Total pod wt	Harvest index (%)	Graded pod harvest index (%)	Days to maturity	Pod length/ 2 seeds (cm)	Pod width/ 2 seeds (cm)	100-seed wt (g)	No. of graded pod/ 500 g
GC 87021-10-B-1-1	6.6	12.0	55.1	30.7	79	5.2	1.3	60.1	172
GC 87010-50-1-15	6.1	12.0	57.6	28.9	77	5.2	1.3	63.7	178
GC 87020-20S-B-5	6.1	10.8	56.1	31.5	78	5.2	1.3	65.4	169
GC 87008-65-2-12	4.8	10.2	50.3	23.9	79	5.2	1.3	72.8	177
KS No. 1 (ck)	5.9	11.7	57.1	28.9	77	5.2	1.3	58.0	188
KS No. 2 (ck)	4.0	8.5	36.6	17.2	79	4.8	1.2	57.4	217
KS No. 3 (ck)	3.8	9.2	44.4	17.9	84	4.9	1.2	52.5	210
Means	5.6	11.3	53.6	26.6	79.7	5.1	1.3	60.8	192.2
CV	14.8	11.1	4.6	9.3	1.0	2.9	3.6	5.4	3.7
LSD (5%)	1.18	1.78	3.5	3.49	1.09	0.21	0.06	4.62	10.13

The yields of the new breeding lines were at par with KS No. 1 (check), but the seed and pod sizes of a few selections are larger than those of the check variety, making them more attractive for the market and the consumers (table 7). The quality characteristics of these lines are not presented here.

Among the intermediate yield trial entries belonging to the ROC Council of Agriculture project, two entries significantly outyielded KS No. 1 (table 8). The highest graded pod yield was 10 t/ha in 83 days, compared to the check variety's 7.5 t/ha in 77 days.

Table 7. Promising selections from IYT vegetable soybean at AVRDC, spring 1994

Entry	Yield (t/ha) Graded pod wt	Total pod wt	Harvest index (%)	Graded pod harvest index (%)	Days to maturity	Pod length/ 2 seeds (cm)	Pod width/ 2 seeds (cm)	100-seed wt (g)	No.of graded pod/ 500 g
GC 89008-3-1-3-2	7.2	12.8	49.4	27.8	83	5.4	1.4	75.9	159
GC 89008-17-1-4	6.0	12.8	46.8	21.8	85	4.8	1.4	77.5	181
GC 89008-7-1-2	5.7	14.8	46.8	17.9	88	5.2	1.5	84.2	165
GC 89017-4	5.5	9.9	54.2	30.0	83	5.6	1.5	95.9	141
GC 89008-5-1-2	5.2	11.2	49.4	23.1	84	5.3	1.4	82.1	183
KS No. 1 (ck)	7.4	12.1	58.3	35.5	76	5.5	1.4	68.6	165
KS No 2 (ck)	4.9	10.9	43.8	19.3	88	4.6	1.2	56.1	213
KS No. 3 (ck)	3.3	9.3	45.9	16.1	91	5.0	1.3	54.3	195
AGS 190 (ck)	4.7	10.1	38.5	17.8	92	4.8	1.3	56.6	218
Ryokkoh (ck)	4.6	10.0	50.9	23.1	83	5.4	1.4	73.0	186
Means	5.5	11.8	47.6	22.1	86.1	5.1	1.3	69.8	187.1
CV	14.7	7.4	4.0	11.2	1.3	3.7	3.6	4.9	3.9
LSD (5%)	1.63	1.77	3.81	4.96	2.20	0.38	0.10	6.84	14.66

Table 8. Promising selections from IYT/COA vegetable soybean at AVRDC, spring 1994

Entry	Yield (t/ha) Graded pod wt	Total pod wt	Harvest index (%)	Graded pod harvest index (%)	Days to matur-ity	Pod length/ 2 seeds (cm)	Pod width/ 2 seeds (cm)	100-seed wt (g)	No. of graded pod/ 500 g
GC 89038-10-1	10.1	16.1	51.3	32.3	83	5.2	1.4	69.6	175
GC 89005-50-1	8.8	15.3	48.9	28.2	83	5.0	1.3	72.8	184
KS No. 1 (ck)	7.5	15.3	54.0	26.9	77	5.0	1.3	67.0	171
KS No. 2 (ck)	7.2	14.0	55.3	28.6	87	4.6	1.3	63.4	197
KS No. 3 (ck)	5.6	11.5	46.5	22.7	87	5.0	1.3	59.8	191
Ryokkoh (ck)	6.5	12.4	51.9	27.0	83	5.1	1.3	66.6	189
Means	6.4	13.1	49.5	24.4	84.4	5.1	0.003	69.4	188.8
CV	11.7	7.6	4.7	9.3	1.1	2.9	3.8	5.2	4.3
LSD (5%)	1.51	1.98	4.65	4.56	1.81	0.3	0.1	7.26	16.22

During summer in AYT 1, Kaohsiung No. 3 was the highest yielder with 5.7 t/ha in 80 days at AVRDC. Four lines significantly outyielded KS No. 1, the check variety (table 9).

Among the IYT entries, at Tainan DAIS in spring 1993, GC 87008-65-2-5 (TS 81-124 V) gave a graded pod yield of 11.6 t/ha in 76 days, compared to 2.2 t/ha for KS No. 1, 8.6 t/ha for KS No. 2, and 11.1 t/ha in 85 days for KS No. 3.

During summer GC 87010-15-1-18 gave a yield of 13.1 t/ha in 73 days, compared to 12.0 t/ha for KS No. 1.

In spring 1994 at Tainan DAIS, eight lines gave a yield of 9 to 13.8 t/ha, compared to 7 t/ha for KS No. 1 (table 10).

Table 9. Promising selections from AYT 1 vegetable soybean at AVRDC, summer 1994

Entry	Yield (t/ha) Graded pod wt	Total pod wt	Harvest index (%)	Graded pod harvest index (%)	Days to matur-ity	Pod length/ 2 seeds (cm)	Pod width/ 2 seeds (cm)	100-seed wt (g)	No. of graded pod/ 500 g
GC 87012-10-B-6	5.2	8.3	62.0	39.0	77	5.1	1.3	60.9	214
GC 87012-10-B-4	5.1	8.3	60.1	36.6	81	4.9	1.3	67.5	218
GC 87021-10-B-1	4.9	8.0	62.4	38.2	77	5.2	1.3	58.0	207
GC 87012-20-B-2-1	4.9	9.4	60.3	31.2	81	5.0	1.3	62.6	225
KS No. 1 (ck)	3.5	6.3	66.1	36.0	77	5.1	1.3	57.5	233
KS No. 2 (ck)	3.3	7.4	48.8	21.5	80	4.7	1.3	62.1	237
KS No. 3 (ck)	5.7	9.4	54.0	32.8	80	4.8	1.3	58.5	224
Means	4.0	7.2	61.0	33.2	78.2	5.0	1.3	59.0	228.6
CV	22.6	17.7	4.7	13.2	0.5	2.9	3.3	6.6	4.3
LSD (5%)	1.26	1.80	4.09	6.19	0.52	0.21	0.06	5.51	14.04

Table 10. Quality characteristics and yield, Tainan DAIS AYT, spring 1994

Entry	No. of graded pod/500 g	Seed wt/ 500 g (g)	100-seed wt (g)	Days to maturity	Harvest no./ 10 m²	Total weight (t/ha)	Graded pod wt (t/ha)	Index (%) ck 1	ck 2
TS 82-01V	173	250.1	72.9	88	209	36.5	13.0	107.2	-
TS 82-02V	163	266.8	75.1	88	208	30.6	13.0	101.2	-
GC 84129-6-1-1 (TS 81-115V)	191	288.5	69.8	77	213	23.3	11.8	-	167.6
GC 87010-2-1-28 (TS 81-108V)	202	281.4	61.1	77	253	23.6	10.7	-	151.8
GC 87008-55-1-24 (TS 81-105V)	189	287.5	69.4	77	223	23.6	10.3	-	145.3
GC 87010-35-2-6 (TS 81-113V)	164	283.1	74.7	77	221	24.8	10.3	-	141.7
GC 87008-65-2-B (TS 81-126V)	157	275.5	82.4	77	224	22.2	9.7	-	137.4
Kaohsiung 3 (ck 1)	163	277.8	79.5	88	218	30.6	12.9	100	-
Kaohsiung San 1 (ck 2)	180	256.9	66.2	74	195	17.3	7.1	-	100
LSD (5 %)	14.8	11.0	4.8	-	ns	4720	1828		
1 %	19.8	14.8	6.4	-	ns	6280	2445		

Evaluation of *lxlx* Gene in Elite Lines

Summary

The incorporation of isozyme lipoxygenase null genes (*lx*) into promising AVRDC lines is expected to make them more attractive due to the nonbeany flavor in soybean milk. The shelf life of soybean oil from lipoxygenase null varieties is also expected to be better. Therefore, a backcrossing program has been started to develop near-isogenic lines of AVRDC-improved materials incorporating triple nulls (*lx1lx2* and *lx3*). The program is in backcross three generation. After backcross five, the lines will be evaluated with parents.

Introduction

In the past, crosses have been made to incorporate lipoxygenase null genes (*lx*) into promising AVRDC-improved breeding lines. *LxLx* genes are responsible for the beany flavor, and the presence of the enzyme makes the shelf life of oil short. The recessive allele *lxlx* minimizes or eliminates the beany flavor and improves shelf life of oil. In South Asian and many Southeast Asian countries,consumers prefer soybean milk without the beany flavor. At AVRDC, it was observed that some vegetable soybeans lack the lipoxygenase enzyme. Three isozymes of *Lx* genes, *Lx1Lx2Lx3*, have been reported in soybean seed.

The objective of this study is to incorporate triple recessive *lx* genes to selected vegetable and grain soybean breeding lines at AVRDC.

Materials and Methods

Three AVRDC elite lines, AGS 129 (Kaohsiung No. 10), AGS 292 (Kaohsiung No. 1), and KVS 39 (Kaohsiung No. 2), were crossed to *lx1lx2lx3* mutant by Dr. Kitamura in Japan in 1992. A total of 22 F_1 seeds were received by AVRDC. In 1993, F_2 population and BC_1F_1 populations were grown. During 1994, three backcrosses were completed. The BC_3F_2 will be ready at the end of 1994.

Seeds from F_2 and backcross F_2 populations were randomly chosen and tested for triple *lx* nulls. For the analytical method and to determine the nulls see AVRDC 1994[5] (p. 307-314). The triple nulls are selected for use in backcrossing with the AVRDC elite parent which is used as the recurrent parent.

Results and Discussion

From 22 F_1 plants, 4328 F_2 seeds were obtained. At random, 1000 seeds were screened for lipoxygenase nulls, of which 73 were found to be triple nulls. They were used to backcross with the recurrent parent: 208 BC_1F_1 seeds were obtained. They were advanced to BC_1F_2. Of the 1542 BC_1F_2, 823 seeds were screened, and 44 were found to be triple nulls. The third backcross has been completed, and a total of 131 seeds will be advanced to F_2 and screened for triple nulls. After five backcrosses, the recurrent parent genes are expected to be mostly combined with the triple null genes so that the resulting lines could be evaluated against the original parents.

International Cooperation

Summary

In 1993, four new AVRDC lines were released and extended to farmers for cultivation in Taiwan (Tainan No. 2), Bangladesh [G 2120 (M7) 69-1], Vietnam (AK-05), and Sri Lanka (AGS 190). As of 1994, 12 countries have released 26 improved varieties using AVRDC soybean selections. Forty cooperators in 26 countries received 12 ASETS and 348 other lines. Another 40 cooperators from 22 countries received 14 AVSETS and 203 other vegetable soybean lines for evaluation. In Vietnam, AGS 314 and SRE-B-15C significantly outyielded local checks. In the Philippines, vegetable soybean G 10142 produced 19 t/ha graded pod in 60 days. A total of 1300 g seeds of F_2 were harvested as SSD at AVRDC during the off-season and airfreighted to Korea.

Introduction

Researchers from the national agricultural research systems (NARS) and from private agencies request germplasm and improved breeding lines from AVRDC, either for direct release to their farmers after evaluation or for use in their breeding programs. Some of the scientists return the data from their trials to AVRDC, with comments on the performance of the AVRDC materials relative to their local check varieties. NARS scientists also periodically raise issues encountered by their farmers in cultivating soybeans efficiently and economically. AVRDC recognizes the ongoing national research activities, anticipates the future needs of different NARS, and tries to undertake research which can assist and strengthen their efforts.

[5] AVRDC. 1994. AVRDC 1993 Progress Report. AVRDC, Shanhua, Tainan, Taiwan.

Distribution of AVRDC Germplasm and Elite Breeding Lines

Forty cooperators from 26 countries received 12 AVRDC ASETs, 20 AGS lines, 187 pedigree selections, 71 accessions, and 70 other selections for evaluation and use in their breeding programs (table 11).

Compared to 1993, the interest in vegetable soybean has increased in 1994. A total of 43 cooperators in 24 countries received 14 AVRDC AVSET, 110 AGS lines, 95 pedigree selections, and 48 accessions for evaluation and use in their breeding programs (table 12).

Table 11. Distribution of AVRDC ASET and other enhanced germplasm accessions to national programs in 1994

Country	No. of cooperators	ASET	AGS line	GC pedigree	Accessions	New accessions	Others
Argentina	1				7	1	
Brazil	1				2		1
El Salvador	1			5			
England	2		2	25			8
Ethiopia	1	1					
India	4			26	49	1	
Indonesia	1			20			
Iran	1	1					
Kenya	1		5				
Korea	2	1	5				35
Lao PDR	1	1					
Liberia	2	2					
Malawi	1			19	1		
Malaysia	1			10			
Papua New Guinea	1	1					
New Zealand	1	1					
Philippines	3	1	1	20			
Suriname	1		1		1		
Tanzania	1	1					
Thailand	2		3	5		1	5
Uganda	1		2				
Vietnam	2			34			
West Indies	1	1					
Zaire	1	1					
Zimbabwe	1			23			1
Taiwan, ROC	5		1		2	6	20
Total	40	12	20	187	62	9	70

ASET = AVRDC Soybean Evaluation Trials
AGS = AVRDC *Glycine max* selection

Table 12. Distribution of AVRDC AVSET and other enhanced germplasm to national programs in 1994

Country	No. of cooperators	AVSET	AGS line	GC pedigree	Accessions	New accessions	Others
Argentina	2	1	4		2	1	
Bangladesh	1		7	3			
El Salvador	1		4		1		
Fiji	1	1					
India	3		12	29	1		
Kenya	2		10		2	3	
Korea	3	1	10		2	3	
Lao PDR	1	1					
Malawi	2		12		2	6	
Malaysia	2		10				
Namibia	1		6		1	3	
Nepal	3	2	6	4			
Pakistan	1	1					
Philippines	2	2					
Suriname	1	1					
Tanzania	1	1					
Thailand	5	1	2		2		
Uganda	1		1				
West Indies	3	2	12	5			
Zambia	1		6		1	3	
Zimbabwe	1		6		1	3	
Taiwan, ROC	2			9			
Total	40	14	108	50	15	22	8

AVSET = AVRDC Vegetable Soybean Evaluation Trial

Grain Soybean Trial

A summary of the results from Vietnam is given in table 13. As in 1993, AGS 314 and SRE-B-15C have done well, compared to one of the local check variety.

In another trial conducted in Vietnam in 1994, the yields of AVRDC lines were not at par with the local check variety MTD 176 (3.5 t/ha in 90-95 days).

Table 13. Summary of international cooperation results, Vietnam

Country/Location/ Cooperator	Entry	Yield (t/ha)	Days to maturity	100-seed weight (g)	Seed quality	Remarks
Vietnam	AGS 314	1.6 a[a]	84 de	5.6 f	1 a	Date planted:
Lat. 10°57'N	AGS 299	1.2 b	83 e	9.8 de	1 a	26 July 1993
71 m	SRE-B-15C	1.2 bc	87 c	13.5 bc	1 a	Varieties: 14
Mr. Ha Huu Tien	Local check	1.1 bcd	82 ef	9.9 de	1 a	Replication: 2
	Local check	0.9 ef	79 g	13.7 bc	1 a	Autumn

[a] Values followed by the same letter are not significantly different from each other at 5% level (DMRT)

Vegetable Soybean Trial

In Thailand during a rainy season trial, a local check (AGS 190) gave a yield of 7.1 t/ha in 80 days while AGS 334 gave a graded pod yield of 6.5 t/ha in 70 days (table 14).

In another rainy season trial, AGS 328, AGS 333 and AGS 335 proved to be promising, compared to Nakhorn Sawan 1, the check variety (table 15).

Table 14. Promising selections from Thailand vegetable soybean trials 1994 rainy season

Entry	Yield (t/ha) Graded pod wt	Total pod wt	Harvest index (%)	Graded pod (%)	Days to matur-ity	Pod length/ 2 seeds (cm)	Pod weight/ 2 seeds (cm)	100-seed wt (g)	No. of graded pod/ 500 g
AGS 334	6.5	11.2	34.4	20.0	70	5.3	1.4	56.0	211
AGS 333	5.4	10.9	30.1	15.2	73	5.1	1.4	63.0	205
AGS 335	4.8	6.5	23.2	16.9	68	5.5	1.4	62.0	188
Local check	7.1	12.2	33.6	19.6	80	5.6	1.5	64.0	183
Means	4.2	8.1	29.3	14.8	71.4	5.3	1.4	58.2	206.9
CV	34.01	11.33	9.6	26.9	0	6.0	3.4	6.4	4.8
LSD (5%)	3.21	2.09	6.36	8.99	0	0.71	0.11	8.47	22.39

Table 15. Promising selections from Thailand vegetable soybean trials, 1993 wet season

Entry	Total pod weight 5 plants (g)	Days to maturity	Pod length/ 2 seeds (cm)	Pod width/ 2 seeds (cm)	Dry 100-seed weight (g)	No. of graded pod/500 g
AGS 328	329	76	4.8	1.4	23.7	195
AGS 333	319	71	4.8	1.5	30.1	162
AGS 335	292	70	5.2	1.5	29.5	159
Nakhorn Sawan 1 (ck)	203	64	4.8	1.2	21.1	294
Means	236.3	70.8	4.8	1.4	27.9	184.9
CV	15.8	1.8	1.8	2.1	1.8	1.8
LSD (5%)	84.32	2.94	0.19	0.07	1.12	7.68

Soybean Physiology

Regeneration System for Soybean and Mungbean

Summary

In this study, we succeeded in establishing tissue culture and regeneration protocols, prerequisites for the gene transformation system, for vegetable soybeans AGS 292 and Kaohsiung No. 2, and mungbeans NM92 and VC 1973A. In vegetable soybean, explants from embryo axes or cotyledonary nodes induced multiple shoots on modified MS media supplemented with 5 mg/l BAP. In mungbean, explants from cotyledons developed multiple shoots on MS or modified MS basal medium with 5 mg/l BAP. All the regenerated shoots developed roots after subculturing in MS media. The gene transformation system, employing established tissue culture protocols, with marker genes is still being developed.

Introduction

Mungbean is an important source of easily digestible, but low overall protein due to low sulfur levels. Vegetable soybean, on the other hand, although rich in proteins has low SS-linked amino acids. Hence, its flavor and texture usually do not meet consumer requirements. Among AVRDC's *Vigna* or *Glycine* germplasm collection, there are no lines with significant levels of sulfur-rich proteins. Therefore, any attempt at varietal improvement for sulfur-rich protein has to rely on other sources of materials.

Sun et al. (1987) of the University of Hawaii have purified from Brazil nut a 2S protein containing high levels of the sulfur amino acids, 18% met and 8% cys; characterized the properties, biosynthesis, and processing of this sulfur-rich protein (SRP); cloned and sequenced several cDNAs encoding the SRP; and transferred the SRP gene into tobacco plants and analyzed its expression in the transgenic seeds. Results from the transgenic study demonstrated that it is feasible to use this molecular approach to enhance the met content in seed protein (by 10 to 30% over that of the normal seeds).

In a cooperative project with AVRDC, Sun et al. of the University of Hawaii attempted to clone cDNAs encoding the 2S proteins from paradise nuts, the 2S protein of which contains 16% met and 8% cys, for protein quality improvement in mungbean and vegetable soybean. In this study, we tried to establish tissue culture and regeneration protocols, prerequisites for the gene transformation system for vegetable soybean and mungbean.

Materials and Methods

Soybean

Seeds of soybean AGS 292 and Kaohsiung No. 2 were surface sterilized with submersion in 70% ethanol for 30 sec, followed by 1.25% NaOCl for 15 min, and then rinsed with sterile water. Sterilized seeds were germinated on MS medium or readied for embryo excision. Embryo axes of sterilized seeds and nodes with part of cotyledons of seedlings were excised as explants. Explants were cultured in test tubes containing 12 ml of medium under 25°C and 16 h 3000 lux light intensity. Two modified MS basal media (i.e., MS salt and B5 compound or 2x of MS salt and B5 compound) supplemented with 5 mg/l BAP, and 2-6% sucrose were used for multiple shooting. The pH of all media were adjusted to 5.8 before 0.7% agar was added. Regenerated shoots were subcultured in MS medium for rooting under the same conditions described earlier.

Mungbean

Seeds of mungbean, cv. NM 92 and VC 1973A, were surface sterilized as described earlier for soybean and imbibed in water for 16, 24, or 26 h in the dark at 30°C. An explant of a cotyledon was separated from the plumule and cultured with the abaxial side in contact with the medium. MS or modified MS basal medium containing MS salt and B5 compound was supplemented with 3 or 5 mg/l BAP and/or 0.1 mg/l NAA and used for shoot regeneration. The pH of all media were adjusted to 5.8 before 0.7% agar was added. Explants were cultured in test tubes containing 12 ml of medium under 25°C and 16 h 3000 lux light intensity. Regenerated shoots were excised and dissected into two pieces about 10 mm from the tip, and then cultured on MS medium to induce more shoots to develop. Regenerated shoots were subcultured in MS medium for rooting under the same conditions described earlier.

Results and Discussion

Soybean

On modified MS basal medium with 6% sucrose or modified 2x MS salt basal medium with 2.5% sucrose supplemented with 5 mg/l BAP, embryo axes proliferated multiple shoots from the petiolar side of plumules. Some of the explants developed calli and regenerated abnormal rosette but showed no shoot elongation, especially on the modified 2x MS salt basal media. The reason for the abnormal rosette is unknown. On the modified MS basal medium supplemented with 5 mg/l BAP and 6% sucrose, four to six multiple shoots were also induced from cotyledonary nodes.

Mungbean

Explants regenerated shoots from the basal adaxial side of cotyledons on MS or modified MS basal medium supplemented with 3 or 5 mg/l within 3 to 4 weeks. Two to five shoots proliferated per explant on MS or modified MS basal medium with 3 or 5 mg/l BAP; no shoots were induced on media containing NAA. Cuttings of the proliferated shoots were able to induce more shoots on MS medium. Roots were induced after being transferred to MS medium.

Tomato Breeding

Crosses and Segregating Populations

A total of 141 crosses were made in 1994 by the AVRDC tomato improvement program. Crossing objectives included incorporation of heat tolerance, bacterial wilt resistance, and geminivirus resistance (30); combining ability tests of F_1 cherry hybrids (86); improvement of provitamin A activity or PVY resistance or insect resistance (25); and backcrosses (BC_1) to improve shelf life (11). DC_1 populations are being advanced by single-seed descent to improve the following characters: heat tolerance, bacterial wilt resistance (9 populations); heat tolerance, bacterial wilt resistance, large fruit size, and quality (19 populations). Three BC_1 populations designed to develop recombinant inbred lines for bacterial spot, heat tolerance, or flood tolerance are currently in the F_4 generation, and advancement by single-seed descent will continue to the F_7 generation. Thirteen F_2 populations were evaluated for heat tolerance, and 237 plants were selected from nine populations. F_3 plants will be tested for bacterial wilt resistance in early 1995.

Evaluation of Bacterial Wilt-resistant Tomato Lines in Southeast Asia

Summary

Bacterial wilt (BW), caused by *Pseudomonas solanacearum* Smith, is the most destructive disease of tomato in the tropics. Twenty tomato lines/accessions previously identified as BW-resistant were evaluated for BW resistance in fields highly infested with *P. solanacearum* at Subang, Indonesia; Los Baños, Philippines; AVRDC and Taiwan Seed Improvement and Propagation Service (TSS), Taiwan. Entries were also tested in the greenhouse at AVRDC using a drench inoculation method with a *P. solanacearum* strain (Pss4) from Taiwan. The objective of the study was to identify the most stable sources of BW resistance. Mean entry survival was 21.6% at Subang, 31.9% at Los Baños, 76.7% at AVRDC, and 93.3% at Taiwan Seed Service, indicating that most entries were resistant at the Taiwan locations but susceptible at Subang and Los Baños. L 285 and CRA 84-58-1 were the most resistant entries over locations. Mean survival and area under the disease progress curve (AUDPC) of entries derived from CRA 66 were significantly better than the mean of entries with resistance derived from PI 129080/UPCA 1169 over locations. Mean survival of AVRDC entries bred in the 1980s was 19% greater than that of AVRDC lines bred in the 1970s. Correlations between entry BW evaluations averaged over the four field trials and entry means from drench inoculation in the greenhouse were highly significant (0.69 and 0.74 for percent survival and AUDPC, respectively), suggesting that the drench inoculation method is effective in selection for BW resistance.

Introduction

Bacterial wilt is the most important disease of tomato in the tropics, and it severely limits tomato production in lowland tropical and subtropical regions. Disease control is difficult because of the broad host range, widespread distribution, and considerable genetic variability of *P. solanacearum*. Identification of BW resistance sources and combining BW resistance with other traits critical to tropical adaptation, such as high temperature fruit-set, have been objectives of the AVRDC tomato improvement program.

AVRDC tomato lines have been widely distributed in Southeast Asia, and it has been observed that some lines selected for BW resistance in Taiwan are susceptible at other locations. Evaluation of BW-resistant tomato germplasm over many environments would be the most effective strategy to identify the best sources of BW resistance. Recognizing the advantage of a regional approach to BW research, AVRDC and several Southeast Asian NARS assembled and tested BW-resistant tomato accessions/lines as part of the Collaborative Vegetable Research Program in Southeast Asia (AVNET). This study aimed to identify the best sources of BW resistance for tomato breeding programs in Southeast Asia.

Materials and Methods

Twenty tomato accessions/lines previously identified as BW-resistant (table 1) were chosen for multilocation BW evaluations. Eleven entries were from the AVRDC tomato program: CL 1131-0-0-13-0-6 (CL 1131), CL 143-0-10-3-0-1-10 (CL 143), CL 8d-0-7-1 (CL 8), CL 9-0-0-1-3 (CL 9), CL 11d-0-2-1 (CL 11), CL 5915-93D_4-1-0 (CL 5915-93), CL 5915-206D_4-2 2-0 (CL 5915-206), CL 6047-1-1-2-3-2-7-0 (CL 6047), CLN 475-BC_1F_2-265-4-19 (CLN 475), CLN 65-349D_5-2-0 (CLN 65), CLN 657-BC_1F_2-285-0-21-0 (CLN 657); five entries were developed by the Institut National de la Recherche Agronomique (INRA) in Guadeloupe, French Antilles; L 285 is a small-fruited *L. esculentum* var. *cerasiforme* landrace. MT 5 and MT 10 are selections made at the Malaysian Agricultural Research and Development Institute from segregating populations sent from AVRDC; Tm2 is a BW-resistant line from the Bureau of Plant Industry, Philippines.

Field trials

Field experiments were established at the Subang Horticultural Research Station, Java, Indonesia (lat. 6°5'S and long. 107°8'E; 110 m asl); the University of the Philippines Los Baños (UPLB), Luzon, Philippines (lat. 14°11'N and long. 121°13'E; 21.7 m asl); Taiwan Seed Improvement and Propagation Station, Taichung, Taiwan (lat. 24°12'N and long. 120°49'E; 470 m asl), and AVRDC, Shanhua, Taiwan (lat. 23°07'N and long. 120°17'E; 14 m asl). Soil pH and textures of the field sites, trial sowing and transplanting dates, total rainfall, and average maximum/minimum temperatures during the trials are presented in table 2.

Prior to initiation of the BW screening trial, the following procedure was carried out at Subang, UPLB, and AVRDC to increase *P. solanacearum* densities and to promote uniform pathogen distribution at field sites. Experimental units consisting of 5.2-m-long, 1-m-wide raised beds, with 0.5 m between beds, were constructed. Three-week-old seedlings of the BW-susceptible tomato accession L 390 (Nan Tzu from Taiwan) were transplanted to each bed and arranged in four rows, spaced 0.2 m between and 0.10 m within rows. When L 390 plants were 30-35 days old, (five- to seven-leaf stage), the upper leaves were cut with knives or scissors and a suspension of *P. solanacearum*, consisting of 1.6×10^8 or 1×10^9 cfu/ml of a virulent *P. solanacearum* isolate, was applied to the fresh wounds by sprayer or by dipping knives or scissors in the inoculum before wounding. *P. solanacearum* isolates used at Subang, UPLB, and AVRDC to prepare inocula were isolated from diseased tomato plants in

Table 1. Tomato accessions/lines evaluated for bacterial wilt reaction in Southeast Asia

Entry	Origin[a]	Growth habit	Resistance source
MT 5[c]	MARDI	sp	PI 129080, UPCA 1169
MT 10[c]	MARDI	sp	PI 129080, UPCA 1169
TM-2	BPI	sp	Unknown
L 285	AVRDC	sp[b]	Taiwan landrace
CRA 84-79	INRA	sp	CRA 66
CRA 84-58-1	INRA	sp	CRA 66
CRA 84-6-3	INRA	sp	CRA 66
CRA 84-26-3	INRA	sp	CRA 66
Caraibe	INRA	sp	CRA 66
CL 1131[d]	AVRDC	sp	PI 129080, UPCA 1169
CL 143	AVRDC	sp	PI 129080, UPCA 1169
CL 8	AVRDC	sp	PI 129080, UPCA 1169
CL 9	AVRDC	sp	PI 129080, UPCA 1169
CL 11	AVRDC	sp	PI 129080, UPCA 1169
CL 5915-93	AVRDC	sp	PI 129080, UPCA 1169
CL 5915-206	AVRDC	sp[b]	PI 129080, UPCA 1169
CL 6047	AVRDC	sp	PI 129080, UPCA 1169
CLN 475	AVRDC	sp	PI 129080, UPCA 1169
CLN 65	AVRDC	sp	PI 129080, UPCA 1169, Kewalo
CLN 657	AVRDC	sp[b]	PI 129080, UPCA 1169

[a] MARDI= Malaysian Agricultural Research and Development Institute; BPI= Bureau of Plant Industry, Philippines; INRA= Institut National de la Recherche Agronomique;
[b] sp[b]=indeterminate, sp=determinate
[c] Introduction from AVRDC or a selection from a segregating population sent from AVRDC
[d] Full AVRDC entry names are given in the Materials and Methods section

Table 2. Selected soil characteristics of test sites, total rainfall, and average temperatures during tomato bacterial wilt evaluations

Location	Soil pH	Soil texture	Transplant date/ Final evaluation	Mean air temperature max./min. (°C)	Rainfall during trial (mm)
Subang, Indonesia	5.3	7.3% sand 29.5% clay 63.2% ash	9 Apr 1992/ 1 June 1992	33.4/23.0	546.6
UPLB, Philippines	5.6	28.8% sand 42.7% silt 28.5% clay	15 Feb 1991/ 13 May 1991	32.2/23.0	141.7
AVRDC, Taiwan	7.4	62.0% sand 32.0% silt 5.0% clay	14 June 1994/ 27 July 1994	33.0/25.2	386.5
Taiwan Seed Service	5.7	58.0% sand 22.0% silt 18.0% clay	3 Aug 1994/ 3 Oct 1994	29.5/20.0	296.6

the respective countries, and all isolates were classified as race 1 biovar 3. About 2 weeks after inoculation, plants were tilled into the soil and beds were rebuilt at the same positions. At TSS, entries were transplanted into soil naturally infested with *P. solanacearum*. The field at TSS had been used in several previous seasons as a "sick plot" for testing tomato lines for BW resistance (Sunny Chen, personal communication).

Three-week-old plants of checks and test accessions were transplanted into the reconstructed beds. At AVRDC, seeds of entries were sown directly in the beds. At UPLB and Subang, three 4-m-long rows were established on each bed. The test accessions were transplanted in the two outer rows, established 0.2 m from the side of the bed. The middle row included resistant (L 285 or Intan Putih) and susceptible (L 390) check plants, transplanted alternately. Spacing between the middle and outer rows was 0.3 m and within-row spacing was 0.2 m. At AVRDC and TSS, only a test accession was transplanted to each bed. Trials were arranged in a randomized complete block design (RCBD) with two replications at Subang and TSS, and three replications at UPLB and AVRDC. The number of wilted plants in each bed was recorded every 7-14 days. Stems of wilted plants were cut, placed in water, and examined for bacterial ooze to confirm the presence of *P. solanacearum*.

Greenhouse trial

Entries were evaluated for BW resistance in an AVRDC greenhouse with a soil drench inoculation method. Individual seedlings were grown in 6-cm-diam plastic pots. Potting soil mixture ratio was 1 sand : 3 soil : 1 rice husk : 1 compost. Entries were replicated twice in an RCBD with 24 plants per replication. At 25 days after sowing (five true-leaf stage), 20 ml of 10^8 cfu/ml *P. solanacearum* (AVRDC isolate Pss4 from Taiwan) was applied to the base of each plant. Plants were evaluated weekly for wilting for 4 weeks.

Data analysis

The percentage of wilted plants was determined for experimental units at each evaluation date, and a standardized AUDPC was calculated. Final percent entry survival was based on the last evaluation at each trial. Survival data were transformed by arcsine of the square root to normalize the scale before carrying out analyses of variance. For combined analyses of variance, locations were regarded as random effects and entries as fixed effects; the location-by-entry mean square was used to test the significance of the mean square for entries and for linear contrasts. Nonindependent, single degree-of-freedom contrasts were constructed to make comparisons among groups of entries: (1) CR66-derived entries vs. entries with BW resistance derived from PI 129080/UPCA 1169; (2) L 285 vs. CRA 66-derived entries; and (3) AVRDC lines bred in the 1970s (CL 11d, CL 8d, CL 9, CL 143, CL 1131) vs. AVRDC lines bred in the 1980s (CL 5915-206, CL 5915-93, CL 6047, CLN 475, CLN 65, CLN 675).

Results and Discussion

BW incidence on the susceptible check, L 390, was almost 100% at UPLB, AVRDC, and TSS (table 3), indicating high disease pressure in the trials. Mean survival of entries, excluding the susceptible check L 390, was dramatically less at Subang and UPLB (21.6% and 31.9% survival, respectively), compared to Taiwan trials (76.7 and 93.3% survival at AVRDC and TSS, respectively). Most entries were resistant in Taiwan but susceptible at Subang and UPLB.

Analyses of variance over locations indicated significant differences among entries in BW resistance. L 285 and CRA 84-58-1 were the most resistant entries at Subang and UPLB, and both demonstrated >80% survival in the Taiwan trials. Entries CL 11, CL 9, and CL 6047, on the other hand,

Table 3. Bacterial wilt reactions of tomato entries evaluated at four Southeast Asian locations and in the greenhouse

Entry	Subang, Indonesia		UPLB, Philippines		AVRDC, Taiwan		TSS, Taiwan		Greenhouse	
	Survival (%)	AUDPC[a]	Survival (%)	AUDPC	Survival (%)	AUDPC	Survival (%)	AUDPC	Survival (%)	AUDPC
L 285	75.3 a[b]	11.6 a	68.1 a	22.0 ab	89.6 a-d	5.5 a	93.8 a	2.9 abc	95.9 abc	3.0 abc
CRA 84-58-1	52.5 ab	23.6 ab	66.0 ab	18.1 ab	84.9 bcd	5.9 a	100.0 a	0.0 a	100.0 a	0.0 a
CRA 84-79	45.8 bc	31.1 bc	53.6 abc	13.6 a	98.3 a	0.2 a	100.0 a	0.0 a	97.9 ab	1.2 ab
CLN 65	42.1 bc	28.3 bc	43.6 a-d	24.2 ab	90.9 a-d	2.9 a	96.5 a	2.1 abc	83.4 cde	11.1 a-f
MT 5	32.9 bcd	27.9 bc	37.5 b-f	25.3 ab	92.7 abc	4.0 a	100.0 a	0.0 a	93.8 a-d	4.7 a-d
CRA 84-6	30.4 b-e	37.3 bf	0.0 h	85.6 f	75.7 def	7.6 a	100.0 a	0.0 a	76.4 def	14.0 c-h
CLN 475	27.5 b-e	33.6 bcd	32.6 c-f	29.4 abc	86.0 bcd	2.1 a	100.0 a	0.0 a	89.2 a-d	6.6 a-e
MT 10	27.3 b-e	34.6 b-e	64.8 ab	19.7 ab	94.8 abc	0.8 a	93.8 a	4.0 abc	73.0 def	18.6 fgh
CL 5915-93	24.2 cde	36.9 bf	59.6 abc	24.8 ab	79.0 cf	8.1 a	100.0 a	0.0 a	87.1 b-e	7.7 a-f
Tm-2	15.3 d-g	47.1 d-h	53.6 abc	20.0 ab	92.3 abc	3.6 a	95.5 a	2.9 abc	70.1 b-e	19.1 fgh
Caraibe	15.3 def	49.6 e-i	43.3 a-e	29.5 abc	86.9 a-d	3.4 a	100.0 a	0.0 a	87.5 b-e	8.1 a-f
CRA 84-26	12.8 d-g	43.7 c-g	47.7 a-d	30.2 a-d	94.6 ab	1.2 a	100.0 a	0.0 a	74.6 def	17.8 e-h
CL 5915-206	10.2 efg	50.4 f-i	13.0 fgh	51.8 e	79.5 b-e	8.6 a	78.5 b	9.3 cd	76.6 def	14.7 c-h
CL 1131	5.7 fgh	55.6 ghi	11.1 fgh	50.4 e	55.9 fg	19.9 bc	100.0 a	0.0 a	56.3 fg	24.8 h
CL 143	5.5 fgh	48.8 d-h	16.9 e-h	34.9 b-e	80.8 b-e	8.0 a	100.0 a	0.0 a	89.4 b-e	6.4 a-e
CL 9	3.4 fgh	58.7 ghi	0.1 h	52.2 e	58.7 efg	10.9 ab	74.0 b	6.4 a-d	84.2 b-e	10.0 a-f
CL 6047	2.8 gh	62.4 hi	0.0 h	83.1 f	38.1 gh	28.6 bc	59.7 b	13.9 d	31.3 gh	45.8 i
CL 8	2.5 gh	58.0 ghi	7.1 gh	45.6 cde	50.5 g	20.9 bc	97.0 a	0.9 ab	83.4 cde	12.2 b-g
CLN 657	0.0 h	57.0 ghi	16.8 d-g	47.8 de	79.6 b-e	9.8 ab	100.0 a	0.0 a	74.2 def	16.5 d-h
CL 11	0.0 h	63.2 i	1.0 h	77.5 f	25.4 h	34.4 d	78.0 b	8.1 bcd	57.7 def	22.4 gh
L 390 (S)[c]			1.0 h	83.3 f	0.0 i	66.5 e	0.0 c	57.0 e	17.1 h	55.3 i
Trial mean	21.6	43.0	31.9	39.3	76.7	9.3	93.3	2.5	79.1	13.2

[a] Area under the disease progress curve

[b] Mean separation by LSD (P = 5%). Data were transformed to the arcsine of the square root for analysis

[c] L 390 not included in Subang trial

ranked consistently among the least resistant entries at all locations. AUDPC values for CL 6047, CL 9, and CL 11 were significantly less than those of L 390 at AVRDC, indicating that these entries did not wilt as quickly as the susceptible check. Most entries performed well in Taiwan but poorly in Subang and UPLB. For example, survival of CLN 657 was about 90% averaged over the Taiwan trials, but was 16.8% at UPLB and 0% at Subang. Significant rank changes and magnitude differences among trials accounted for the highly significant location-by-entry interaction in the analyses of variance (table 4).

Table 4. Mean squares from the combined analysis of variance and contrasts of tomato entries evaluated for bacterial wilt reaction in Southeast Asia

Source of variation	DF	Mean square
% Survival[a]		
Locations (L)	3	34025**
Entries	19	1746**
L x Entries	57	236**
Contrasts		
#1 CRA 66-derived (65.3)[b] vs. PI 129080-derived (47.4)	1	7450**
#2 L 285 (81.1) vs. CRA (65.3)	1	588[ns]
#3 AVRDC: 1970s (37.1) vs. 1980s (44.3)	1	3251**
AUDPC[c]		
Locations (L)	3	19913**
Entries	19	1134**
L x Entries	57	272**
Contrasts		
#1 CRA 66-derived (19.1) vs. PI 129080-derived (28.0)	1	2526**
#2 L 285 (11.1) vs. CRA 66-derived (19.1)	1	586[ns]
#3 AVRDC: 1970s (33.3) vs. 1980s (25.9)1	1	1318*

[a] Data were transformed to the arcsine of the square root for analysis
[b] Number in parentheses is the group mean
[c] Area under the disease progress curve
[ns,*,**] Nonsignificant or significant at 5% and 1%, respectively

When the Subang and UPLB trials were analyzed together without the Taiwan trials, a significant location-by-entry effect was found (data not shown), but, generally, the most resistant entries at Subang were also the most resistant at UPLB. The survival mean of CRA 66-derived entries was almost 40% greater than the mean of entries with resistance derived from PI 129080/UPCA 1169 (contrast no. 1). Mean survival of L 285 was not significantly higher than the group mean of CRA 66-derived lines (contrast no. 2). The group survival mean of AVRDC lines bred in the 1980s was 19% greater than the mean of 1970 lines, indicating that AVRDC has made progress in selection for BW resistance (contrast no. 3). However, BW resistance in most AVRDC entries is derived from only two sources, and progress probably could be improved by exploiting new resistance sources.

An objective of the AVRDC tomato improvement program is the development of stable, BW-resistant lines with a survival greater than 90%. However, no entry in this experiment achieved this resistance level at Subang or UPLB. It is clear that different BW resistance sources must be combined with the objective of achieving higher resistance levels or with L 285. Advanced lines derived from CRA and AVRDC lines are undergoing preliminary testing at AVRDC. Although most CRA lines carry excellent BW resistance and have large fruit size, most need improvement for high temperature fruit-set.

Routine BW screening at AVRDC includes inoculation of tomato lines in the greenhouse with a virulent strain of *P. solanacearum* from Taiwan (Pss4) with a drench technique. Correlations between entry BW evaluations averaged over field trials and entry means from drench inoculation in the greenhouse were highly significant (0.69 and 0.74 for percent survival and AUDPC, respectively). This suggests that the drench method is effective in selection for BW resistance. The drench method is advantageous compared to field BW screening because cost per entry is less and results are more quickly available. Detection of susceptible lines and entries with low BW resistance such as CL 6047 or CL 11 is probable at AVRDC with the drench inoculation method. However, AVRDC screening may not detect lines like CL 143 or CL 5915-206, which are resistant in Taiwan but perform poorly in the Philippines and Indonesia. Establishment of a two-stage testing program where entries resistant at AVRDC are sent to the Philippines and Indonesia for further evaluation would also help identify weak entries.

Routine Bacterial Wilt Evaluation

Summary

Over 400 tomato entries from were tested in the plastic house for bacterial wilt resistance during late 1993 to mid-1994 in 11 BW trials. BW resistance levels were generally high among fresh market (FM) entries. The 16 best fresh market entries demonstrated BW resistance equal to the resistant check. BW resistance, overall, was less among the processing and cherry entries tested; the best entries in these market classes showed about 50% survival. Crosses to improve BW resistance in processing and cherry market classes will be planned.

Introduction

Bacterial wilt is the most important tomato disease in the tropics and, consequently, BW resistance testing is a major effort of the AVRDC tomato improvement program.

Materials and Methods

BW evaluation trials conducted during late 1993-94 are listed in the following table.

Types of materials	No. of entries	Sown	Inoculated	Recorded
Fresh market inbreds (Group 1)	40	5/12/94	6/9/94	6/16-6/23/94
Fresh market inbreds (Group 2)	46	5/12/94	6/9/94	6/16-6/23/94
Fresh market inbreds (Group 3)	49	6/8/94	7/5/94	7/12-7/19/94
Fresh market inbreds (Group 4)	48	6/8/94	7/5/94	7/12-7/19/94
Cherry tomato inbreds	24	8/3/94	8/31/94	9/7-9/14/94
Cherry tomato inbreds	42	8/3/94	8/31/94	9/7-9/14/94
Processing tomato inbreds (Group 1)	63	8/3/93	8/31/94	9/7-9/14/94
Processing tomato inbreds (Group 2)	51	8/10/94	9/7/94	9/14-9/21/94
PVY-resistant line	15	8/10/94	9/7/94	9/14-9/21/94
CLN 1305 recombinant inbreds	68	8/10/94	9/7/94	9/14-9/21/94
ß-carotene FM	40	8/10/94	9/7/94	9/14-9/21/94

Entries were tested in summer 1994 in the plastic house using a soil drench inoculation method. Individual plants were grown in 6-cm-diam pots in an AVRDC soil medium. Experimental units consisted of 24 plants and entries were replicated twice in a randomized complete block design. About 25 days after sowing (five true-leaf stage), 20 ml of 10^8 cells/ml inoculum of *Pseudomonas*

solanacearum strain Pss4 was poured into each pot. Plants were observed weekly for wilting until 4 weeks after inoculation.

The fresh market lines evaluated for BW resistance were derivatives from crosses of AVRDC tropical lines and CRA lines from Guadeloupe, French West Indies. The objective of this study is to broaden the genetic base for BW resistance in AVRDC germplasm and improve BW resistance. The best FM entries are given in table 5, several of which demonstrated BW resistance equal to the resistant check, L 285. Trial means of the processing and cherry lines were low. Two best cherry tomato entries were CLN 1561-128-37-11-11-6 (46.9% survival) and CLN 1561-128-37-7-4-3 (41.9%). The best PT entries were PT 4658BC_1F_3-4-0-1 (52.1% survival), PT 4664BC_1F_3-3-0-1 (47.9%), PT 4675BC_2F_3-2-0-4 (47.9%), PT 4679BC_2F_3-67-0-6 (46.7%), PT 4716BC_1F_3-1-0-8 (45.9%), and PT 4671BC_1F_3-18-0-1 (45.8%). Future crosses are planned to improve the BW resistance in processing and cherry tomato market classes.

Table 5. Bacterial wilt reactions of selected fresh market lines inoculated by drenching, plastic house, summer 1994

	BW survival (%)
Group 1	
CLN 1460-162-16-52-0-0	90.5a
CLN 1463-245-14-0-0-0	73.9a-c
CLN 1462-20-10-25-1-0	68.5a-d
L 285 (R ck)	84.5ab
L 390 (S ck)	0.0g
Mean of all entries	44.6
Group 2	
CLN 1466-65-40-30-0-12	77.9a
CLN 1464-46-11-30-0-7	77.1ab
CLN 1466-65-40-30-0-12	76.2ab
CLN 1466-65-40-49-0-0	75.0a-c
L 285 (R ck)	71.9a-d
L 390 (S ck)	2.2g
Mean of all entries	50.9
Group 3	
CLN 1462-140-31-42-0-0	83.4a
CLN 1464-46-11-2-0-1	80.9a-b
CLN 1461-125-3-46-0-4	76.7a-c
L 285 (R ck)	78.3ab
L 390 (S ck)	6.3d
Mean of all entries	53.0
Group 4	
CLN 1466-65-40-25-1-1	91.6a
CLN 1466-16-16-16-0-5	81.3ab
CLN 1466-65-40-49-0-7	81.1ab
CLN 1461-125-3-46-0-4	80.0ab
CLN 1466-65-40-30-0-2	77.3a-c
CLN 1466-140-31-42-0-0	75.0a-c
L 285 (R ck)	72.9a-c
L 390 (S ck)	4.2
Mean of all entries	53.2

* Means within groups followed by the same letter are not significantly different at the 5% level (DMRT)

F_7-derived recombinant inbred lines from Ohio MR-13 x L 285 were evaluated for BW resistance. Among the 65 recombinant inbreds, CLN 1305F_7-87S, CLN 1305F_7-136M, CLN 1305F_7-178M, CLN 1305F_7-228 ML, CLN 1305F_7-293M, and CLN 1305F_7-307S had reactions comparable to the resistant parent, L 285. These lines may be useful in the breeding program. Future plans include evaluating the recombinant inbreds for BW resistance at several locations and to use the data to map BW resistance genes in L 285 with molecular markers as well as to characterize the contribution of different loci to BW resistance.

Genetic Improvement of Fresh Market Tomato

Summary

Eight fresh market tomato trials were conducted at AVRDC during late summer 1993 through early summer 1994; however, five trials were destroyed by flooding and geminivirus infection. Yields of the best FM inbreds in the 1993 summer observational trial (OT) were exceptionally high, ranging from 24.6 to 42.4 t/ha. Similarly, yields of experimental hybrids in the second 1993 summer OT ranged from 21.7 to 56.1 t/ha. Most entries in both OT showed excellent bacterial wilt resistance. Even though high temperatures and relative humidities prevailed during summer 1993, there was an absence of significant flooding which may help explain the unusually high yields. Additional evaluation of these entries is needed to confirm their performance.

Introduction

The large-fruited fresh market tomato is the most important market class in the tropics. The characteristics of a good tropical fresh market tomato variety include high temperature fruit-set and high marketable yields, bacterial wilt resistance, good fruit quality, and firmness.

Materials and Methods

Eight trials were established during late summer 1994 until early summer 1995. Trial type, entry number, and sowing, transplanting, and harvest dates are given below:

Types of materials	No. of entries	Sown	Transplanted	Harvest
OYT 1	52	6/29/93	7/28/93	10/5-10/28/93
OYT 2	130	7/01/93	7/29/93	10/14-11/5/93
OYT 3	44	4/12/94	5/10/94	no harvest
OYT 4	45	5/17/94	6/17/94	no harvest
OYT 5	49	5/17/94	6/17/94	no harvest
PYT 1	10	4/12/94	5/10/94	no harvest
PYT 2	40	4/28/94	5/26/94	7/20/94
PYT 3	46	5/17/94	6/17/94	no harvest

Observational trials were made up of two-row unreplicated plots. Rows were 4.8 m long, and between and within-row spacing was 0.6 m and 0.4 m, respectively. Distance between plots (beds) was 1.5 m. The purpose of the OT is to identify promising lines or hybrids. Plot size and spacing in the preliminary yield trial (PYT) is identical to the OT, but entries are replicated twice in a randomized complete block design. Prior to forming beds, 2000 kg/ha of organic compost, 5 kg/ha borax, and 40-80-60 kg/ha of N-P_2O_5-K_2O were broadcasted in the field. About 10 days after transplanting, a side-dressing of 20-0-30 kg/ha of N-P_2O_5-K_2O was applied. A second side-dressing, applied 3-4 weeks after transplanting, included 60-20-60 of N-P_2O_5-K_2O kg/ha. The third side-dressing, applied 6 weeks after transplanting, included 60-20-30 of N-P_2O_5-K_2O kg/ha. Plots were furrow-irrigated as required.

Results and Discussion

Entries in all FM trials consisted of advanced lines or hybrids from crosses of AVRDC tropical lines and CRA lines from INRA, Guadeloupe. CRA lines carry high levels of bacterial wilt resistance and are large-fruited, but tend not to set well under high temperatures.

OT 2 included indeterminate inbreds. Average maximum/minimum temperatures during the trial were 30.7/23.4°C; total precipitation and mean relative humidity were 411 mm and 78.9%, respectively. Marketable yields and other characteristics of the nine best entries and a hybrid check, FMTT 22, are presented in table 6. Yields were remarkably high, ranging from 24 to 42 t/ha. Six entries yielded more than FMTT 22. Although warm temperatures prevailed during the trial, the absence of significant flooding may have allowed entries to express their yield potential. Fruit color of all entries was poor and most entries need better vine cover. All entries showed good bacterial wilt resistance.

Indeterminate FM hybrids were evaluated in OT 1. Maximum/minimum temperatures during the trial were 31.6/24.1°C. Total rainfall and mean relative humidity were 411 mm and 78.2%, respectively. Information regarding the eight best hybrids are given in table 7. Marketable yields were extraordinarily high, possibly because of excellent heat tolerance in the hybrids and lack of flooding during the trial. Brix contents were above 5, fruit colors were generally fair, and vine cover of entries was good. Since these entries were tested at one location without replication, further testing is needed to confirm their high yield potential.

The 1994 summer trials suffered from a combination of flooding and geminivirus infection; no yield was harvested from five trials. However, several entries in PYT 3 and OT 2 were noted for having fruit-set percentage greater than the heat-tolerant check (CL 5915-93D_4-1-0-3): CLN 1460-220-10-0-0-5, CLN 1462-220-10-0-9-0, CLN 1462-125-3-46-3-0 (PYT 3); CLN 1462-140-31-10-0-4, CLN 1460-42-20-56-0-0, and CLN 1462-140-31-42-0-0.

Yields were collected from PYT 2 (indeterminate entries), but were abysmally poor (<2 t/ha). The best entries were CLN 1464-111-30-2-0-0, CLN 1464-111-30-45-0-0, CLN 1462-220-10-8-1-0, and CLN 1462-111-30-30-39-0-0. Some flooding occurred, but all plants of every entry were infected by geminivirus (Taiwan tomato leaf curl virus) vectored by whiteflies. Average maximum/minimum temperatures during PYT 2 were 32.7/25.0°C; total precipitation was 585 mm.

Table 6. Yield and other horticultural characteristics of selected indeterminate fresh market breeding lines and checks in observational trial, AVRDC, summer 1993

Entries	Mkt. yield (t/ha)	Fruit-set (%)	Fruit size (g)	Days to flower-ing	Days to first ripe fruit	Days to maturity	Acidity	°Brix	Color (a/b)	Sugar/ acid ratio	Cracking[a]	Firmness[b]	Vine cover[c]	Size uniformity w/in cluster	BW survival (%)
CLN 1463-160-40-16	42.4	28	85	17	69	85	0.30	4.9	0.85	16.3	SLR/SA	MF	F	G	95
CLN 1462-220-10-0	39.4	45	86	17	66	85	0.29	4.4	1.02	15.2	0	MS	F	E	92
CLN 1463-245-14-0	38.4	33	75	22	69	85	0.49	4.8	1.75	9.8	MC/R	F	E	F	100
CLN 1464-111-30-2	37.9	22	84	17	73	86	0.34	4.4	0.92	12.9	0	VF	F	E	80
CLN 1464-61-31-18	32.8	26	77	20	66	85	0.43	5.1	1.71	11.9	0	MF	G	F	96
CLN 1462-140-31-10	31.7	40	73	20	66	85	0.43	4.9	1.00	11.4	MC/RSA	MF	F	G	95
CLN 1466-65-40-25	28.3	36	113	22	79	87	0.32	4.8	1.10	15.0	VSLR/SA	F	F	G	90
CLN 1464-111-30-39	26.8	17	77	20	73	86	0.32	4.5	1.42	14.1	SLSA	MF	G	E	100
CLN 1466-65-40-49	24.6	28	85	17	69	85	0.31	4.4	1.07	14.1	0	VF	F	E	92
FMTT 22 (HT ck)	31.5	31	77	10	66	85	0.42	5.4	1.15	12.9	SLR	MF	E	G	89

[a] 0 = none; VSLRSA = very slight radial cracking and superficial abrasion; SLR = slight radial cracking; SL/A = slight superficial abrasion; SLR = slight radial cracking; MC/R = moderate concentric and radial cracking

[b] MS = moderately soft; MF = moderately firm; F = firm; VF = very firm

[c] F = fair; G = good; E= excellent

Table 7. Yield and other horticultural characteristics of selected indeterminate fresh market hybrids and checks in observational trial, AVRDC, late summer 1993

Entries	Mkt. yield (t/ha)	Fruit-set (%)	Fruit size (g)	Days to flowering	Days to first ripe fruit	Days to maturity	Acidity	°Brix	Color (a/b)	Sugar/acid ratio	Cracking[a]	Firmness[b]	Vine cover[c]	Vine compactness	BW survival (%)
CLN 1463-160-40-16	42.4	28	85	17	69	85	0.30	4.9	0.85	16.3	SLR/SA	MF	F	G	95
FMTT 574	56.1	36	89	13	66	79	0.56	6.5	1.46	11.7	0	F	E	E	95
FMTT 573	42.2	26	93	13	66	79	0.33	5.1	1.58	13.0	0	VF	E	E	92
FMTT 559	33.7	28	96	15	66	79	0.52	5.6	2.06	10.8	MR	MF	F	F	100
FMTT 572	27.4	23	65	13	66	79	0.49	5.1	1.78	10.4	0	VF	G	E	80
FMTT 557	25.3	24	95	20	62	78	0.47	5.2	1.92	11.9	MR	F	G	G	96
FMTT 558	23.9	31	77	20	66	79	0.54	5.1	2.03	9.4	MR/C	F	G	F	95
FMTT 560	22.6	33	88	15	66	79	0.44	5.1	1.50	11.6	0	VF	F	F	90
FMTT 566	21.7	36	74	15	66	79	0.43	5.0	1.67	11.6	0	F	F	F	100
FMTT 22 (CH ck)	15.6	26	78	17	66	79	0.46	5.1	1.16	11.0	0	F	G	G	92
Double Fortune	4.6	17	79	20	79	86	–	–	–	–	SVR	S	G	E	89

[a] 0 = none; MR = moderate radial cracking; MR/C = moderate radial and concentric cracking; SVR = severe radial cracking

[b] MF = moderately firm; F = firm; VF = very firm

[c] F = fair; G = good; E= excellent

Genetic Improvement of Processing Tomato

Summary

One processing tomato (PT) yield trial was conducted in the cool season (October 1993-February 1994) to identify high-yielding entries with good processing fruit qualities and machine harvest characters. The trial mean was 49.3 t/ha and the seven best entries yielded from 54.3 to 69.5 t/ha, but none were significantly better than the check varieties. Fruit of the highest-yielding entry, CLN 1356-1088-1-22-0, were very firm.

Introduction

Products made from processed tomato such as catsup or paste are popular in some tropical countries. Special fruit characters are required for PT varieties including deep-red color, high solids content, low pH, and firmness. Machine-harvestable PT should have a concentrated fruit-set, compact vine, and a jointless pedicel.

Materials and Methods

One trial of PT lines was conducted during fall 1993. Entry number, trial type, sowing, trains planting, and harvest dates are given below:

Types of materials	No. of entries	Sown	Transplanted	Harvested
Preliminary	19	9/15/93	10/14/93	12/28/93-2/10/94

The preliminary yield trial was planted in RCBD with two replications. Each plot was comprised of two 4-m-long beds spaced 1.5 m apart; within-row spacing between plants was 40 cm. Fertilizer was broadcasted into the field prior to forming beds at 42-100-120 kg/ha of N-P_2O_5-K_2O. Organic compost (2000 kg) and borax (5 kg/ha) were also applied prior to bed formation. Plots were furrow-irrigated.

Results and Discussion

Mean maximum and minimum temperatures during the preliminary yield trial were 26.1 and 16.2°C, respectively. Total rainfall was 42.0 mm and mean relative humidity was 73.5%. The objective of the PYT was to identify PT lines with machine-harvest characters, particularly the jointless pedicel and compact vine, as well as good fruit quality, firmness, and high yield. The seven best entries in the PYT are presented in table 8. Marketable yields of the best entries ranged from 54.3 to 69.5 t/ha, but none yielded significantly more than checks UC 204A and PT 4454. Fruit of the highest-yielding entry, CLN 1356-1088-1-22-0, were very firm. Color and Brix of the experimental lines were less than the checks. The best entries will be further evaluated for heat tolerance and bacterial wilt resistance.

Table 8. Yield and other horticultural characteristics of selected processing breeding lines and checks in the PYT, AVRDC, cool season 1993

Entries	Mkt. yield (t/ha)[a]	Fruit-set (%)	Fruit size (g)	flower-ing	Days to first ripe fruit	maturity	Acidity	Brix (%)	Color (a/b)	Sugar/ acid ratio	Crack-ing[a]	Firm-ness[b]	Vine cover[c]	Vine compact-ness	Joint-less
CLN 1356-1088-1-22-0	69.5a	62	54e	17a-c	61b	100a	0.31e-g	4.6d-f	1.96e-h	14.8	SLR	VF	G	G	+
CLN 1349-1001-5-10-0	64.6ab	55	60de	19a-c	63b	101a	0.36e-g	4.7c-f	1.64i	13.1	0	MF	G	G	+
CLN 1349-1001-5-14-0	61.5ab	71	60de	17a-c	61b	100a	0.31d-g	4.20f	1.97e-h	13.1	SLC/R	MF	G	G	+
CLN 1356-1058-18-14-0	59.9ab	77	75b-d	17a-c	63b	101a	0.32c-g	4.5d-e	2.14b-g	14.1	0	MF	F	F	+
CLN 1350-1039-1-0	57.3a-c	71	68c-e	19a-c	63b	101a	0.27g	4.6d-e	2.10b-h	17.0	0	F	G	G	+
CLN 1349-1014-5-1-0	57.1a-c	64	65c-e	23a	63b	101a	0.28fg	4.3ef	2.15b-f	15.3	MC	MF	F	F	+
CLN 1349-1001-20-3-0	54.3a-d	64	76bc	22ab	67ab	102a	0.32c-g	4.4ef	1.93f-h	13.8	SLR	F	E	E	+
PT 4454 (ck)	65.1ab	67	73b-d	16c	63b	101a	0.33b-f	4.9b-f	2.26a-c	14.8	0	MF	G	G	–
UC 204A (ck)	56.8a-c	68	74b-d	19a-c	70a	103a	0.38a	5.4a-c	2.18b-e	14.2	0	F	E	E	–
TK 18 (local ck)	43.4b-e	59	102a	17a-c	63b	101a	0.35a-e	4.9b-f	2.42a	14.0	0	S	F	F	–
Mean of all entries	49.3	66	71	19	64	101	0.32	4.8	2.1		–	–	–	–	–
CV (%)	19.1	14.4	8.6	10.8	5.6	0.9	6.5	6.4	4.5		–	–	–	–	–

[a] 0 = none; SLR = slight radial cracking; SLC/R = slight concentric and radial cracking; MC = moderate concentric cracking

[b] S = soft; MF = moderately firm; F = firm; VF = very firm

[c] F = fair; G = good; E= excellent

[d] Means in the same column followed by the same letter are not significantly different at the 5% level (DMRT)

Genetic Improvement of Cherry Tomato

Summary

Two cool season and four summer cherry tomato (CT) trials were established at AVRDC in late 1993 and 1994. Cool season yields of the best indeterminate cherry hybrids ranged from 33 to 43 t/ha, and yields of the best indeterminate lines ranged from 35 to 52 t/ha. Summer trials were heavily damaged by a combination of flooding and geminivirus infection. Yields of the best entries in both summer trials were below 5 t/ha.

Introduction

Cherry tomato (*L. esculentum* var. *cerasiforme*), the probable ancestor of the large-fruited cultivated tomato, is relatively unknown in tropical Asia and sub-Saharan Africa. It has become popular in the USA, Japan, Taiwan, and Europe. In general, cherry tomato is better adapted to warm, humid conditions compared to the large-fruited tomato, so it may have potential for lowland summer production. High temperature fruit-set; resistance to TMV, bacterial wilt, and geminivirus; and good fruit qualities are major selection criteria.

Materials and Methods

Six CT trials were conducted from September 1993 to June 1994, two of which were destroyed by flooding and geminivirus infection. Trial type, entry number, and dates of sowing, transplanting, and harvest are listed below:

Types of materials	No. of entries	Sown	Transplanted	Harvested
Observational 1	14	4/12/94	5/10/94	no harvest
Observational 2	40	5/17/94	6/24/94	no harvest
Preliminary 1	19	9/10/93	10/14/93	10/25/93-2/1/94
Preliminary 2	22	5/17/94	6/24/94	8/14/94-8/24/94
Advanced 1	10	9/14/93	10/14/93	12/14/93-1/31/94
Advanced 2	10	4/12/94	5/10/94	6/30-7/13/94

OT plots consisted of one double-row bed, each 4 m long and spaced 60 cm apart within the beds. Plants within the row were 40 cm apart. Entries were not replicated. Prior to forming beds, 2000 kg/ha organic compost, 5 kg/ha borax, and 40-80-60 kg/ha of N-P_2O_5-K_2O were broadcasted in the field. About 10 days after transplanting, a side-dressing of 20-0-30 kg/ha of N-P_2O_5-K_2O was applied. The second side-dressing, applied 3-4 weeks after transplanting, included 60-20-60 kg/ha of N-P_2O_5-K_2O. A third side-dressing, applied 6 weeks after transplanting, included 60-20-60 kg/ha of N-P_2O_5-K_2O. Plots were furrow-irrigated as needed. Plot size of the PYT was identical to the OYT, but entries were replicated twice in RCBD. Experimental units of Advanced Yield Trials (AYT) consisted of two beds, each 4 m long, spaced 60 cm apart within the beds, with plants within the row spaced 40 cm apart. Entries were replicated thrice and arranged in RCBD.

Results and Discussion

Cool season trials. The 1993 PYT included indeterminate cherry tomato hybrids; data on the best entries are presented in table 9. Mean maximum/minimum temperatures during the trial were 25.7/16.0°C. Mean relative humidity was 72.6% and total precipitation was 42 mm. The best entries yielded as well as AVRDC check hybrids CHT 261 and CHT 264 and three to four times more than the

Table 9. Yield and other horticultural characteristics of selected cherry tomato hybrids and checks in PYT, AVRDC, cool season, 1994

Entries	Mkt. yield (t/ha)[d]	Fruit-set (%)	Fruit size (g)	Days to flowering	Days to first ripe fruit	Days to maturity	Acidity	°Brix	Color (a/b)	Sugar/ acid ratio	Crack-ing[a]	Firm-ness[b]	Vine-cover[c]
CHT 720	40.3a	77d-f	14bc	10a	50a-c	79b	0.66ab	4.7d-f	1.58ab	7.1	0	MF	E
CHT 621	39.6a	74ef	15bc	10a	50a-c	79b	0.59a-e	4.4gf	1.63a	7.5	0	S	E
CHT 957	36.9ab	89ab	12ef	8a	48bc	79b	0.53ef	4.8b-f	1.36a-e	9.1	0	MF	E
CHT 659	35.1ab	90a	13cd	10a	50a-c	79b	0.65ab	4.9b-d	1.40a-d	7.5	0	VF	E
CHT 961	34.3a-c	83a-d	9g	7a	46c	79b	0.58b-f	4.8b-e	1.38a-e	8.3	MR	MF	G
CHT 691	34.1a-c	72f	15b	7a	50a-c	79b	0.64a-c	4.7c-f	1.64a	7.3	MR	MF	E
CHT 649	33.7a-c	89a	22a	9a	50a-c	79b	0.55d-f	5.1b	1.11ef	9.3	o	MF	E
CHT 959	33.0a-c	81b-e	12ef	8a	46c	79b	0.59a-e	4.7c-f	1.49a-c	7.9	MR	F	E
CHT 261 (HT ck)	38.9a	84a-c	11e-g	10a	53a	80a	0.53ef	4.5ef	1.48a-b	7.6	N	F	G
CHT 264 (HT ck)	35.5ab	77d-f	11e-g	8a	50a-c	79b	0.49f	4.1g	1.53ab	8.4	N	F	G
Santa (local ck)	11.8f	82a-e	10fg	10a	52ab	79b	0.63a-d	6.2a	1.16ef	9.8	SVR	VF	F
Mean of all entries	30.8	80	12.5	8.4	49	79	0.58	4.87	1.37	8.4	–	–	–
CV (%)	12.3	4.3	5.8	21.4	2.5	0.2	5.7	3.2	8.5		–	–	–

[a] 0 = none; MR = moderate radial cracking; SVR = severe radial cracking
[b] S = soft; MF = moderately firm; F = firm; VF = very firm
[c] F = fair; G = good; E= excellent
[d] Means in the same column followed by the same letter are not significantly different at the 5% level (DMRT)

commercial hybrid Santa (Known-You Seed Company). Most developed excellent vine cover as well. However, entries produced significantly lower soluble solids content than Santa.

The AYT included cherry inbred lines developed from Santa. Mean temperatures, relative humidity, and precipitation were identical to the PYT. The best lines, SN-240SD-2-0 and SN-664-1-0, produced marketable yields of 51.2 and 49.1 t/ha, respectively, comparable to AVRDC hybrid checks CHT 261 and CHT 264 but superior to Santa (table 10). The Brix content of Santa was superior to other entries. Fruit sizes of all entries were small.

Summer trials. The summer PYT cherry trial (indeterminate lines) was heavily damaged by flooding and geminivirus infection and lasted only 62 days. Average maximum/minimum temperatures during the trial were 32.5/25.2°C. Total precipitation was 1769 mm; mean relative humidity was 68.9%. The trial mean was 1.2 t/ha, and the best entry, CLN 1560-4-3-16-3-3, yielded only 4.4 t/ha but fruit-set was 40%, indicating that it carries some heat tolerance. Almost one-third of the plants of this entry were infected by geminivirus. Similarly, the AYT suffered heavy flooding and geminivirus infection. Mean yield was 3.2 t/ha. SN-60-1-0, SN-60-10-0, SN-60-2-0, and SN-240SD-4-0 achieved fruit-sets greater than 30% and may have some heat tolerance.

High Vitamin A Tomato

Summary

Diets in most tropical countries are deficient in vitamin A which is crucial for good human nutrition. A tropically-adapted tomato variety with high provitamin A activity (high fruit beta-carotene content) is valuable for home gardens. Seventy-one advanced lines derived from crosses segregating for genes (*dg* or *B*) that increase beta-carotene content were evaluated for fruit quality and bacterial wilt resistance in the field. Seven entries with either *dg* (dark-green) or *B* (Beta) genes produced an average of 6.7 times more beta-carotene than the normal-fruited check. Internal color of *dg* entries was deep red, but Brix content tended to be low. Fruit of *B* entries were yellow-orange. One *B* entry, CLN 1315F_1-57A-30-26, was bacterial wilt resistant.

Introduction

Vitamin A is crucial for good human nutrition, particularly for growth and proper eyesight. Tomato can be an important source of vitamin A. This year we have renewed efforts to develop tomato varieties high in beta-carotene, the fruit pigment responsible for provitamin A activity. Our objective is to develop lines high in beta-carotene content suitable for home gardens.

Several genes are known to elevate beta-carotene contents, including Beta (*B*), dark-green (*dg*), and high pigment (*hp*). Advanced lines from populations developed in the mid-1980s at AVRDC, segregating for *dg* or *B*, were evaluated for fruit quality characters and bacterial wilt resistance. The objective was to identify tropically adapted indeterminate lines carrying high beta-carotene genes for further testing or for use as parents in crosses.

Materials and Methods

Seventy-one were sown on 30 December 1993 and transplanted to the field on 4 February 1994. Plots included 12 plants in a 6-m row, spaced 0.6 m between and 0.5 m within rows. Entries were not replicated. Lines with dark-green or yellow fruit were tested for fruit quality by the AVRDC Analytical Laboratory.

Table 10. Yield and other horticultural characteristics of selected cherry tomato inbred lines and check cultivars in the AYT, AVRDC, cool season, 1993

Entries	Mkt. yield (t/ha)[a]	Fruit-set (%)	Fruit size (g)	Days to flower-ing	Days to first ripe fruit	Days to maturity	Acidity	°Brix	Color (a/b)	Sugar/ acid ratio	Vit. A	Vit. C	Crack-ing[a]	Vine cover[b]	Firm-ness[c]	Foliar diseases[d] (BLM)
SN-240SD-2-0	51.2a-c	72	9	16a	56a	81a	0.48ef	5.2c	1.50d	10.8	1.04b	15.0a-c	0	G	VF	3
SN-664-1-0	49.1a-d	70	7	7b	46c	80b	0.54bc	5.2c	1.75ab	9.6	1.16b	15.8ab	0	F	MF	3
SN-60-1-0	48.5b-d	72	7	10b	49bc	80b	0.52cd	5.3bc	1.71a-c	10.2	1.09b	17.8a	SLR	F	VF	4
SN-275-1-0	48.1b-d	70	7	8b	47c	80b	0.47f	5.3bc	1.70a-c	11.3	1.52a	15.8ab	0	G	VF	3
SN-60-2-0	46.7cd	75	7	10b	47c	80b	0.52cd	5.7ab	1.67a-c	10.9	1.00b	16.9a	0	F	VF	3
SN-240SD-4-0	41.4de	72	9	9b	46c	80b	0.48ef	5.2c	1.50bc	10.8	1.08b	14.6a-c	0	F	VF	4
SN-60-10-0	35.1e	73	7	8b	46c	80b	0.48ef	5.2c	1.84d	10.8	0.99b	16.3a	0	F	F	4
CHT 261 (ck)	55.8ab	72	10	10b	54c	81a	0.56ab	4.5d	1.63a	8.0	1.13b	12.0bc	0	F	F	5
CHT 264 (ck)	57.1a	71	10	11b	54a	81a	0.50de	4.6d	1.73b-d	9.2	1.01b	12.5bc	0	G	F	6
Santa (ck)	20.5f	74	10	10b	51b	80b	0.58a	6.0a	1.31a-c	10.3	0.96b	15.9ab	SVR	G	VF	4
Mean of all entries	45.4	72	8.4	10.0	49.7	80	0.51	5.2	1.64e	10.2	1.10	15.8	–	–	–	–
CV (%)	9.6	5.9	2.9	16.7	3.7	0.2	3.7	4.1	4.8	–	9.9	8.5	–	–	–	–

[a] 0 = none; SLR = slight radial cracking; SVR = severe radial cracking
[b] F = fair; G = good
[c] MF = moderately firm; F = firm; VF = very firm
[d] Rating scale of 1 = none; 2-3 = slight; 4-5 = moderate; 6-7 = severe; 8-9 = very severe
[e] Means in the same column followed by the same letter are not significantly different at 5% level (DMRT)

Results

Entries carrying *B* or *dg* contained about 6.7 times more beta-carotene than the check FMTT 33 (table 11). The two *dg* entries showed slightly higher beta-carotene levels than *B* lines; Brix content of *dg* entries was lower than *B* lines. Because the *B* gene increases beta-carotene content at the expense of lycopene, *B* fruit were yellow or yellow-orange. The *dg* gene increases both beta-carotene and lycopene contents, and internal color of *dg* entries was excellent. Lines with *dg* may be more acceptable to consumers accustomed to red-fruited tomato.

Entries did not differ appreciably in fruit pH or acidity. Only CLN 1315F_3-57A-30-26 demonstrated significant bacterial wilt resistance. This line may be the most tropically adapted *B* entry. All the lines need improvement for high temperature fruit-set and vine cover. Lines carrying *hp* will be evaluated in 1995.

Table 11. Fruit quality and bacterial wilt reactions of tomato lines with *B* or *dg* genes

Entry	Gene	pH	Acidity	°Brix	Color (a/b)	ß-carotene mg/100 g	BW (% survival)
CLN 1308F_3-25-0-29	dg	4.3	0.34	3.9	1.50	3.28	10.4
CLN 1312F_3-98-5-15	dg	4.4	0.30	3.7	1.76	3.63	2.1
CLN 1313F_3-68-1-8	B	4.3	0.45	4.4	1.91	2.84	6.5
CLN 1314BC_1F_3-32-4-4	B	4.2	0.49	4.6	0.54	2.85	2.2
CLN 1314BC_1F_3-32-30-15	B	4.4	0.40	4.6	0.65	2.11	4.2
CLN 1315F_3-9-9-22	B	4.2	0.52	5.1	0.55	2.85	0
CLN 1315F_3-57A-30-26	B	4.2	0.39	4.7	0.49	3.52	79.2
FMTT 33	–	4.4	0.36	5.1	2.15	0.45	–

Development of Multivirus-resistant Tomato

Geminivirus Resistance

Summary

Collaboration among the AVRDC plant physiology, tomato breeding, and virology units was initiated to introgress genes from *L. chilense* conditioning geminivirus resistance into cultivated tomato. Interspecific crosses involving 11 geminivirus-resistant *L. chilense* accessions and three tomato varieties were carried out at AVRDC, beginning in April 1993. Embryo rescue of the F_1 was necessary because of sexual incompatibility between *L. chilense* and *L. esculentum*. F_1 plants were successfully obtained from 20 cross combinations, but most have not been tested for reaction to Taiwan tomato leaf curl virus (TTLCV), the local geminivirus strain. A grafting technique was used initially to screen plants for virus resistance, but testing by exposure to viruliferous whiteflies was carried out as well. Pollen from resistant plants was collected and used to backcross to *L. esculentum*. In one cross, two backcrosses were performed.

Introduction

Geminivirus, vectored by the sweet potato whitefly (*Bemisia tabaci*), is an important production constraint of tomato in many parts of the world. Yield loss can be 100% if young plants become infected. Since one viruliferous whitefly can cause infection, host resistance would be the best control method. Currently, there is little geminivirus resistance available in *L. esculentum*; however, some accessions of *L. chilense*, a green-fruited wild tomato species, are immune to the virus. The objective of this subproject is to introgress genes conditioning geminivirus resistance from *L. chilense* into tropical tomato.

Materials and Methods

The Virology Unit has identified 40 *L. chilense* accessions immune to the local geminivirus strain, Taiwan tomato leaf curl virus (TTLCV). Interspecific crosses involving 11 *L. chilense* accessions and 3 tomato varieties (33 cross combinations) were carried out at AVRDC, beginning in April 1993. Embryo rescue of the interspecific hybrids was required 3-4 weeks after pollination because *L. esculentum* and *L. chilense* are normally incompatible.

Embryo rescue. Fruit from each of the crosses were harvested 3 to 4 weeks after pollination and surface sterilized. These were dissected under sterile conditions and the immature seeds excised. Immature seed coats were removed and embryos at the heart stage were placed on MS basal medium supplemented with IAA, BA, GA_3, NAA, kinetin, zeatin, 2 IP, or 2, 4-D and kept at room temperature under 12 h light. Regenerated plantlets were dissected into various segments and cultured in the MS basal medium without hormones for multiplication. After multiplication, the small plantlets were transferred from MS medium to sterile vermiculite and kept in the culture room. When the plants showed shoot elongation and leaf expansion, they were transferred to a growth chamber and maintained at 25/20°C (day/night) and 75% relative humidity. When the plantlets were strong enough, they were transferred to clay pots containing the AVRDC compost mixture.

Virus resistance evaluation. Initially, F_1 and BC_1F_1 plants obtained from embryo rescue were screened for TTLCV resistance by a grafting technique. The top 10 cm of the plants to be tested was excised and grafted to TTLCV-infected tomato. Six weeks after grafting, leaf tissue of the grafted scion was removed and tested for the presence of TTLCV by nonradioactive DNA-hybridization, using a TTLCV-specific probe previously developed at AVRDC.

Pollen was collected from resistant plants, bulked, and used for backcrossing to *L. esculentum* to produce BC_1F_1 seed. Embryo rescue was necessary to obtain about 50-60 BC_1F_1 plants per cross. Embryo rescue is not required after the BC_2.

Results and Discussion

Interspecific F_1 plants from 20 crosses were obtained, including at least one cross with each *L. chilense* accession. Although hundreds of embryos per cross were rescued, the number of clones per cross was low due to incompatibility between the two species. Crosses derived from LA 1932 and LA 2737 produced relatively more successful embryos and were more amenable to crossing with tomato compared to other *L. chilense* accessions.

Of the 20 successful crosses, backcrosses of F_1 plants to tomato have been accomplished for 17 crosses, but most BC_1F_1 plants are still in tissue culture. BC_1F_1 and BC_2F_1 plants have been developed from two crosses involving LA 1969. It appears that TTLCV resistance in LA 1969 (source I_6 from Dr. Rick, University of California-Davis) may be dominant, but the number of genes conditioning resistance is not clear. However, most F_1 and BC_1F_1 plants from LA 1969 (I_4) are susceptible, indicating variability for virus resistance within LA 1969.

During most of 1994 we evaluated plants for TTLCV reaction by grafting, but since August 1994 we have shifted to screening plants with a combination of viruliferous whiteflies and TTLCV probes. Plants at about the five-leaf stage were exposed to viruliferous whiteflies in the greenhouse or screenhouse, and susceptible plants developed symptoms about 2-3 weeks after exposure. Symptomless plants were tested for virus infection and also observed longer for symptom expression. Plants free of symptoms and without virus infection were given priority for backcrossing to tomato.

An objective of this subproject is to introgress genes from *L. chilense* into tomato which confer resistance to a wide range of geminiviruses. Presently, we are evaluating plants with only the TTLCV strain and assume that TTLCV resistance will hold up against geminivirus strains at other locations. We have sent the 11 *L. chilense* accessions and BC_1F_1 plants from crosses involving *L. chilense* LA 1969 for geminivirus evaluation to the AVRDC Africa Regional Program (ARP) in Tanzania.

In 1995, we intend to work out the screening technique so that resistant plants are identified more quickly, permitting rapid backcrossing and reducing the workload involved in the management of many crosses. We hope to determine if introgressed genes from *L. chilense* condition immunity or delayed symptom expression, and to better understand the inheritance of TTLCV resistance.

Potato virus Y (PVY)

PI 247087 (*L. hirsutum*) is resistant to PVY, an important disease of tomato in the tropics. In 1986, crosses were made at AVRDC to introgress PVY resistance from PI 247087 into tomato. Four backcrosses to *L. esculentum* were performed, followed by inbreeding. Presently, we have six BC_4F_5 lines resistant to PVY. In 1995, we intend to reconfirm PVY resistance in these lines, preferably at other locations as well as AVRDC, to establish crosses to improve tropical adaptation of these lines and determine the inheritance of PVY resistance.

Cucumber mosaic virus (CMV)

Two single-plant selections from *L. chilense* accession LA 1969 (LA 1969-9 and LA 1969-11) were identified by the AVRDC Virology Unit as CMV-resistant. Interspecific F_1 plants from *L. esculentum* x LA 1969-9 have been obtained but have not been tested for CMV resistance. We will continue backcrossing to *L. esculentum*.

International Cooperation

Cultivar Releases

CL 32D-0-1-1-P-0-0-0-1, CL 119-1-2-0, and CL 143-0-4B-1-0-0 were officially released as Samtom 13, Samtom 14, and Samtom 15, respectively, in the semiarid zones of Nigeria in 1993. This brings the total of released AVRDC tomato lines since 1978 to 80 in 34 countries.

Feedback from Collaborative Trials

Africa. Dr. Brian Hilton, of Food for the Hungry Institute Afrique, tested AVRDC indeterminate breeding lines in 1992. The best line, CL 5915-206D_4-2-2-0, outyielded the local check varieties, but the fruit size of CL 5915-206D_4-2-2-0 was slightly smaller than the checks. All AVRDC lines showed less flooding tolerance and lower fruit acid content than the check cultivar, Marmande.

India. Dr. P. Vidyasagar, of Khatau Junker Limited, India, tested AVRDC fresh market, processing, and cherry tomato lines and hybrids. AVRDC hybrids FMTT 22 and PT 4121 yielded more than Rashmi, the local check.

Guam. Ms. Mari Marutani, Mssrs. Frank Cruz, Vince Santos, and George Wall of the College of Agricultural and Life Sciences, University of Guam, tested eight AVRDC tropical fresh market hybrids in 1992. The best three hybrids, FMTT 22, FMTT 138, and FMTT 267, produced nearly 2.0 kg fruit per plant, better than the best check, Chandelier. FMTT 268, FMTT 269, and FMTT 301 produced 1.4, 1.2, and 0.8 kg of fruit per plant, respectively, and showed highly resistant bacterial-wilt reactions.

Philippines. Dr. J.L. San Pedro, University Taro Production Offices, tested 10 AVRDC fresh market breeding lines and five hybrids in 1991. FMTT 33, FMTT 3, FMTT 22, FMTT 260, and CLN 657BC$_1$F$_2$-274-0-15-4 outyielded check hybrids Hope No. 1 and Tropic Boy on the basis of 10 harvests, but differences were not significant. Seed multiplication and on-farm tests are planned for 1995.

Thailand. Mr. Roengchai Chumpirom, Chiang Rai Horticultural Research Center, Thailand, evaluated AVRDC cherry tomato hybrids, fresh market hybrids, 10 indeterminate tropical breeding lines, and 8 processing tomato entries.

The best fresh market hybrid, FMTT 105, yielded 27.6 t/ha, but was susceptible to fusarium wilt and bacterial wilt. Two other hybrids, FMTT 95 and FMTT 114, yielded 15.1 and 12.0 t/ha, respectively, and were resistant to bacterial wilt (FMTT 95--80% survival and FMTT 114–91.7% survival) and fusarium wilt. Five processing hybrids, PT 4454, PT 4563, PT 4285, PT 4225, PT 4121, and PT 4446, had high yields, and were free of fusarium and bacterial wilt infection. PT 4225 showed good heat tolerance at both Nokornphanorm and Chiang Rai locations in April and May.

Vietnam. Dr. Mai Van Quyen, Deputy Director of Institute of Agricultural Science of Vietnam at Binh Quoi, Binh Thanh Dist, tested eight AVRDC processing hybrids in the 1992 rainy season. Three hybrids, PT 4110, PT 4121, and PT 4026, outyielded the check N°12 (CL 5915-223D$_4$-2-1-0), and produced good soluble solids contents. He also evaluated seven AVRDC tropical tomato breeding lines in spring 1989. The small-fruited line, CL 5915-153D$_4$-3-3-NSB, was the highest-yielding entry, and he requested more seed for additional testing in 1995.

Tomato Entomology

Screening and Selection of *Lycopersicon pennellii*-based Resistance Breeding Progeny for Resistance to Tomato Fruitworm

Summary

A *Lycopersicon pennellii* accession is being used as a source of resistance to breed tomato fruitworm (*Helicoverpa armigera* Hübner)-resistant cultivars at AVRDC. Resistance in *L. pennellii* is due to the high acylsugar content in the leaves; the higher the acylsugar, the higher the insect mortality, and the greater is the insect resistance. In spring, we screened 10 families of BC_1F_1 consisting of 232 plants for concentration of acylsugars in leaves. Only four plants having greater than 27 mg acylsugar/cm^2 leaf area were selected. The BC_1F_2 progeny planted in autumn showed varying concentrations of acylsugar. Six BC_1F_2 plants containing exceptionally high acylsugar concentration (48 to 97 mg/m^2 leaf area) were selected for making crosses to improve insect resistance and horticultural characteristics.

Introduction

Tomato fruitworm (TFW), *Helicoverpa armigera* (Hübner), is the most destructive insect pest of tomato in Asia. The adults of this polyphagous insect lay eggs on tomato foliage. Neonate larvae feed on leaves, but second instar and older ones bore into tomato fruit and feed concealed inside the fruit until ready for pupation. Full-grown larvae leave the damaged fruit and pupate in the soil. The concealed nature of the larval and pupal stages make the control of this insect by conventional means difficult. We have been using a wild tomato species, *Lycopersicon pennellii*, which is resistant to a wide range of insect pests, to breed tomato breeding lines resistant to TFW. The resistance of this accession is due to the presence of high concentrations of acylsugars in the foliage. TFW larvae crawling or feeding on the foliage which come in contact with this chemical soon die. The higher the acylsugar content, the greater is the insect mortality, and the higher is the level of resistance. The measurement of acylsugar content by chemical assay gives an accurate measurement of the level of insect resistance. In 1994, we evaluated 10 BC_1F_1 and 4 BC_1F_2 families for high concentration of acylsugars.

Materials and Methods

In spring, we planted in a greenhouse 10 families of BC_1F_1 population consisting of 232 plants. Each plant was raised in an individual clay pot. When plants were 14 weeks old, we extracted two fully opened, topmost leaflets from each plant and extracted acylsugars using methylene chloride by a routine procedure shown in fig. 1. Extraction lasted over 1 week during which samples were maintained at -20°C. Each extract was further processed and concentration of acylsugar was measured by a chemical analysis illustrated in fig. 2. The same analytical procedure was used to screen four BC_1F_2 families in subsequent tests.

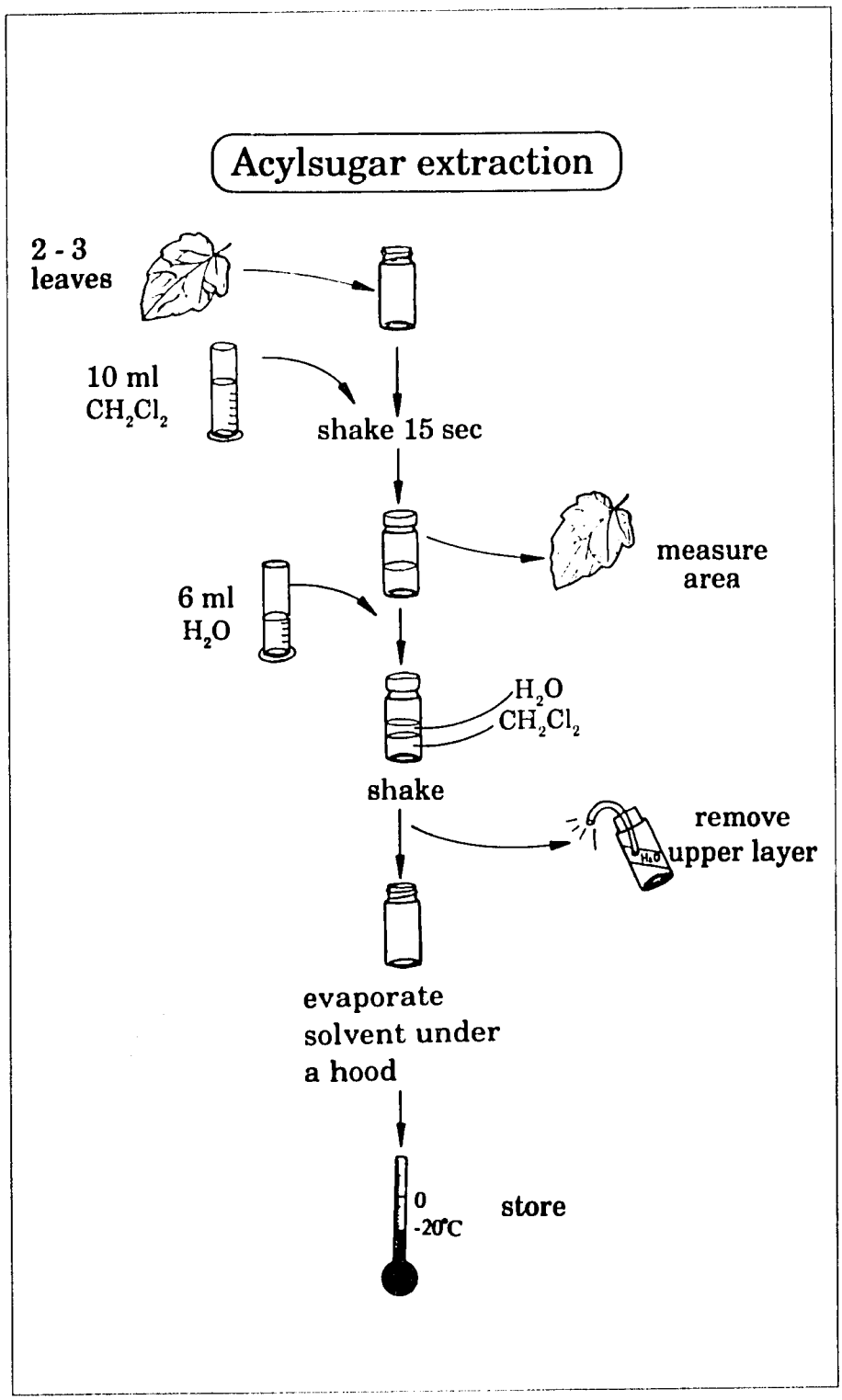


Fig. 1. Methodology used for extraction of acylsugars from tomato leaves

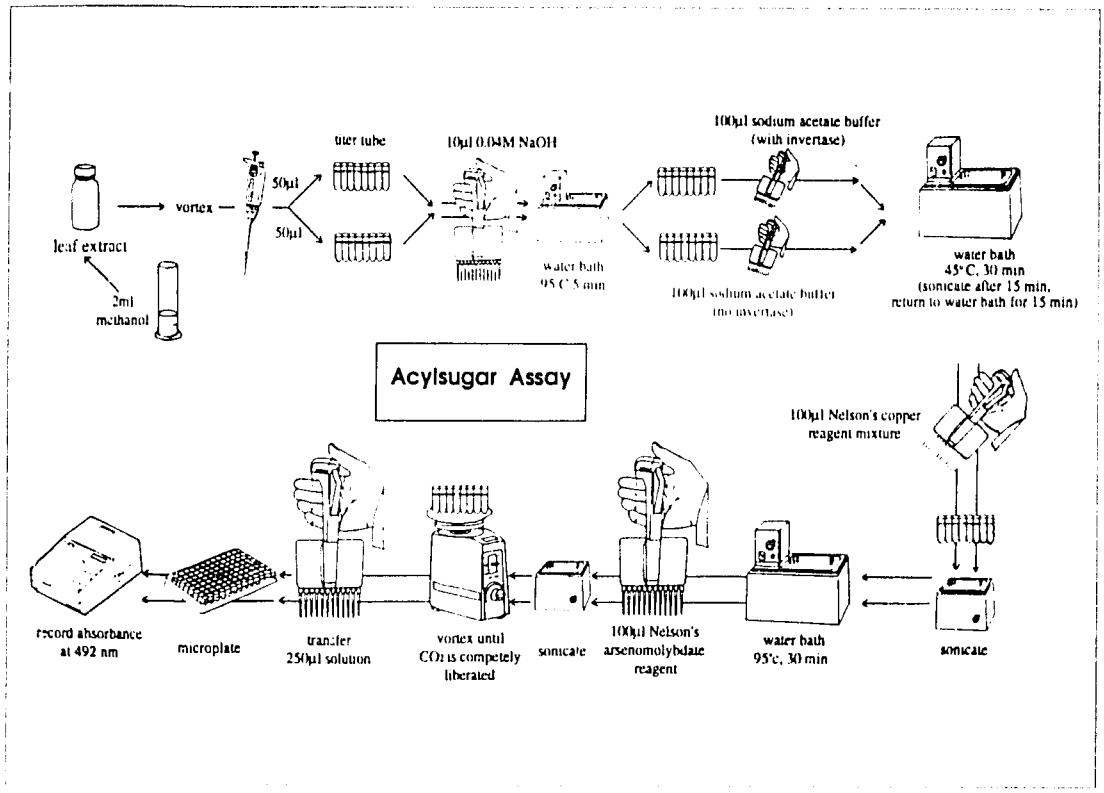


Fig. 2. Procedure used for chemical estimation of acylsugars from tomato leaves

Results

The acylsugar concentration in the foliage of BC_1F_1 progeny varied considerably (table 1).

Straight cross F_1 progeny (family no. 4) had the highest acylsugar concentration compared to the rest. Among the 10 families, four plants containing more than 27 mg acylsugar/cm^2 of leaf surface were selected for further study by our tomato breeder.

The BC_1F_2 seeds of four plants selected earlier were planted in clay pots in a greenhouse. When plants were 12 weeks old, we sampled some foliage from each plant and extracted and analyzed acylsugar content using a routine analytical procedure described earlier. The results are summarized in table 2. Six BC_1F_2 plants, containing exceptionally high acylsugar concentration ranging from 48 to 97 mg/cm^2 leaf surface, have been selected for making crosses to improve insect resistance and horticultural characteristics. The BC_2F_1 and BC_2F_2 progenies will be tested in 1995. A heat-tolerant tomato breeding line, 941586 (CL 5915-93D$_4$-1-0-3), showed an unusually high concentration of acylsugar. The relationship between heat tolerance and insect resistance will also be investigated in 1995.

Table 1. Concentration of acylsugars in foliage of BC_1F_1 progeny

Designation	No. of plants	Acylsugar concentration (mg/cm^2) Mean	Range
1. 89-1047-9 x 892079 (group 1)	28	1.3	0.4 - 6.7
2. 89-1047-9 x 892079 (group 2)	45	3.3	0.7 - 27.9
3. 89-1047-7 x 892079	18	5.1	0.9 - 11.6
4. 89-1047-1 x 894101-28 2F (LP) F_1	2	26.5	25.6 - 27.4
5. 89-1047-7 x 892081	33	10.0	1.5 - 33.4
6. 89-1047-3 x 892081	9	5.6	1.1 - 21.2
7. 89-1047-4 x 892081	18	10.6	1.3 - 33.5
8. 89-1047-12 x 892087	13	2.3	0.6 - 5.7
9. 89-1047-8 x 892087 2F Ys	5	1.7	0.9 - 3.4
10. 89-1047-12 x 892087	52	3.2	0.7 - 15.9
11. TA 209 (ck)	3	1.4	0.8 - 1.8
12. CL 5915-93 (ck)	3	1.5	1.2 - 1.4
13. *L. pennellii*	3	72.1	47.9 - 89.7

Table 2. Acylsugar concentration in the foliage of BC_1F_2 progeny

Designation	No. of plants	Acylsugar concentration (mg/cm^2) Mean	Range
941581	47	6.6	2.3 - 29.8
941582	46	8.5	2.1 - 35.9
941583	68	21.9	3.5 - 78.4
941584	77	12.0	3.2 - 96.9
941584 (F_1 ck)	10	39.3	19.7 - 52.3
941586 (ck)	23	7.1	4.1 - 11.7

A New Source of Resistance to Tomato Fruitworm

Summary

The effect of an insect-resistant *Lycopersicon hirsutum* accession, LA 1777, on tomato fruitworm larval development, adult oviposition preference, and survival of eggs was studied in laboratory experiments. All neonate larvae feeding on leaves of LA 1777 died within 4 days whereas only 10% of those feeding on susceptible breeding line CL 5915 died. TFW laid three to six times more eggs on CL 5915 than on LA 1777. More than 31% of the eggs laid on CL 5915 hatched into larvae whereas less than 8% of those laid on LA 1777 did. These results indicate that LA 1777 has certain antibiotic principles in the foliage where TFW lays eggs and first instar larvae feed.

Introduction

A wild tomato, *Lycopersicon hirsutum* f. *typicum*, accession LA 1777, has been reported to be resistant to various caterpillar species in the U.S. and Israel. The mechanism of resistance is different from the mechanism that imparts insect resistance in *L. pennellii*, the current source of resistance in

AVRDC's research program. A research group at Cornell University in the U.S. is using this wild accession to breed insect-resistant tomato. They offered to share progeny from their molecular marker-assisted breeding program for testing for tomato fruitworm resistance at AVRDC. Since we are working on breeding tomato fruitworm resistance, we sought the materials from the Cornell group. However, before screening large segregating populations, we investigated whether LA 1777 is indeed resistant to TFW.

Material and Methods

Plants of LA 1777 and a known susceptible breeding line, CL 5915, were raised in clay pots in a greenhouse. When plants were 6 weeks old, we sampled fully opened young leaves and placed them in plastic cups, with one leaflet per cup. There were 30 cups each for LA 1777 and for CL 5915. They were arranged in three replicates of 10 cups each for each accession. We then introduced two neonate TFW larvae in each cup and allowed them to feed. Everyday for 4 consecutive days we observed each cup and recorded insect mortality.

A preliminary experiment was conducted to find out whether there is any difference in preference by TFW to lay eggs on LA 1777 and CL 5915. In each of two trials, we confined four pairs of TFW adults in two 50 x 50 cm x 50 cm nylon net cages, each containing two potted plants of LA 1777 or CL 5915. Insects were allowed to lay eggs for 4 consecutive days. The total number of eggs were recorded. These eggs were then observed daily and the number of those that hatched into neonate larvae were recorded; those that failed to hatch for up to 6 days were considered dead. The percentage eggs successfully hatched into larvae was computed.

Results

There was considerably greater larval mortality when insects were fed on leaves of LA 1777 than on CL 5915 (fig. 3). The trend was observed daily. All insects feeding on LA 1777 died within 4 days whereas a maximum of only 10% of larvae died on CL 5915. This shows that there are certain strong antibiotic principles in the foliage of LA 1777 that kill pest larvae.

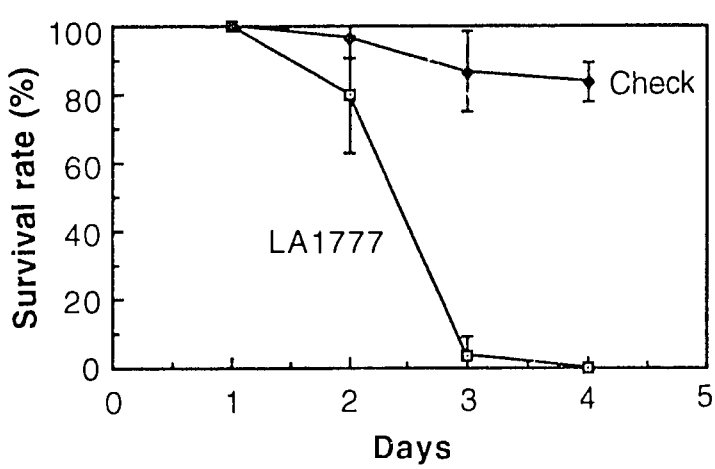


Fig. 3. Survival of TFW larvae fed on LA 1777, a wild tomato, and CL 5915, a check cultivar

In the first oviposition preference study, insects laid 298 eggs on CL 5915 as against only 5 on LA 1777. In the second test, TFW laid 286 eggs on CL 5915 but only 12 on LA 1777. TFW, thus, prefers the susceptible cultivar over LA 1777 for oviposition. In the first test 39% and in the second 31% of eggs laid on CL 5915 hatched into neonate larvae, but in LA 1777 only 2% and 7%, respectively, did. A drastic reduction in egg hatching on LA 1777 indicates the presence of a powerful antibiotic principle that prevents hatching of eggs.

Screening and Selection of *Lycopersicon hirsutum*-based Resistance Breeding Progeny for Resistance to Tomato Fruitworm

Summary

BC_1F_1 and BC_2F_2 progenies derived from crosses between insect-resistant *Lycopersicon hirsutum* f. *typicum* accession LA 1777 and a commercial tomato cultivar were screened for resistance to tomato fruitworm. Higher mortality of larvae feeding on foliage and lower percent of damaged fruit were the criteria used for selecting resistant progeny. Nineteen plants that had more than 70% larval mortality and lower than 20% damaged fruit were selected for further improvement.

Introduction

Accession LA 1777 shows high levels of resistance to a wide range of insect pests in the U.S. and Israel, including tomato fruitworm and beet armyworm, *Spodoptera exigua*, two important polyphagous pests of tomato in Asia. The mechanism of resistance is different from the acylsugar-mediated and 2-tridecanone-mediated resistance of *L. pennellii* and *L. hirsutum* f. *glabratum*, respectively. We are now using *L. pennellii* in our resistance breeding program; in the past we used *L. hirsutum*. A source with a different mechanism of resistance is useful in strengthening the breeding for insect-resistant cultivars that can withstand infestation by various pests and potential pest biotypes. The accession LA 1777 is being used by a Cornell University research group to breed a pest-resistant tomato cultivar. We are now collaborating with this group and testing their resistance breeding progeny for resistance to TFW.

Materials and Methods

Twenty seedlings each of 62 BC_1F_1 and BC_2F_2 selections were transplanted in rows set 1.5 m apart with a plant-to-plant distance of 1 m in the field. We bioassayed plants for TFW resistance. We sampled fully opened young leaves, and put them in a 500-ml plastic container in the laboratory. Five neonate larvae were released on the leaves and their growth and mortality were monitored for 8 days. At the start of first flowering, we released TFW adults in the field. The insect was released at irregular intervals thereafter, depending upon the availability, and the releases were continued until harvest. At harvest, we recorded the total number and insect-damaged fruit on each plant. The percent fruit damaged and mortality of tomato fruitworm larvae in the leaf bioassay were used in selecting plants for fruitworm resistance.

Results

The data on percent fruit damage and mortality of TFW larvae for all 62 families are summarized in table 3. Within each family both larval mortality and fruit damage varied considerably. Therefore, we selected 19 individual plants, without consideration of the performance of the family they belonged to, which had greater than 70% larval mortality and less than 20% fruit damage. These plants will be evaluated further for insect resistance and horticultural characters.

Table 3. Tomato fruitworm larval mortality on foliage and damage to fruit of *L. hirtusum*-based resistance breeding progeny

Entry	Identification	Avg. larval mortality (%)	Avg. fruit damage (%)
1	93T522-3	63.4	30.2
2	93T522-4	60.2	39.2
3	93T522-10	50.2	26.3
4	93T522-12	88.5	26.6
5	93T523-4	68.4	34.1
6	93T523-7	67.2	35.6
7	93T523-8	62.9	54.7
8	93T523-11	71.9	38.3
9	93T525-5	74.5	54.3
10	93T525-7	47.9	37.6
11	93T525-15	54.7	45.1
12	93T526-2	55.4	55.2
13	93T526-3	68.1	64.9
14	93T526-4	60.5	64.5
15	93T526-6	52.4	84.8
16	93T526-8	65.6	49.4
17	93T526-9	53.0	37.6
18	93T526-10	43.1	60.9
19	93T526-11	58.0	53.3
20	93T526-12	65.4	61.4
21	93T526-15	69.6	57.4
22	93T527-2	65.8	37.6
23	93T527-3	61.5	39.4
24	93T527-5	84.7	63.4
25	93T527-6	66.0	54.0
26	93T527-8	57.3	65.2
27	93T527-13	55.4	37.1
28	93T528-4	51.7	75.3
29	93T528-8	56.6	64.9
30	93T529-8	73.5	0
31	93T529-12	49.3	58.3
32	93T530-8	54.5	54.1
33	93T530-11	36.7	52.5
34	93T530-15	46.6	67.0
35	93T531-10	64.6	78.6
36	93T532-2	45.0	61.2
37	93T532-5	40.4	48.8
38	93T532-8	52.2	63.7
39	93T533-14	49.7	65.9
40	93T534-3	0.5	61.4

Table 3. continued.

Entry	Identification	Avg. larval mortality (%)	Avg. fruit damage (%)
41	93T534-5	68.6	54.8
42	93T534-6	44.1	66.8
43	93T534-7	40.9	85.6
44	93T534-8	50.7	69.6
45	93T534-12	64.0	75.1
46	93T535-4	48.1	86.5
47	93T535-7	51.5	85.7
48	93T535-9	58.9	55.1
49	93T535-10	44.6	47.2
50	93T536-1	45.9	72.4
51	93T536-2	37.9	74.2
T 52	93T536-4	47.5	75.3
53	93T536-6	44.9	77.3
54	93T536-15	56.2	94.3
55	93T537-4	65.7	77.3
56	93T537-9	64.2	67.3
57	93T537-15	56.7	65.0
58	93T538-5	45.4	86.4
59	93T539-4	60.0	71.1
60	93T539-6	55.1	78.8
61	93T539-8	66.9	83.7
62	93T540-4	58.9	61.6
63	TA 209[a]	63.0	87.3
64	TA 209[a]	58.8	72.8

[a] Susceptible check

Toxicity of Selected *Bacillus thuringiensis* Strains to Tomato Fruitworm

Summary

In a series of laboratory tests the toxicity of eight *Bacillus thuringiensis* (Bt) formulations to the larvae of tomato fruitworm was investigated. EGX, Florbac, Biobit, Delfin, Dipel, and MVP were equally toxic. Cutlass and Condor were slightly less toxic.

Introduction

Tomato fruitworm is considered as the most destructive pest of tomato in Asia. Insect larvae bore inside tomato fruit making even slightly damaged fruit unmarketable. AVRDC research on the control of this pest emphasizes breeding tomato cultivars resistant to TFW. We are using conventional breeding techniques to introduce general purpose insect resistance genes in tomato. This, however, is a slow process. Recent advances in biotechnology have made it possible to introduce a specific toxic crystal protein gene from *Bacillus thuringiensis* into plants, allowing the gene recipient plants to synthesize their own toxin and for protection from the insect. Before undertaking the research, we want to find out which of the crystal protein strains are indeed toxic to TFW. In this laboratory test, therefore, we investigated the efficacy of several commercially available Bt strains against TFW.

Materials and Methods

Eight commercially available formulations, presumably containing different serotypes of Bt, were obtained from Taiwan representatives of various manufacturers. A preliminary test was conducted to find out the appropriate concentrations of each formulation to obtain approximately 50% insect mortality. In the final replicated test, we used two dosages higher and two lower than the probable LC_{50} concentration. Appropriate quantities of each product were diluted in distilled water. Freshly harvested tomato leaves were dipped in each solution for 30 sec. The leaves were air-dried and confined in petri dishes. Ten second-instar TFW larvae were then introduced in the petri dishes. Insects were observed daily for 5 consecutive days. During this period, dried or consumed tomato leaves were replaced by identically treated fresh leaves. Based on insect mortality, LC_{50} values were calculated.

Results

Practically all formulations of Bt were toxic to TFW larvae (table 4).

Cutlass and Condor were relatively less toxic than the other formulations. In the field, because of the cryptic nature of the TFW larvae which minimizes contact between the insect and Bt-treated leaf surface, this insect is not easily controlled by any of the Bt formulations. If they are sprayed frequently, early instars can feed on treated foliage before entering the fruit. However, frequent applications are not economical. In our tests, because of prolonged contact between TFW larvae and Bt-treated foliage, we were able to achieve the desired level of insect mortality. The presence of the Bt crystal protein in tomato foliage resulted in the death of the insect before it entered the fruit.

Table 4. Toxicity of various Bt strains to TFW

Product	Strains	LC_{50} (ppm)
EGX	[a]	10.89
Cutlass	Kurstaki EG2371	54.30
Condor	Kurstaki EG2348	71.51
Florbac	Aizawai	17.31
Biobit	Kurstaki	18.83
Delfin	Kurstaki 3a.3b.SA-11	23.27
Dipel	Kurstaki	30.15
MVP	Kurstaki	27.27

[a] Information not yet available

Tomato Pathology

Screening for Resistance to Bacterial Wilt and Detection of Latent Infection in Resistant Lines

Summary

A total of 313 accessions of wild tomatoes and 21 resistant cultivars were screened for resistance to bacterial wilt in the greenhouse using the soil drenching method. Six accessions with the least disease severity and with similar resistance level as the resistant control, L 285, were selected for further study. They were L 180 (*Lycopersicum pimpinellifolium*), four accessions from University of the Philippines Los Baños (UPLB), i.e., TML 114-48-5-N-spreading, TML 46-N-12-N-early N.T., R 3034-3-10-N-UG, F 7-80-465-10-pink, and one accession from the Malaysian Agricultural Research and Development Institute (MARDI), MT-11. They were planted in the artificially infested soil at AVRDC and in naturally infested soil at the Taiwan Seed Service (TSS). When data of percentage of wilt were analyzed, factors of entry, and entry by environment were significant. The environment, entry, and entry by environment were all significant when disease rate data were analyzed. Means of percentage of wilt and disease rate of entries at AVRDC field and greenhouse screening were both significantly higher than those at TSS. The wilting percentages of accessions from UPLB were significantly less than L 285 under different environments. Latent infection was present in all selected resistant lines as well as in L 285 at the end of the greenhouse screenings. Direct plating had higher sensitivity than ELISA for detecting *P. solanacearum* in plant tissues.

Introduction

Bacterial wilt caused by *Pseudomonas solanacearum* E. F. Smith (Ps) is an important disease in tomato in the tropics and subtropics. Resistance breeding has been the major emphasis for controlling the disease in tomato. However, it has been observed that some resistant varieties are site-specific. Thus, several resistance sources are usually used in the breeding process to obtain better adaptation. Therefore, it is important to continue screening for resistance sources with diverse genetic backgrounds. It has been reported that many resistance sources of tomato harbor the pathogen without symptoms. Presently, selection based on visual symptoms is the only criteria used at AVRDC. Thus, detection of latent infection is necessary to further characterize resistance sources. The objectives of this study were to screen for resistance to bacterial wilt and characterize the resistant accessions.

Materials and Methods

Entries

A total of 313 accessions of wild tomato from AVRDC's Genetic Resources and Seed Unit (GRSU) were screened. There were 2 accessions of *Lycopersicum cheesmanii*, 15 of *L. hirsutum*, 1 of *L. pennellii*, 63 of *L. peruvianum*, and 232 of *L. pimpinellifolium*. Another 21 resistant tomato cultivars were collected from Malaysia, Indonesia, Philippines, Taiwan, and USA. L 285 and L 390 were used as resistant and susceptible controls, respectively, in the screening.

Greenhouse evaluation

Tomato seedlings were grown individually in 9-cm-diam plastic pots and screened 28 days after sowing by soil drenching inoculation method. A strain of *Pseudomonas solanacearum*, Pss4 (race 1, biovar 3), was used as inoculum. A volume of 30 ml of bacterial suspension (A_{600} = 0.3, about 10^8 cfu/ml) was poured into each pot. Ten plants of each variety were inoculated and placed in the heated greenhouse at 25 to 35°C. Percentage wilt was recorded up to 21 days after inoculation (DAI). Those accessions with more than 30% survival were selected for further screening. To confirm resistance in the selected entries, 12 plants of each entry were used and three replications arranged in a randomized complete block design (RCBD). After inoculation, plants were placed in a growth room at 28°C. Percentage of wilted plant and disease rating based on Winstead and Kelman's rating scale were recorded up to 21 DAI.

Detection of Ps from symptomless plants

At the end of the greenhouse screening, detection of Ps was conducted on plants without visual symptoms by plating in selective media and ELISA. All symptomless plants were cut at the base of the stem, washed with tap water, air-dried, and surface-sterilized with 70% alcohol. Two grams of fresh tissue were taken from the collar portion of each plant and divided into half vertically. One part of the sample was incubated in 5-ml sterile water at 20°C for 16 h. One hundred microliters of the suspension was used in plating on triphenyl tetrazolium chloride (TTC) medium using a Spiral Plating System. Plates were incubated at 30°C for 2 days. Samples with typical *P. solanacearum* colonies were scored as positive.

The other part of the collar portion from the same plant was macerated with 1 ml extraction buffer, pH 9.6. A volume of 100 µl of each extract was added to each well. The samples were incubated at 37°C for 1 h, flicked out from the plate, and wells were washed three times with PBS-T, leaving the last change of buffer for at least 5 min. A volume of 200 µl of blocking buffer was added to each well, and the samples were incubated at 37°C for 1 h. Wells were washed as previously described. Subsequently, 100 µl of a specific polyclonal antibody IgG R303 (provided by Dr. J. Elphinstone) diluted 1:2000 in blocking buffer was added to each well and incubated at 37°C. Washing with PBS-T was repeated, and 100 µl of second goat antirabbit antibody-alkaline phosphatase (GAR-AP) conjugate diluted 1:3000 in blocking buffer was added to each well. These were then incubated for 1 h at 37°C and washed again with PBS-T. Two hundred microliters of freshly prepared peroxidase substrate solution (1 mg/ml *p*-nitrophenyl phosphate in substrate buffer) was added to each well and the samples were incubated at room temperature for 5-30 min until reaction was completed. Reaction was stopped by the addition of 50 µl of 3 M NaOH to each well. Absorbance was measured by an ELISA reader at 405 nm and 490 nm. Values of 2.5 times the mean of the healthy tissue were used as cut-off points for positive and negative reaction.

Field evaluation of selected tomato lines

Resistant entries selected from greenhouse screening were tested in the fields at AVRDC and Taiwan Seed Service. The field at AVRDC was infested with Ps before transplanting following the disease nursery protocol; the TSS field was naturally infested with Ps. Trials were arranged in RCBD with three replications at AVRDC and two replications at TSS. Experimental units consisting of raised beds, 3 m long, 1 m wide, with 0.5 m between beds were constructed. A total of 20 plants arranged in two rows were transplanted to each bed. Disease severity rating was conducted every 7 to 14 days at least five times. The AVRDC trial was conducted from 14 June to 27 July, and the TSS trial from 3 August to 3 October.

The percentage of wilted plants was calculated based on the last evaluation of each trial and transformed by the arcsine of the square root before carrying out analyses of variance. Disease index was transformed by ln (1/1-x), where x is the disease index. The regression coefficient of the transformed data was considered as the disease rate. Both percentage data of wilted plants and disease rates of two greenhouse screenings and field trials were used in a combined analysis. For combined analyses of variance, locations were regarded as random effects and entries as fixed effects.

Results and Discussion

Greenhouse evaluation

Most of the tested accessions were susceptible to bacterial wilt. Accessions with perentages of survival higher than 30% were L 1947, L 3387 (*L. peruvillorum*), L 141, L 180, L 4159, L 4237, L 4422, L 4445, L 4449, L 4450, L 4451 (*L. pimpinellifolium*), Fla7421, Ranti, Iatan putih, Magic, MT-1, MT-11, TML 114-48-5-N-spreading, TML 46-N-12-N-early N.T., R 3034-3-10-N-UG, and F 7-80-465-10-pink (cultivated tomato). After further screening, six accessions with more than 80% survival and with similar resistance level as the resistant control were selected. They were L180, four accessions collected from UPLB, (TML 114-48-5-N-spreading, TML 46-N-12-N-early N.T., R 3034-3-10-N-UG, F 7-80-465-10-pink), and one accession collected from MARDI, MT-11. These accessions were used for further field evaluation and latent infection studies.

Detection of Ps from symptomless plants

At the end of the greenhouse screening, the pathogen was detected from all selected resistant entries regardless of their degree of resistance to bacterial wilt (table 1). This confirmed previous reports on latent infection in tomatoes. Direct plating on TTC had higher sensitivity than ELISA for detecting *P. solanacearum* in plant tissues. This is probably due to the insufficient specificity of the polyclonal antibody. Colonization frequency of the four accessions collected from UPLB were less than those of the other resistant entries. These accessions were also more resistant than others over screenings conducted at different locations where lower percentages of wilted plants and disease rates were observed (table 2). More studies should be conducted to determine the relationship of colonization frequency and resistance level. This will show whether the percentage of colonization in the stem of symptomless plants can be a complementary criterion for evaluating tomato germplasm for resistance or obtaining more stability resistance to bacterial wilt.

Table 1. Disease severity of bacterial wilt on resistant tomato accessions in a greenhouse screening and detection of *Pseudomonas solanacearum* in symptomless plants by direct plating on TTC and ELISA

Entries	Wilt (%)	TTC (%)	ELISA (%)
TML 46-N-12-N-early	0.0	63[a]	32[a]
TML 114-48-5-N-spreading	2.8	66	14
R 3034-3-10-N-UG	11.6	74	26
F 7-80-465-10-pink	0.0	58	11
MT-11	23.2	89	39
L 180	0.0	89	33
L 285	14.1	83	60
L 390	100.0	-	-

[a] Percentages of colonization = No. of positive sample/No. of tested symptomless plants

Table 2. Percentage of wilted plant and disease rates (x 100) under different environments

Entries	GR1[b]		GR2[b]		AVRDC[c]		TSS[c]		Mean[d]	
	Wilt (%)	Disease rate	Wilt (%)	Disease rate	Wilt (%)	Disease rate	Wilt (%)	Disease rate	Wilt (%)	Disease rate
TML 46-N-12-N-early	0.0	0.0	0.0	0.0	14.0	0.3	0.0	0.0	3.8 c	0.1 b
TML 114-48-5-N-spreading	0.0	0.0	5.6	0.2	4.3	0.0	0.0	0.0	3.0 c	0.1 b
R 3034-3-10-N-UG	5.6	0.1	18.0	0.5	13.6	0.7	0.0	0.0	10.1 c	0.4 b
F7-80-465-10-pink	8.0	0.3	0.0	0.0	13.4	0.2	0.0	0.0	5.9 c	0.1 b
MT-11	23.6	0.6	28.6	1.3	16.7	0.4	20.2	0.2	22.5 b	0.6 b
L 180	26.1	1.4	0.0	0.0	26.3	0.6	20.8	0.3	18.1 b	0.6 b
L 285	19.2	0.5	21.4	0.4	18.0	0.3	10.4	0.2	17.9 B	0.4 b
L 390	84.4	79.2	90.0	97.4	90.0	45.9	90.0	25.2	88.5 a	65.2 a
Mean[a] (wilt %)	21.8 ab		20.5 ab		24.5 a		17.7 b			
Mean[a] (disease rate)		10.7 a		12.5 a		6.1 ab		3.2 b		

[a] Means of % wilted plants or disease rates of different accessions under each environment. Values followed by the same letter are not significantly different according at 5% level (DMRT)

[b] Results of repeated seedling screenings conducted in 28°C growth room

[c] Results of field screenings conducted at AVRDC and Taiwan Seed Service

[d] Means of % wilted plants or disease rates of each accession under different environments. Values followed by the same letter are not significantly different at 5% level (DMRT)

Field evaluation of selected tomato lines

The percentages of wilted plants and disease rates of two greenhouse trials at 28°C and two field trials at AVRDC and TSS are listed in table 2. The lines identified as resistant in the greenhouse were also resistant in the field. When data of percentage of wilt were analyzed, factors of entry (P = 0.0001) and entry by location (P = 0.0198) were significant. The factors of location (P = 0.00194), entry (P = 0.001), and entry by location (P = 0.0002) were all significant when disease rate data were analyzed (table 3). Since only eight resistant lines were tested, the data may be insufficient to determine the effect of location. However, the disease pressure was low at TSS. This was indicated by the significantly less percentages of wilted plant and disease rates at the TSS trial than those at the AVRDC field and two greenhouse trials. The wilt percentages of the four entries from UPLB were significantly less than those of L 285 under different environments. However, their disease rates were similar with those of L 285. These four entries are useful sources of bacterial wilt resistance for Southeast Asia.

Table 3. Combined analyses of variance of percentages of wilted plants and disease rates using the data listed in table 2

Source of variation	DF	MS	
		Wilt (%)	Disease rate
Locations (L)	3	160.4^{ns}	0.03***
Entries	7	8518.9****	0.50***
L x Entries	21	128.2**	0.03***

ns = not significant

*, **, ***, **** = significant at the 5, 1, 0.1,0.01% levels, respectively

Detection of *Pseudomonas solanacearum* in Plant Tissue Using Polymerase Chain Reaction

Summary

Oligonucleotide primers AU759 and 760 from clone 092-03B col.5 (a biovar nonspecific probe) were used in polymerase chain reaction (PCR) to test their specificity on *Pseudomonas solanacearum* isolated from Taiwan. Eighty-one fluidal and 11 nonfluidal strains of *P. solanacearum* isolated from 13 different host plants were tested. A unique product of 281bp can be amplified from the genomic DNAs by primers AU759 and 760 but not from the other tested plant pathogenic bacteria. Plant assays were developed to detect the presence of *P. solanacearum* in symptomless plants using PCR, and the sensitivity was compared with direct plating on selective media. PCR was more sensitive when samples were taken from tomato extract than from direct maceration. In inoculated plants, *P. solanacearum* was detected 2 days after inoculation when no visual symptom was observed using both plating and PCR methods. The latter is faster and more sensitive.

Introduction

Pseudomonas solanacearum (Ps) is the causal agent of bacterial wilt, one of the most destructive plant diseases in tropical and subtropical areas of the world. The disease can affect more than 44 families of plants, and most of the economic damage is caused in solanaceous plants, including potato, tomato, tobacco, eggplant and pepper, and some nonsolanaceous hosts such as banana, peanut, and ginger.

Because of the soil-borne nature of the pathogen, its diversity, and the possibility of spreading through planting materials, there is no universal way to control the disease. Host resistance breeding, use of clean seed, crop rotations, and other measures may be used under different conditions[1]. It is thus important to have better screening methods other than visual observation to evaluate resistance level and to detect the presence of the pathogen in planting material. Therefore, it is necessary to develop sensitive and specific detection methods from plant samples.

[1] Hayward, A.C. 1991. Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. Ann. Rev. Phytopathol. 29: 65-87.

Traditionally, selective agar plates (such as SM1) are used to detect the pathogen by observing the colony morphology. The method is very time-consuming and laborious. Serological approaches (such as ELISA) are easy to apply, but are not very specific. Nucleic acid-based techniques, which require the development of a specific probe (as in hybridization or blotting methods) or a set of specific primers (as in polymerase chain reaction amplifications), have been developed recently and are fast, specific, and sensitive. Polymerase chain reaction has been used to detect pathogens from plant samples, such as Ps latent infection in potato tubers (Seal et al. 1992)[2], *Xylella fastidiosa* in grapevine (Minsavage et al. 1994)[3], and *Verticillium tricorpus* in potato (Moukhamedov et al. 1994)[4].

The biovar nonspecific DNA probe 092-03B col.5 was shown to hybridize with all Ps strains tested. The DNAs were sequenced and two oligonucleotide primers, AU759 and 760, developed from this probe were used in the PCR tests.

The objectives of this study are to: (1) determine the specificity of primers AU759 and 760 to Ps strains in Taiwan, and (2) develop a detection method using PCR from tomato stem samples and compare the sensitivity of the PCR detection system with the plating method.

Materials and Methods

Bacterial strains and DNA sequences

A total of 81 fluidal Ps strains and 11 nonfluidal mutants were used in PCR. The fluidal strains were isolated from tomato, sweet pepper, tobacco, eggplant, potato, bird of paradise, peanut, radish, strawberry, wax apple, clustered apple, perilla, and *Cyperus iria* L. in Taiwan. The nonfluidal mutants were isolated from Ps-stored cultures. Other pathogenic bacteria used in this study were *Xanthomonas campestris* pv. *vesicatoria* (Xcv) race 1 (XvT28), 2 (XvT48), and 3 (XvT70) isolated from tomato; Xcv strains isolated from pepper (XvP40 and 49); *X. campestris* pv. *campestris* (Xcc60 and 63); *X. campestris* pv. *glycenia* (Xcg2 and 5); *Erwinia carotovora* subsp. *carotovora* (Ecc); *E. chrythanthemi* (Ech); *P. cepacia* (Pc5); and *P. gladioli* (Pc10). Oligonucleotide primers AU759 and 760 were synthesized by the Quality Systems, Inc. in Taiwan. The sequences (AU759: 5'-GTC GCC GTC AAC TCA CTT TCC- 3'. AU760: 5'-GTC GCC GTC AGC AAT GCG GAA TCG- 3') were provided by Dr. J. Timmis of the University of Adelaide, Australia.

Genomic DNA isolation

Bacteria were grown overnight in nutrient broth at 30°C, harvested, washed twice, and resuspended in TEN buffer (10 mM Tris, pH8, 1 mM EDTA, 100 mM NaCl). The bacterial suspension was incubated at 56°C for 1 h with proteinase K and SDS at 0.15 mg/ml and 1%, respectively. Phenol and chloroform extraction were performed and the DNA was precipitated by isopropyl alcohol and ammonium acetate. The pellet was washed in ethanol, dried, and dissolved in water.

[2] Seal, S., Elphinstone, J.G., Skoglund, L., and Berrios, D. 1992. Comparison of new techniques for the detection of latent infection in seed potatoes during multiplication in Burundi. Bacterial Wilt Newsletter 8: 2-3.

[3] Minsavage, G.V., Thompson, C.M., Hopkins, D.L., Leite, R.M.V.B.C., and Stall, R.E. 1994. Development of *Xylella fastidiosa* in plant tissue. Phytopathology 84: 456-461.

[4] Moukhamedov, R., Hu, X., Nazar, R.N., and Robb, J. 1994. Use of polymerase chain reaction-amplified ribosomal intergenic sequences for the diagnosis of *Verticillium tricorpus*. Phytopathology 84: 256-259.

PCR amplification and gel electrophoresis

PCR amplification of DNA was done in the PTC-100 Programmable Thermal Controller (MJ Research, Inc.). Individual PCR samples (25 µl) contained 1x PCR buffer (BRL), 1.5 mM $MgCl_2$ (BRL), 200 µm of each dNTP (Promega), 50 ng of each primer, template DNA, and 0.5 unit *Taq* DNA polymerase (BRL). The polymerase was added at 72°C after the initial 15-min denaturation at 96°C when plant samples were tested. This step was omitted when Ps genomic DNAs were used as templates. Each reaction was overlaid with 25 µl of mineral oil. Thirty PCR cycles of 93°C for 30 sec, 55 (for Ps genomic DNA) or 65°C (for plant samples) for 30 sec, and 72°C for 1 min were performed after the initial denaturation. The samples were cooled to 4°C after a final step of 72°C for 9 min. Two-step PCR was performed by substituting the annealing temperature from 55 (or 65) to 72°C. Gel tracking dye (2.5 µl) was added directly to each reaction tube and 10 µl of the reaction mixtures were electrophoresed at 50 volts using 1.5% agarose gel in 0.5x TBE. The gels were stained with ethidium bromide and photographed with a Polaroid MP4 Camera System using 667 Polaroid film.

Preparation of plant samples

One month-old susceptible tomato plants (L390) were inoculated with 30 ml bacterial suspension (Pss4, OD_{600} = 0.3) by soil drenching method. The inoculated plants were incubated in the greenhouse at 28°C. The symptomless plants were sampled every 2 days after inoculation for both PCR and plating detection methods. Two grams of the stems (collar regions) were removed from the pots and the stem segments were split longitudinally. One half was incubated in 5 ml sterilized water at 20°C for about 8 h (modified from Grimault and Prior 1993)[5]. The other half was macerated in 1 ml SCPAP buffer [disodium succinate, 1 g/l; trisodium citrate, 1 g/l; K_2HPO_4, 1.5 g/l; and KH_2PO_4, 1 g/l; pH 7.0; 0.02 M sodium ascorbate, and 5% acid-washed insoluble polyvinylpyrrolidone (PVPP)] and low speed centrifuged for 30 sec to remove insoluble PVPP and plant debris. Different amounts (2 and 100 µl) of samples were taken from both extracts of incubated and macerated samples for PCR. The 2 µl samples were added directly to PCR reaction tubes. The 100 µl samples were centrifuged at 12,000 rpm for 5 min, the supernatants removed by a pipetman, and the PCR reaction mixture added to each tube. One hundred microliters of tomato extracts were also used to spread on SM1 agar plates. Plates were incubated at 30°C for 2 days.

Results

Specificity of primers AU759 and 760

Oligonucleotide primers AU759 and 760 were used in PCR amplification to test on Ps strains and some other pathogenic bacteria isolated from Taiwan. All genomic DNAs isolated from 81 Ps strains tested showed a single product of 281 bp after 30 cycles of PCR at the annealing temperature of 55°C. Some of the DNAs from other pathogenic bacteria such as XvT28, 48, 70, and Eec, can be amplified by AU759 and 760. However, none of them had the same size product as Ps strains (fig. 1a). When two-step PCR was applied, all these nonspecifically amplified products disappeared (fig. 1b). Eleven nonfluidal Ps mutants isolated from stored culture had the same product as the fluidal strains. These DNAs can still be amplified to produce the same 281 bp product when the two-step PCR technique was used. Results suggest that primers AU759 and 760 are specific to Ps but cannot be used to differentiate fluidal and nonfluidal strains of Ps.

[5] Grimault, V. and Prior, P. 1993. Bacterial wilt resistance in tomato associated with tolerance of vascular tissues to *Pseudomonas solanacearum*. Plant Pathology 42: 589-594.

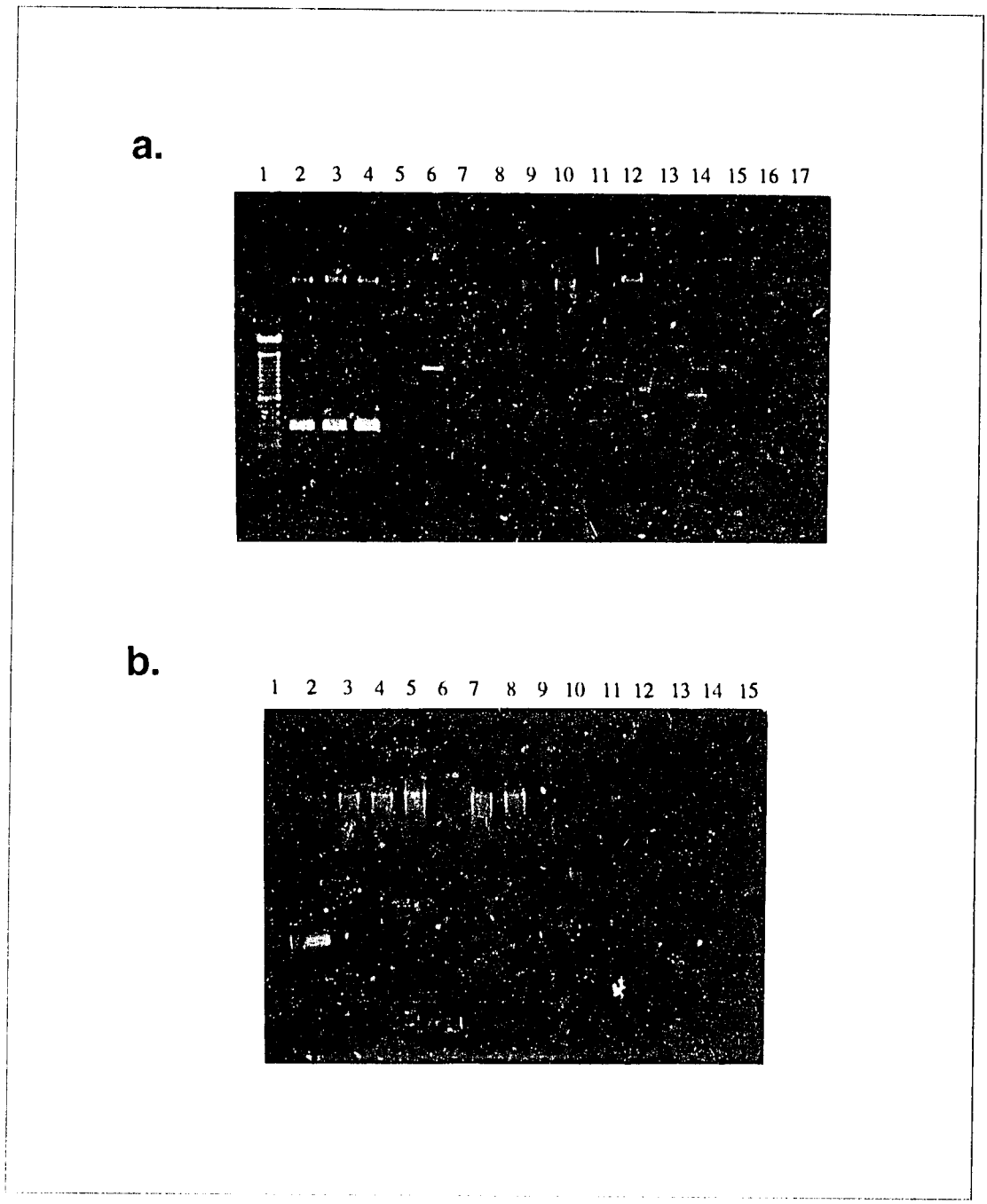


Fig. 1. Agarcse gel electrophoresis of the products of PCR amplification of DNAs from Ps and various pathogenic strains using primers AU759 and 760 at annealing temperatures of (a) 55°C and (b) 72°C (two-step PCR)
a. Lanes 1: 100 bp ladder DNA; 2-4: Ps strains; 5: XvT28; 6: XvT48; 7:XvT70; 8: XvP40; 9: XvP49; 10: Xcc60; 11: Xcc63; 12: Xcg2; 13: Xcg4; 14: Ecc; 15: Ech; 16: Pc5; and 17: Pc10
b. Lanes 1: negative control; 2: Ps; 3: XvT28; 4: XvT48; 5: XvT70; 6: XvP40; 7: XvP49; 8: Xcc60; 9: Xcc63; 10: Xcg2; 11: Xcg4; 12: Ecc; 13: Ech; 14: Pc5; and 15: Pc10

Detection of *P. solanacearum* in plant tissue using PCR and plating

The presence of *P. solanacearum* in susceptible tomato plants 2 and 4 days after inoculation was detected using PCR at the annealing temperature of 65°C. Thirty-nine plants were inoculated at the same time and 13 symptomless plants were assayed each time. Some plants started wilting 4 days after inoculation. All plants were wilted 6 days after inoculation and, thus, no PCR or plating was performed then.

When tissues were macerated in SCPAP buffer, nothing was amplified using either the 100 µl sample or the same sample with purified Ps DNA as an internal control. SCPAP buffer was used because acid-washed PVPP and sodium ascorbate were reported to inactivate the inhibitors of PCR in plant tissues (Minsavage et al. 1994). However, this result suggests that enough inhibitors are present in the 100 µl sample. When purified DNA was added to the 2 µl macerated sample as the template, the 281 bp product was observed. Therefore, only 2 µl of the maceration sample was used for later PCR detection. No obvious PCR inhibitors were present even when 100 µl extracts were used as the template.

At 2 days, four plants were PCR-positive using 100 µl extracts from incubated samples. Only one of them was detected by PCR when 2 µl was applied. However, all were PCR-negative when macerated samples were tested. At 4 days, 11 plants were PCR-positive with 100 µl extracts. Ten of them were positive but weaker than when 2 µl was used. Three macerated samples were PCR-positive (table 4). Comparing the three different sampling methods (2 and 100 µl by incubation and 2 µl by maceration), 100 µl of the incubation appeared to have the highest sensitivity.

Incubation samples were also used to spread on SM1 agar plates to compare sensitivity. At 2 days, one sample had typical Ps colonies growing on SM1. At 4 days, eight samples had Ps growing on them (table 4). Based on these results, PCR was considered more sensitive than the traditional plating method. Also, PCR can be finished in 1 day while it takes at least 2 days for the bacteria to grow on SM1 agar plates.

Table 4. Positive results of PCR and plating using different sampling methods

	PCR		Plating	
	2 DAI[a]	4 DAI	2 DAI	4 DAI
Incubation 100 µl	4/13	11/13	1/13	8/13
Incubation 2 µl	1/13	10/13	NT[b]	NT
Maceration 2 µl	0/13	3/13	NT	NT

[a] DAI: days after inoculation
[b] NT: not tested

Discussion

Primers AU759 and 760 can be used to detect Ps in tomato tissues directly. There is no need to isolate DNA from the tissues. Subjecting samples to 96°C for 15 min is sufficient to break bacterial cells to release DNA.

Different sampling methods were used to compare the sensitivity of PCR. When stem segments were incubated in sterilized water, bacteria can be released from the vascular system into the water. Theoretically, PCR can detect as low as one cell in the reaction. If the plant was heavily infected, the water became cloudy after 8 h of incubation. There was no problem detecting Ps when 2 µl was used

as the template for PCR. When there were only small amounts of bacteria released from plants, the chance of picking up one cell (2 µl) from the total 5-ml extracts would be nil. The reason for using 100 µl as the template was to increase the chance of picking up at least one bacterial cell. However, if 100 µl was used, more reagents are needed (25 µl in total). Thus, the samples were first centrifuged to concentrate the bacteria, and the PCR mixture was added to each tube so that the final volume would still be 25 µl. An internal control Ps DNA was added to see if anything in the concentrated extracts inhibited PCR. No apparent inhibitor was observed when comparing the intensity of PCR products with the pure Ps DNA and the internal control.

Macerated samples, on the other hand, had PCR-inhibiting substances. When tissues were macerated, many plant composition, including PCR inhibitors, were released together with the bacteria. The samples were sometimes diluted for use in PCR. However, both the bacteria and inhibitors were diluted and the sensitivity of PCR decreased. Ascorbate and acid-washed PVPP were reported to inactivate inhibitors in plant samples. Tissues macerated in buffer containing these compounds can be used directly for PCR, which was true when only 2 µl was used. When sample volume was increased to 100 µl to increase sensitivity, it was found that PCR was inhibited.

PCR is more sensitive and faster compared with the traditional plating method. More samples from symptomless plants had positive reactions using PCR both 2 and 4 days after inoculation. The plating method is time-consuming and identification of colonies requires some experience. Sometimes, the bacteria from agar plates need to be inoculated onto host plants for identification. However, since fluidal colonies are mostly virulent, it is easier to tell if the isolated bacteria were virulent by the plating method. This is the advantage of plating, because the set of PCR primers used in this study cannot distinguish between fluidal and nonfluidal strains. Our observations show no nonfluidal mutant from any of the SM1 plates when extracts were spread. This suggests that the PCR-positive result is mostly due to the presence of fluidal Ps strains but not nonfluidal mutants. PCR is a powerful tool for detecting the presence of Ps. However, the protocol used in this study still requires an 8-h incubation before PCR can be applied. Other faster sampling methods need to be developed to facilitate the detection of Ps latent infection in plant tissues.

It has been shown that most of the resistance sources of tomato to bacterial wilt are carriers of the pathogen, and the internal population of the pathogen is significantly less than that in the susceptible lines. Thus, this detection method can facilitate the study on the correlation of internal bacterial population and resistance level. A more efficient screening method for bacterial wilt based on the internal bacterial population rather than visual symptoms may be developed. This detection method can be used to detect latent infection of *P. solanacearum* in symptomless plants and applied in epidemiological studies. A similar detection method can also be developed for soil samples.

Studies on Leaf Curl Virus and SAVERNET and CONVERDS Support

Summary

AVRDC, since 1991, has accorded high priority to leaf curl virus of tomato, with emphasis on breeding for resistance. Similarly, leaf curl virus of tomato and peppers are major targets of the South Asian Vegetable Research network (SAVERNET), the Collaborative Vegetable Research Program for Southeast Asia (AVNET), and the Collaborative Network for Vegetable Research and Development in Central America (REDCAHOR). Leaf curl virus of tomato is also an issue in the Collaborative Network for Vegetable Research and Development in Southern Africa (CONVERDS). Because of the

absence of specific diagnostic probes, most surveys and assessments in the past have been done by symptom observation. However, insect-feeding damage, synergistic effects by coinfection with several viruses, and other viruses such as the leafhopper-transmitted curly top geminivirus or the tomato yellow top luteovirus may cause similar symptoms.

Surveys were, therefore, conducted to determine the distribution and confirm the importance of whitefly-transmitted geminiviruses on tomato and peppers in the above regions.

We have previously developed a nucleic acid probe against the Taiwan tomato leaf curl virus (TTLCV) and this year have obtained plasmids containing DNA fragments of several other whitefly-transmitted geminiviruses, such as TYLCV-Egypt and TLCV-India — both monopartite geminiviruses — and TYLCV-Thailand, a bipartite geminivirus. These plasmids have been further processed into nonradioactive diagnostic probes and were used in the surveys.

Surveys in Asia have confirmed the presence of geminiviruses in both tomato and peppers in Nepal, India, Sri Lanka, Pakistan, Thailand, and Taiwan. Surprisingly, overall incidence was higher in peppers (19%) than in tomato (7%). With the probes used, we were, however, not able to detect any geminivirus in Bangladesh, Bhutan, Indonesia, and the Philippines. There is strong indication that geminiviruses, distinct from those known so far, may occur in all of these countries.

Eight percent of the tomato samples collected from East Africa gave a positive reaction with the TYLCV-Egypt probe, strongly suggesting that this virus, or a closely related variant, is present there. However, the fact that not all samples which showed very strong and distinct leaf curl and yellowing symptoms reacted with the Egypt probe also suggests that another geminivirus may also be present there.

An extensive survey in Taiwan during 1993-94 indicated that the TTLCV is present throughout the island, with incidences as high as 64% in some farmers' fields.

We also assisted the breeder in screening (by whitefly or graft inoculation and subsequent rating by DNA hybridization) 949 plants of 26 lines comprising parentals and crosses of leaf curl virus-resistant materials.

Introduction

Whitefly-transmitted geminiviruses cause serious diseases on peppers and tomatoes worldwide, mostly in tropical and subtropical regions. Enormous yield losses, sometimes complete crop failure, have been attributed to these viruses. The prevalence and distribution of these viruses have been increasing in recent years for reasons not yet understood. Our partners in the national programs of SAVERNET, AVNET, CONVERDS, and REDCAHOR, aware of the threat or potential threat of these diseases, have declared geminiviruses of tomato and peppers their main research priorities.

In Taiwan, the virus and the disease it causes were not known prior to 1983. In the following 9 years, it was only rarely encountered in farmers' tomato fields. However, in spring 1994 severe epidemics occurred in the southern parts of Taiwan and throughout the island. AVRDC, consequently, has intensified its research on geminiviruses, with emphasis on the development of diagnostic probes, the geographic distribution of these viruses in our mandate areas, and on developing stable resistance, particularly in tomato. During 1993, we have identified several sources of resistance, mainly in *Lycopersicon chilense* and have developed a nucleic acid probe for the Taiwan tomato leaf curl virus. In 1994, our focus was on identifying additional sources of resistance and on screening and testing progenies of resistant materials and on conducting surveys.

Materials and Methods

Surveys

Samples were collected either by us or by our cooperators from tomato and peppers showing symptoms typical of geminivirus infection, such as yellowing and leaf curling, in Bangladesh, Bhutan, India, Indonesia, Nepal, Pakistan, the Philippines, Sri Lanka, Taiwan, Thailand, Tanzania, and Uganda.

Leaf disks of approximately 1-cm diam were squashed onto nylon membranes. The air-dried membranes were treated as follows: 0.5 N NaOH, 1 M Tris pH 7.5, 2xSSC, each for 5 min, followed by a rinse in ethanol 95%. After fixing the nucleic acid to the membrane in the UV Strata Linker box at UV 254 nm for 5 min, the membranes were treated with 5xSSC prehybridization buffer for 1 h at 60°C. For hybridization, 5-6 µl labeled DNA probe was added, and the membrane was incubated overnight at 60°C in a waterbath shaker. After washing the membranes twice each with 2xSSC (0.1% SDS) for 5 min at RT and 0.1xSSC (0.1% SDS) for 15 min at 60°C, the membranes were air-dried and readied for digoxigenin (DIG) detection, which proceeded following suppliers' instructions: washing in Buffer 1, blocking in Buffer 2, addition of anti-DIG alkaline phosphatase conjugate, and washing in Buffer 1 (0.3% Tween 20). After equilibrating the membrane in Buffer 3, the membrane was incubated with lumigen PPD (1:100) at 37°C/10 min, at RT/3 min, followed by 37°C, 1 min. The membrane was then placed on the intensity plate for subsequent photographic processing.

The following probes were used: M3 (TLCV-Taiwan) Eg (TYLCV-Egypt), 16 i (TLCV-India), Thai A (TYLCV-Thailand). The first probe was previously developed by us; the latter three probes were received from D.P. Maxwell, University of Wisconsin, Madison, USA, in the form of transformed *Escherichia coli*, from which the plasmid-containing geminivirus DNA had to be extracted and digested by *PstI* restriction enzyme. Following agarose gel electrophoresis and ethidium bromide staining, the geminivirus DNA fragments were excised, recovered by the Geneclean II Kit, and labeled with DIG, following suppliers' instructions. The probes were either used singly or in mixture. Some of the samples were probed repeatedly with individual probes after deprobing with NaOH-SDS.

Results and Discussion

Surveys

Asia. The results of the surveys conducted in Asia are shown in table 5. Fifteen percent of all samples tested were infected with a geminivirus. The data suggest that both peppers as well as tomato are infected with geminiviruses, with infection in peppers (19%) even higher than that in tomato (7%). Samples from Bangladesh, Bhutan, Indonesia, and the Philippines did not react with any of the nonradioactive probes used that would detect the Taiwan, Egypt, and India tomato leaf curl viruses. Similarly, none of the samples from Sri Lanka reacted with those probes nor with the Thailand tomato yellow leaf curl virus. This was somewhat surprising because the Thailand TYLCV probe is highly unspecific and is known to react with a number of different geminiviruses, not only those affecting solanaceous crops. However, when tested at the University of Wisconsin with a P_{32}-labeled probe mixture (TYLCV-Egypt, TLCV-Taiwan, TLCV-India, MYMV-Thailand, and ToMoV-Florida), a positive reaction was obtained in some of the Sri Lankan samples. This may be due to the slightly higher sensitivity of radioactive probes. However, it may also be possible that a virus present in Sri Lanka is different from the Taiwan, Egypt, Indian, or Thailand leaf curl virus, and more similar to the Florida tomato mottle geminivirus (ToMoV-Florida), a bipartite geminivirus. Of the total 130 tomato

and pepper samples tested, only six (4.6%) gave a positive reaction with the Indian tomato TLCV probe. Forty-five of the negative samples were reprobed with the broad-spectrum TYLCV-Thai probe, but were also negative. This might indicate that another geminivirus or some other virus may be present in these samples.

Table 5. Leaf curl virus survey of tomato and peppers in Asia

Country	Crop[a]	No. samples tested	No. samples positive	Probe used[b]
Bangladesh	T	24	0	E, I, T*
	P	20	0	E, I, T
Bhutan	T	10	0	I
	P	34	0	I
India (South)	T	16	0	I, T, Th
	P	30	0	I, Th
	T, P	38	6 (I)	E, I, T
	?	46	0	I
Indonesia	T, P	46	0	I, T
Nepal	T, P	34	1	I
	T	44	1 (I), 6 (Th)	I, Th
	P	40	19 (I), 31 (Th)	I, Th
	?	7	0	E, I, T
Pakistan	T	20	2	I
	P	6	3 (I, Th)	I, Th
Philippines	P	85	0	I
Sri Lanka	P	27	0	E, I, T, Th
Thailand	P, T	46	23	T
	P	46	21	T
Total		619	93 (15%)	
	T	114	8 (7, 7%)	
	P	288	54 (19%)	
	T + P	164	30 (18%)	
	?	53	0	

[a] T = tomato, P = pepper, ? = crop unknown

[b] Probe used for hybridization: digoxigenin-labeled probes of E = TYLCV-Egypt, T = TLCV-Taiwan, I = TLCV-India, Th = TYLCV-Thailand; the probes were used independently or in mixture (indicated by *)

The results of the Nepal survey strongly indicated the presence of more than one geminivirus. When tested with TLCV-India probe, a positive reaction was obtained with only 1 of 44 tomato samples and with 19 of the 40 pepper samples. However, when tested with the Thailand tomato yellow leaf curl virus probe, 6 of the tomato and 31 of the pepper samples gave a positive reaction. Samples from Thailand had the highest infection rate, with nearly 50% of the samples positive.

The Taiwan survey (table 6) showed that the Taiwan tomato leaf curl virus is present in tomato almost throughout the island, i.e., in eight of nine counties. Although average disease incidence was low (9%), it was quite high in some of the areas, ranging from 22 (Shoufeng, Hualien county), 33 (Matou, Tainan county), to 64% (Hsiangshan, Hsinchu county). Tomato was the main crop surveyed in Taiwan. However, peppers were also found to be infected with the Taiwan tomato leaf curl virus or a closely related virus. Twelve weed samples comprised of *Ocimium basilicum*, *Erigeron bonariensis*, *Ageratum houstonianum*, *Euphorbia hirta*, and *Emilia sonchifolia*, showing yellowing and curling symptoms, were collected in and around tomato fields where incidence of leaf curl virus was high. However, none of these reacted with the Taiwan tomato leaf curl virus. More weed samples have to be collected to determine whether these weed species are alternate hosts of TTLCV, or whether they harbor another type of geminivirus causing similar symptoms.

Table 6. TLCV survey of tomato and peppers in Taiwan

County	Township	Date[a]	Crop[b]	No. fields surveyed	No. samples collected	No. (%) positive samples
Hualien	Muhguachi	10/93	T	3	18	0
	Shoufeng	10/93	T	4	27	6 (22)
Taitung	Kuanshan	10/93	T	10	17	1 (5.8)
Pingtung	Linlo	9/93	T	1	6	0
	Likang	1/94	T	2	11	1 (9)
	Likang	1/94	P	2	15	0
	Kaoshu	1/94	T	1	10	0
Kaohsiung	Mito (1)	11/93	T	2	33	0
	Mito (2)	1/94	T	1	8	1 (12)
	Yung-an	11/93	T	2	55	0
	Luchu	11/93	T	3	29	0
Tainan	Anting	10/93	T	3	10	1 (10)
	Shanhua	10/93	T	2	6	1 (17)
	Matou	10/93	T	3	21	7 (33)
	AVRDC	5/94	P	5	62	13 (21)
Chiayi	Minhsiung	9/93	T	2	22	1 (4.5)
Changhua	Chihu	1/94	T	7	194	19 (9.8)
Hsinchu	Hsiangshan	12/93	T	1	14	9 (64)
	Hsiunglin	12/93	T	9	60	1 (2)
	Chupei	12/93	T	4	17	0
Nantou	Chingjing	6/94	T	1	16	0
	Hsin Yi	6/94	T	8	27	0
Total					678	61 (9)
			T		601	48 (8)
			P		77	13 (17)

[a] Date of collection
[b] T = tomato, P = pepper
[c] Probe used for DNA hybridization: TLCV-Taiwan

Africa. Samples were collected from Tanzania and Uganda (table 7) and tested with a probe against the Egyptian isolate of TYLCV, since that geminivirus was considered most likely to be present in that region. Some samples were also tested with the Indian tomato leaf curl virus and TTLCV probes. Out of a total of 420 samples tested, only 7.6% gave a positive reaction. Three percent reacted with the EIT probe mixture and 4% reacted only with the broad-spectrum TYLCV Egypt probe, but not with the Indian or Taiwan leaf curl virus probe. This suggests that TYLCV-Egypt or a very closely related geminivirus is present in Tanzania. Recently, the broad spectrum TYLCV-Thai probe was also used because positive signals were rarely obtained with the other three probes. Since many samples from plants with typical yellowing and leaf curl symptoms reacted with none of the probes, there is strong indication that the leaf curl virus present in Tanzania may not be the same as TYLCV-Egypt (strain) and or that TYLCV-Egypt may not be the only geminivirus present. Additional geminiviruses may be infecting the tomato crop in the region. AVRDC, together with the AVRDC Africa Regional Program (ARP), will investigate this further by testing additional samples by the polymerase chain reaction (PCR) and by sequencing any newly isolated geminivirus DNA.

Table 7. Leaf curl virus survey of tomato and peppers in Africa

Country	Crop[a]	Date received	No. samples tested	No. samples positive	Probe[b]
Tanzania	T	5/93	30	15 (E, I, T)	E, I, T*
	T	8/93	40	0	E, I
	T	8/93	56	0	E, I
	T	10/93	9	0	E, I, T
	T	10/93	36	0	E, I, T
	T	3/94	21	15 (E)	E
	?	6/94	43	0	E, I, T
	?	6/94	17	0	E, I, T
	?	6/94	8	2 (E)	E, I, T
	?	7/94	41	0	E
	?	7/94	43	0	E
	T	10/94	46	0	E, Th
Uganda	P	6/94	30	0	E, I, T
Total				**420**	**32 (7.6%)**

[a] T = tomato, P = pepper, ? = unknown, probably tomato

[b] Probe used for hybridization: E = TYLCV-Egypt, T = TLCV-Taiwan, I = ITmLCV-India, Th = TYLCV-Thailand; the probes were used independently or in mixture (indicated by *)

Conclusions

The results of the surveys indicate that geminiviruses infect not only tomato but also peppers in Nepal, Pakistan, India, Thailand, and Taiwan. The presence of several different geminiviruses is suggested. Geminiviruses other than the ones known so far to occur on solanaceous crops may also be present in these countries. This should be further investigated. It was interesting to note that the Taiwan tomato leaf curl virus probe reacted with peppers collected from farmers' fields in Taiwan. We had repeatedly tried to transmit the TTLCV from infected tomato or tobacco to pepper by grafting or whitefly inoculation but were never successful. It is possible that whiteflies transmit the virus more efficiently in nature, or that a slightly different variant of the virus is present in farmers' fields.

The Eastern Mediterranean strain of TYLCV appears to infect leaf curl-diseased tomato in East Africa. However, since many samples with very pronounced leaf curl and yellowing symptoms did not react with the TYLCV-Egypt probe, nor with TTLCV, TLCV-India, or TYLCV-Thai, it is likely that still another type of geminivirus may be present in that region.

Inheritance of Black Leaf Mold Resistance in Tomato

Summary

Inheritance of black leaf mold (BLM) (caused by *Pseudocercospora fuligena*) resistance was studied in four crosses involving two resistant Lycopersicon accessions (PI 134417, *L. hirsutum* and PI 254655, *L. esculentum*) and four susceptible AVRDC tomato lines (CLN 657BC$_1$F$_2$-267-0-3-12-7, CL 143-0-10-3-0-1-10, CLN 698BC$_1$F$_2$-358-4-13, and CL 5915-93D$_4$-1-0-3). For each cross, six generations, i.e., P$_1$, P$_2$, F$_1$, F$_2$, BC$_1$F$_1$, and BC$_1$F$_2$, were evaluated. Chi-square analyses of the data based on the ratio of resistant to susceptible plants in the F$_2$ with three of four crosses gave a good fit to a segregation ratio of 1R : 15S. BC$_1$F$_2$ data from three of four crosses gave an acceptable fit to the segregation ratio of 1R : 63S. The results indicate that resistance to BLM may be conditioned by two recessive genes acting epistatically in both PI 134417 and PI 254655.

Introduction

Black leaf mold, also known as Cercospora leaf mold, [caused by *Pseudocercospora fuligena* (Roldan) Deighton (=*Cercospora fuligena* Roldan)] was first reported on tomato (*Lycopersicon esculentum* Mill.) in the Philippines in 1938. The fungus is reported to be widespread in tropical and subtropical Asia and is also known to occur in Nigeria and southern USA. Previous studies at AVRDC failed to identify useful levels of BLM resistance among cultivars commonly grown in Taiwan. However, sources of resistance to BLM have been identified in four *L. esculentum* accessions: PI 180721, 205037, 254655, and 296363; and six *L. hirsutum* accessions: PI 126434, 126445, 127827, 134417, 134418, and 308182. In this study, two accessions, PI 254655 and PI 134417, were selected for crossing studies with BLM-susceptible tomato lines. The objective of the study was to determine whether resistance is heritable and, if so, to determine the mode of inheritance of BLM resistance. A longer term objective is to develop BLM-resistant tomato lines through breeding.

Materials and Methods

Six parents were chosen for this study based on their BLM responses in previous assays. Two accessions, PI 254655 (*L. esculentum*, AVRDC accession L 1777) and PI 134417 (*L. hirsutum*, AVRDC accession L 1064), were selected as resistant parents. Four AVRDC lines, CLN 657BC$_1$F$_2$-267-0-3-12-7 (SP-1), CL 143-0-10-3-0-1-10 (SP-2), CL 5915-93D$_4$-1-0-3 (SP-3), and CLN 698BC$_1$F$_2$-358-4-13 (SP-4), were chosen as susceptible parents. The study involved four crosses between susceptible and resistant parents: SP-1 x PI 134417, SP-2 x PI 134417, SP-3 x PI 254655, and SP-4 x PI 254655. The F$_2$ plants were obtained by selfing 10 F$_1$ plants from each cross. The F$_1$ plants of each cross were crossed to their respective susceptible parent to develop backcross F$_1$ (BC$_1$F$_1$) and respective BC$_1$F$_2$ populations were obtained by selfing 30 BC$_1$F$_1$ plants derived from each cross.

Plants representing all generations derived from each cross, i.e., parents, F$_1$, F$_2$, BC$_1$F$_1$, and BC$_1$F$_2$, were grown individually in 9.5-cm-diam plastic pots in the greenhouse. When plants were 24 to 31 days old, they were moved into the growth room at 28±2°C and 95±2% RH with a 14-h light period, 49.3 µE/m^2/s. Plants were inoculated by spraying the foliage to the point of run-off with a conidial suspension, 5 x 10^3 conidia/ml, that was obtained by washing 10-day-old colonies of *P. fuligena*, isolate PF-2, grown on tomato-oatmeal agar. Generations from each cross were evaluated at the same time in a single inoculation experiment.

Fifteen days after inoculation, the disease reaction of each plant was determined by visual estimation of the percentage of leaf area affected. The disease severity rating scale was 0 to 100, which was equal to the percentage of leaf area affected. Plants with disease severity ratings below the midparent value were considered resistant. In all cases, the midparent value was below the range of severity ratings for plants of the susceptible parent. Chi-square tests were used to determine goodness-of-fit of the observed to the expected ratio of resistant to susceptible plants in F_2 and BC_1F_2 populations.

Results and Discussion

BLM severity ratings ranged from 10 to 40 for individual plants of the resistant parents and 50 to 90 for plants of the susceptible parents. The F_1 were as susceptible as the respective susceptible parent, indicating that resistance was recessive. The four F_2 populations segregated for BLM resistance. The F_2 plants from crosses involving resistant parent PI 134417 with severity ratings of 40 or less were considered resistant. The F_2 plants from crosses involving resistant parent PI 254655 with severity ratings of 50 or less were considered resistant. Segregation patterns in the three F_2 populations were similar and appear to fit a ratio of 1R : 15S (table 8). F_2 segregation of SP-4 x PI 254655 did not fit a 1R : 15S ratio (X^2 = 56.96) because only 3 of the 948 F_2 plants had BLM severity scores below the mid-parent value (≤ 51). Of the 945 F_2 plants classified as susceptible, 902 received severity scores of 70 or greater, well within the range of the susceptible parent. However, 43 F_2 plants had a score of 60, just above the mid-parent value but below the range of the susceptible parent. It is possible that these 43 plants carry the resistant genotype but environmental effects may have caused susceptible phenotypes.

BC_1F_2 populations also segregated for BLM resistance. The BC_1F_2 plants from crosses involving resistant parent PI 134417 with disease severity ratings of 40 or less were considered resistant. The BC_1F_2 plants from crosses involving PI 254655 with disease severity ratings of 50 or less were considered resistant. Segregation patterns in the three BC_1F_2 populations appeared to closely fit a ratio of 1R : 63S (table 9). SP-1 x PI 134417 does not show good fit for BC_1F_2 (X^2=6.54). This may be due to sampling error since only 253 plants were evaluated. Our data show that the BLM resistance found in PI 134417 and PI 254655 may be conditioned by recessive genes at two loci acting epistatically.

Table 8. Segregation of tomato F_2 progenies from four different crosses into resistant and susceptible classes based on their reactions to black leaf mold

Cross	No. of F_2 plants	Reaction[a] R	S	X^2 value 1:15	P values
SP-1 x PI134417	271	14	257	0.341	0.5 - 0.75
SP-2 x PI134417	905	43	862	3.032	0.05 - 0.1
SP-3 x PI254655	322	16	306	0.645	0.25 - 0.5
SP-4 x PI254655	948	3	945	52.208	<0.001

[a] S = susceptible, R = resistant

Table 9. Segregation of tomato BC_1F_2 progenies from four different crosses into resistant and susceptible classes based on their reactions to black leaf mold

Cross	No. of BC_1F_2 plants	Reaction[a] R	S	X^2 value 1:63	P value
SP-1 x PI134417	253	9	244	5.063	0.01 - 0.025
SP-2 x PI134417	920	20	900	1.806	0.1 - 0.25
SP-3 x PI254655	317	4	313	0.050	0.75 - 0.9
SP-4 x PI254655	895	9	886	1.446	0.1 - 0.25

[a] S= susceptible, R = resistant

Fungicidal Control of Black Leaf Mold on Tomato

Summary

Four fungicide treatments were evaluated on tomato variety Taichung ASVEG No. 4 for the control of black leaf mold under field conditions. Score alone (1 g of 10% W.P. per liter) and benomyl (1 g of 50% W.P. per liter) combined with mancozeb (2.5 g of 80% W.P. per liter) were the most effective fungicide treatments.

Introduction

Black leaf mold of tomato caused by *Pseudocercospora fuligena* has been shown recently to cause extensive damage to tomatoes in Taiwan. The disease has been reported to cause damage to tomato crops in the Philippines, Japan, Taiwan, India, southern USA, Malaysia, and Thailand. Cultivars tolerant or resistant to black leaf mold have been identified and used in Florida to minimize losses from the disease. Previous studies at AVRDC failed to identify useful levels of black leaf mold resistance among cultivars commonly grown in Taiwan. There is only little information on chemical control of the disease. The objective of this study was to evaluate and assess fungicide treatments in the field for black leaf mold control.

Materials and Methods

Tomato variety Taichung ASVEG No. 4 was sown on sterilized soil media in black plastic pot (9.5-cm-diam) on 7 April 1994. The media composition was soil:rice hull:sand:compost = 3:1:1:1. Each pot was thinned to one plant after germination. Seedlings were transplanted to the field on 6 May in two-row plots 5 m long with 0.75 m between rows and 0.5 m between plants in the row. There were five fungicide treatments arranged in a randomized complete block design with four replications. Treatments were: (1) Bayleton: triadimefon (1 g of 5% W.P. per liter); (2) Score: difenconazol (1 g of 10% W.P. per liter); (3) Sporgon: prochloraz + manganese (II) chloride complex (0.25 g of 50% W.P. per liter); (4) Benlate + Dithane M-45: benomyl (1 g of 50% W.P. per liter) plus mancozeb (2.5 g of 80% W.P. per liter); and (5) Control (no fungicide). The first fungicide application was made on 23 June and, thereafter, at 2-week intervals. A total of three applications were made.

Disease was assessed five times at 14-day intervals from 9 June (2 weeks prior to the first fungicide application) to 4 August (2 weeks after the final application). Disease severity was visually estimated by recording the percentage of leaf area infected after walking around the perimeter of 20 plants of each plot. Data were analyzed by Duncan's multiple range test (DMRT) ($p < 1\%$).

Results and Discussion

At the time of the first fungicide applications, black leaf mold was present in the experimental field, at which time less than 10% of the leaf area was affected (table 10). Percentage of leaf area affected was similar among the plots predesignated to receive the fungicidal treatments ($P < 1\%$).

Percentage of leaf area affected by black leaf mold was reduced in the diphenconazol, prochloraz, and benomyl+ mancozeb treatments 2 weeks after the first applications. Two weeks after the second application, some reduction in disease was observed in triadimefon, but less than with the other fungicide treatments. On the final rating, 2 weeks after the third and final fungicide applications significant reductions in percent leaf area affected were found in all fungicide treatments. The diphenconazol and the benomyl+mancozeb treatments were the best, followed by prochloraz and triadimefon, respectively.

Table 10. Fungicidal control of black leaf mold on tomato[a], AVRDC 1994

	Percent leaf area affected				
Treatment	9 Jun	23 Jun	7 Jul	21 Jul	4 Aug
		Fungicide applications			
		1st	*2nd*	*3rd*	
Triadimefon (Bayleton)	2 a[b]	7 a	26 a	31 b	39 b
Diphenconazol (Score)	2 a	7 a	8 c	10 c	13 d
Prochloraz (Sporgon)	3 a	5 a	10 c	15 c	25 c
Benomyl+Mancozeb[c] (Benlate) (Dithane M-45)	3 a	6 a	7 c	10 c	11 d
Nontreated	2 a	8 a	38 a	55 a	73 a

[a] Cultivar: Taichung ASVEG No. 4
[b] DMRT (P = 1%)
[c] Tank mix, full rate of each fungicide

Tomato Late Blight Resistance Studies

Summary

Six commercial tomato cultivars with reported late blight resistance and 37 accessions of wild *Lycopersicon* spp. were evaluated for their reactions to a Taiwan isolate of *Phytophthora infestans*. All of the cultivars were susceptible, but seven of the wild tomatoes showed an intermediate level of resistance. In a separate study, *Lycopersicon* spp. accessions L 3683, L 3684, L 3707, and L 3708, previously identified in the laboratory at AVRDC to be late blight-resistant, were found to be resistant in out-field tests near Puli in Taiwan and at Iringa in Tanzania.

Introduction

Late blight caused by *Phytophthora infestans* (Mont.) de Bary is one of the most destructive diseases of tomato in wet temperate climates and tropical highlands. The pathogen attacks all aboveground parts of the plant causing rapid defoliation and fruit rot. Durable host resistance to late blight is not available in commercial cultivars, thus chemical control is the only alternative for disease management when the tomato crop is grown under conducive conditions. One objective of this study was to test additional wild tomato accessions and commercial tomato cultivars for their reactions to Taiwan isolates of *P. infestans*. A second objective was to test the durability, under field conditions, of late blight resistance sources previously identified at AVRDC.

Materials and Methods

Disease assessment scale

Disease severity ratings (DSR) were made according to the following scale: 0 = no visible symptoms; 1 = 1-10% leaf area affected; 2 = 11-20% leaf area affected; 3 = 21-40% leaf area affected and/or 1-10% stem area affected; 4 = 41-70% leaf area affected and/or 11-50% stem area affected; 5 = 71-90% leaf area affected and/or 51-100% stem area affected; and 6 = 91-100% leaf area affected and/or plant dead. Late blight reaction categories for tomatoes were based on their DSRs as follows: DSR 0-2.0 = highly resistant; DSR 2.1-4.0 = moderately resistant; and DSR 4.1-6.0 = susceptible.

Laboratory inoculations

Isolate Pi-1 of *P. infestans* obtained from diseased tomato plants in the Hsin Yi area in Taiwan was used for the laboratory inoculation studies. The isolate was maintained on rye A agar slant tube cultures at 18°C. Sporangia for inoculum were produced on detached tomato leaves maintained in 15-cm-diam petri dishes on moist filter paper with wet cotton covering the cut end of the petiole. Detached leaves were spray-inoculated with a sporangial suspension from rye A agar or detached leaf cultures of *P. infestans*. The petri dishes were sealed with parafilm and incubated at 18°C with a 12-h light period for 7 to 14 days. Infected leaves were transferred to a beaker of sterile distilled water and agitated to dislodge the sporangia. The sporangial suspension was filtered through three layers of cheesecloth to remove leaf fragments and then diluted, based on hemacytometer counts, to the desired concentration. Tomato plants were inoculated by spraying the foliage to the point of run-off with a sporangial suspension, then incubated in a growth room maintained at 22±2°C and 98±2% RH with 14 h of light (68 $\mu E/m^2/s$) per day. The plant foliage was kept wet by an overhead water system that emitted a fine mist for 30-sec periods twice each hour.

Field evaluations

Tomato plantings which included the AVRDC late blight-resistant accessions L 3683 and L 3684 (*L. hirsutum*), and L 3707 and L 3708 (*L. pimpinellifolium*) were planted at tropical highland locations where late blight occurrence is common. Four replications of four plants each in 20-cm-diam clay pots were arranged in RCBD in the field near Puli, Taiwan during September 1993 and rated for their late blight disease reactions at weekly intervals until mid-December 1993. A similar study was conducted at Iringa, Tanzania from March to May 1994 in which accessions L 3683, L 3684, and L 3707 were evaluated for their late blight responses. In both cases, late blight development was from natural inoculum.

Results and Discussion

Laboratory inoculations

Seven of the 37 wild tomato accessions received mean DSRs of 2.7 to 4.0 which placed them in the moderately resistant category (table 11). The other 30 wild tomato accessions received DSRs of 4.4-6.0 placing them in the susceptible category. All of the cultivars were susceptible to isolate Pi-1, receiving DSRs of 4.8 to 6.0. Individual plants in some of the moderately resistant wild tomato accessions were rated highly resistant and were selected for multiplication. Progeny from these plants will be tested in the future for their reactions to *P. infestans*.

Table 11. Late blight reactions of wild tomato accessions and tomato cultivars inoculated with isolate Pi-1 of *Phytophthora infestans* from Taiwan

Line/Cultivar	Species	Disease severity rating[a]	Disease reaction category[b]
LA 473	*L. esc.* primitive cv.	2.7	MR
LA 1033	*L. hirsutum*	3.0	MR
LA 1469	*L. esc.* v. *cerasiforme*	3.3	MR
LA 1468	*L. esc.* v. *cerasiforme*	3.6	MR
LA 2080	*L. esc.* v. *cerasiforme*	3.6	MR
LA 1712	*L. esc.* v. *cerasiforme*	3.9	MR
LA 1206	*L. esc.* v. *cerasiforme*	4.0	MR
(30 additional lines)	*Lycopersicon* spp.	(4.4-6.0)	S
New Yorker	*L. esculentum*	4.8	S
Pieralbo	*L. esculentum*	5.3	S
Pieraline	*L. esculentum*	4.8	S
Piline	*L. esculentum*	6.0	S
Pusa Ruby	*L. esculentum*	5.0	S

[a] DSR scale is 0-6; see text for details

[b] MR = moderately resistant, DSR 2.1-4.0; S = susceptible, DSR 4.1-6.0

Field evaluations

Late blight disease pressure was high in both out-field locations due to sufficient natural inoculum and conditions that were conducive to disease development. In field plantings near Puli in the central highlands of Taiwan, all four of the AVRDC late blight-resistant accessions (L 3683, L 3684, L 3707, and L 3708) were resistant with low AUDPC values based on weekly DSRs throughout the evaluation period (table 12). None of the other accessions demonstrated an effective level of resistance at this location.

Only three of the AVRDC late blight-resistant accessions (L 3683, L 3684, and L 3707) were included in the test at Iringa in the southern highlands of Tanzania. All three were rated highly resistant along with two late blight-resistant tomato lines (WV 36 and WV 106) from West Virginia University (table 13). The WV lines received susceptible ratings in Taiwan, suggesting that distinct tomato races of *P. infestans* are present at these two locations.

Table 12. Field evaluation of late blight-resistant tomato lines near Puli, central highlands of Taiwan, September - December 1993

Accession/Cultivar	PI/Line	Resistance		
		Species	Gene	AUDPC[a]
L 3683	PI 365904	*L. hirs.*	Unk.	25
L 3684	PI 365905	*L. hirs.*	Unk.	20
L 3707	PI 365951	*L. pimp.*	Unk.	50
L 3708	PI 365957	*L. pimp.*	Unk.	68
L 1497	PI 204976 (WV36)	*L. esc.* x *pimp.*	Ph 1	189
L 1501	PI 204980 (WV106)	*L. esc.* x *pimp.*	Ph 1	189
Caline		*L. esc.*	Ph 1 & 2	164
Mecline		*L. esc.*	Ph 1 & 2	174
L 3975 (S)		*L. esc.*	None	172

[a] Area under the disease progress curve, based on weekly disease severity ratings

Table 13. Field reactions of tomato accessions to late blight in out-field plantings in Taiwan and Tanzania

Accession/Cultivar	Species	Mean disease severity rating[a]	
		Taiwan (AVRDC)	Tanzania (Iringa)
L 3683	*L. hirs.*	2.3	1
L 3684	*L. hirs.*	2.3	0.5
L 3707	*L. pimp.*	1.8	0
L 3708	*L. pimp.*	1.4	-[b]
WV 36	*L. esc.* x *pimp.*	4.4	1
WV 106	*L. esc.* x *pimp.*	4.4	1
Nova	*L. esc.*	6.0	6
Fline	*L. esc.*	6.0	6

[a] Disease severity scale: 0 = no symptoms; 6 = 91-100% leaf area affected
[b] Not included at this location

Future tomato late blight research plans

During 1995, laboratory inoculation experiments will be continued to screen additional wild tomato accessions for late blight resistance and to screen breeding populations derived from late blight-resistant parents. Field studies designed to test durability and geographical effectiveness of resistant lines will be continued with additional plantings in Thailand, Tanzania, and Taiwan. A planting is also planned in the Cameron Highlands of Malaysia.

Biological Control of Tomato Fusarium Wilt

Summary

Seven bacterial isolates, two *Bacillus subtilis*, four *Pseudomonas cepacia*, and *P. aeruginosa*, that are highly antagonistic to *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) have been isolated and identified. An additional 14 *Fol*-antagonistic isolates have been selected but not yet identified. Greenhouse studies are under way to test the potential of these bacteria to serve as biological control agents of tomato fusarium wilt.

Introduction

Fusarium wilt caused by *F. oxysporum* Schlechtend.: Fr f. sp. *lycopersici* (Sacc.) W.C. Snyder & H.N. Hans. (*Fol*) is a potential threat to tomato production worldwide. It is effectively controlled by single gene resistance which has been somewhat durable, but three races of the bacterium are now known to occur. Recent evidence shows that race 2, which overcomes many of the AVRDC tomato lines, is prevalent in Taiwan. Among 16 isolates of *Fol* recovered from various locations in Taiwan, 13 were identified as race 2 and three as race 1. Since new races do evolve, approaches such as biological control should be investigated in addition to the introduction of resistance genes through traditional breeding.

Materials and Methods

Bacteria antagonistic to *Fol* have been isolated from field soils and plant roots using an in vitro biological assay. A series of soil dilutions were made with water containing a suspension of *Fol* conidia (10^4 conidia/ml) and aliquots (0.1 ml/plate) of the soil suspensions were spread over the agar surface of PDA plates. Bacterial colonies associated with zones of fungal inhibition were selected from the PDA plates and dilution streaked onto nutrient agar. Individual bacterial colonies were selected for additional tests. Spot transfers of the bacteria were placed 2.5 cm from the center of the antagonism test plate. A 4-mm-diam inoculum plug from a PDA plate culture of *Fol* was placed in the center of each antagonism test plate. The degree of antagonism by each bacterial isolate was estimated by the size of the inhibition zone, i.e., the distance between the bacterial colony and the margin of the fungal colony after 6 days of incubation at 24°C (fig. 2).

Results and Discussion

Seven of the most antagonistic bacterial colonies were chosen for further laboratory and greenhouse studies. Among them are two *Bacillus subtilis*, one *Pseudomonas aeruginosa*, and four *Pseudomonas cepacia* isolates. More recently an additional 14 bacterial isolates, highly antagonistic to *Fol* but not yet identified, have been selected from AVRDC tomato field soils and placed in long-term storage for future biological control studies. Preliminary greenhouse studies have been conducted to test the seven identified antagonistic bacteria as potential candidates for biological control of fusarium wilt of tomato. The bacteria have been applied as a seed soak, root dip, and soil drench prior to inoculation of 2-week-old Fantastic tomato seedlings with *Fol*. All seven bacterial isolates delayed wilting of tomato plants when applied as a root dip; four of them delayed wilting when applied as a soil drench, but none were effective as a seed soak (table 14).

Plans for further work include: testing additional methods for application of the antagonistic bacteria for control of fusarium wilt, collection of additional antagonist bacteria from a wide range of sources, testing of *Fol*-antagonistic bacteria against other tomato fungal pathogens, and providing antagonistic bacterial isolates to the AVRDC Plant Physiology Unit as possible sources of inhibitory genes that can be cloned and used to develop transgenic tomato plants that can resist invasion by *Fol*.

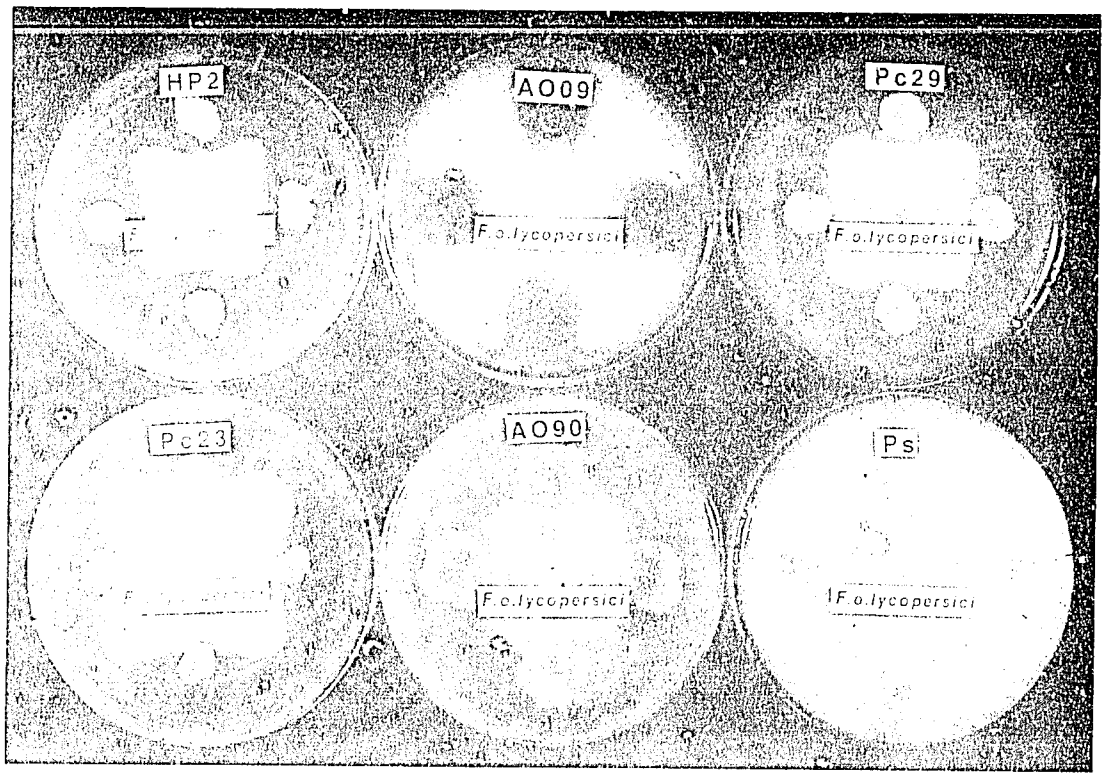


Fig. 2. Inhibition of *Fusarium oxysporum* f. sp. *lycopersici* growth on PDA medium by bacterial isolates HP2 (*Bacillus subtilis*), A 009, Pc 29, and Pc 23 (*Pseudomonas cepacia*), and A090 (*P. aeruginosa*)
(Isolate Ps (*P. solanacearum*) was included as a noninhibiting control)

Table 14. Effect of treatments with fungal antagonistic bacteria on survival of Fantastic tomato seedlings inoculated with *Fusarium oxysporum* f. sp. *lycopersici*[a]

Bacterial Isolate	No. surviving plants after 14 days[b] Application of bacteria			
	Seed soak	Soil drench	Root dip	None
B. subtilis DP2	1	5	7	3
B. subtilis HP2	2	4	7	2
P. cepacia, PC23	1	5	6	4
P. cepacia, PC29	1	6	7	2
P. cepacia, PC30	0	2	7	2
P. cepacia, A009	2	3	5	2
P. aeruginosa, A090	3	3	6	5

[a] Isolate *Fol*-4, tomato race 2

[b] 10 plants per treatment

Tomato Physiology

Cloning of Bacterial Chitinase Genes for Fusarium Wilt Resistance

Summary

Chitinases, partially purified from *Aeromonas hydrophila* JP101, *Escherichia coli* JM109 (pCH1001), and 100 newly-isolated chitinolytic bacterial strains, were assayed for their effects on *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*). All chitinase samples expressed their ability to degrade colloidal chitin, but none of them had an inhibitory effect against *Fol*.

In a related experiment, seven strains of bacteria isolated by AVRDC's Mycology Unit and identified to have strong inhibitory effects on *Fol* were tested against other fungal pathogens of tomato, i.e., *Rhizoctonia solani*, *Sclerotium rolfsii*, *Phytophthora capsici*, *P. infestans*, *Alternaria solani*, and *Stemphylium lycopersici*. Genomic libraries were constructed from the two *Bacillus subtilis* strains, which had the strongest and widest inhibitory effects among the seven identified strains by means of shot gun strategy. Approximately 20,000 transformants each were harvested from these libraries and are presently being screened for antifungal gene(s) in a biological assay involving *Fol*.

Introduction

Fusarium wilt of tomato, caused by *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*), is one of the major diseases of tomato in the tropics and subtropics. Thus, the establishment of a controlling system for *Fol* is one of the priorities in tomato production in these areas. Applying fungicide is a direct and instant approach, but provides only short-term benefit and stimulates the development of new physiologic races that are resistant to fungicide. Because of the concern for the environment, there is a tendency to minimize chemical usage. Another alternative and preferred approach is to find and/or develop *Fol* -resistant varieties. Theoretically, a variety with complete resistance will eliminate the pathogen, and thus, potentially prevent the development of new physiologic races through hybridization or mutation.

Chitin is the major component of fungal cell wall. Many reports have suggested that chitinase derived from either bacteria or plants expressed inhibitory effects on fungal growth, which contributed to the current hypothesis that chitinase may play a role in fungal disease resistance. For example, transgenic tobacco and canola expressing higher level of chitinase enhanced resistance to fungal diseases. One of the objectives of this study was to test whether or not chitinolytic *Aeromonas hydrophila* JP101 (isolated from shrimp shell-enriched soil, and with digestive effects on crab shell) and *Escherichia coli* JM109, harboring plasmid pCH1001 containing the chitinase gene cloned from *A. hydrophila* JP101, have antifungal effects on *Fol*.

Efforts were also made to clone genes that encode for chitinase or other fungal inhibitory factors from antifungal bacteria. Seven strains of bacteria that strongly inhibited growth of *Fol* had been isolated by the AVRDC Mycology Unit and identified as *Bacillus subtilis*, *Pseudomonas cepacia*, and *P. aeruginosa*. They were tested against *Rhizoctonia solani*, *Sclerotium rolfsii*, *Phytophthora capsici*, *P. infestans*, *Alternaria solani*, and *Stemphylium lycopersici*. The cloning of antifungal gene(s) was then attempted with bacterial strains that have the strongest and widest ranging antifungal activities. This study is part of the overall strategy to develop fusarium wilt-resistant tomato varieties with cloned *Fol* resistant gene(s), which will be transferred to tomato plants via the transformation system that has been previously established.

Materials and Methods

Chitinase preparation and analysis

Chitinolytic *Aeromonas hydrophila* JP101 and 100 bacterial strains newly isolated from shrimp shell-enriched soil, and *Escherichia coli* JM109 harboring plasmid pCH1001 that contain the cloned chitinase gene from *A. hydrophila* JP101 (provided by Dr. M.C. Chang) were cultured in M9 medium containing 50 mg/ml ampicillin, 0.5% colloidal chitin but no glucose for 3 days. Colloidal chitin was prepared. Cells were removed by centrifugation at 10,000 g for 10 min. The supernatant was brought to 75% ammonium sulfate at 0°C to precipitate, and the precipitant was redissolved in 50 mM Tris buffer at pH 7.2 and dialyzed against the same buffer overnight. The samples were then assayed for chitinase activity by the turbidimetric method. One milliliter of protein sample was incubated in 1 ml colloidal chitin solution (0.1% colloidal chitin, 0.6 M citric acid, and 1.2 M $NaHPO_4$ at pH 5.1) for 2 h at 37°C. Chitinase activity was determined by absorbency measured at 610 nm.

Assay for antifungal activity

Antifungal activity was estimated using mycelium extension-inhibition assay. A disk of filter paper dipped in fungal conidia suspension was placed on the center of a potato dextrose agar plate, and disks dipped in protein (extracted as described earlier) were placed on the periphery. Antifungal activity was determined by whether or not the hyphae grew over peripheral disks. Based on this assay system, seven strains of *Bacillus subtilis*, *Pseudomonas cepacia*, and *P. aeruginosa* were isolated and identified as having strong inhibitory effect on the mycelium growth of *Fol*. They were then further tested against *Rhizoctonia solani*, *Sclerotium rolfsii*, *Phytophthora capsici*, *P. infestans*, *Alternaria solani*, and *Stemphylium lycopersici*.

Genomic libraries construction and antagonistic assay

Genomic DNA from *B. subtilis* HP2 and DP2, the strains that expressed highest antifungal activities in response to *Fol* and other tomato pathogens, were isolated and partially digested with *Sau*3AI. The resulting fragments ranging from 4 to 15 kb were isolated by sucrose density gradient centrifugation, and ligated to *Bam*HI-digested plasmid pMK4, and then transferred into *E. coli* MC1061. About 20,000 transformants per library were selected on LB plates with a supplement of 50 mg/ml ampicillin. Antagonistic assay was carried out by the modified hyphae extension-inhibition assay described earlier. A mass of fungal mycelium was placed in the center of the plate and 16 *E. coli* clones including 15 transformants and the control (*E. coli* MC1061 harboring pMK4) were inoculated peripherally.

Results and Discussion

Chitinases, partially purified from *A. hydrophila* JP101, *E. coli* JM109 (pCH1001), and 100 newly isolated strains, were assayed for chitinase activities and inhibitory effects on *Fol*. All chitinase samples purified from these chitinolytic strains expressed the ability to digest colloidal chitin; however, none of them inhibited the growth of the *Fol* hyphae. There are several possible explanations for this difference. First, the isolated chitinases may be specific to colloidal chitin, which is oligomer. Second, there are several isomers in the chitinase family, which can be classified as endoenzyme and exoenzyme. Most of the chitinases with antifungal activities appeared to be endoenzymes. In this connection, chitinases from *A. hydrophila* JP101, *E. coli* JM109 (pCH1001), and 100 newly isolated strains may be exoenzymes. Third, crab/shrimp chitin and fungal chitin may have different conformations.

Another experiment was set up to isolate antifungal gene(s) from antifungal bacteria specific to *Fol*. First, seven strains of bacteria isolated and identified to have inhibitory effects on *Fol* by the Mycology Unit were tested against other fungal pathogens of tomato, i.e., *Rhizoctonia solani*, *Sclerotium rolfsii*, *Phytophthora capsici*, *P. infestans*, *Alternaria solani*, and *Stemphylium lycopersici*. Table 1 shows the effects of antifungal bacteria on different tomato fungal pathogens. *Pseudomonas aeruginosa* (A090) inhibited mycelia growth of *F. oxysporum*, *R. solani*, and *P. capsici* grown on the edges of the crescents. The affected mycelia could not restore growth when they were transferred to the newly prepared potato dextrose agar plate.

Results suggest that *P. aeruginosa* (A090) has a fungicidal effect on the tested fungi. Likewise, *Bacillus subtilis* (DP2) has a similar fungicidal effect on *R. solani*, and *Pseudomonas cepacia* (PC29) on *R. solani* and *P. capsici*. On the other hand, *P. aeruginosa* (A090) inhibited the mycelium growth of *S. rolfsii*, but did not kill the mycelium. The inhibited mycelia grew again when they were transferred to the newly prepared potato dextrose agar plate. Results suggest that *P. aeruginosa* (A090) has a fungistatic effect on *S. rolfsii*. Likewise, *Bacillus subtilis* (DP2) had fungistatic effects on *F. oxysporum*, *S. rolfsii*, and *P. capsici*; and *Pseudomonas cepacia* (PC29) on *F. oxysporum* and *S. rolfsii*.

Table 1. Fungicidal and fungistatic effects of antifungal bacteria on different tomato fungal pathogens [a]

Bacteria	Fungal pathogens			
	Fusarium oxysporum	*Sclerotium rolfsii*	*Rhizoctonia solani*	*Phytophthora capsici*
P. aeruginosa (A090)	- [b]	+	-	-
B. subtilis (DP2)	+	+	-	+
P. cepacia (PC29)	+	+	-	-

[a] Initially inhibited mycelia of specific fungal pathogens were subcultured in fresh media

[b] - = no mycelia growth in subculture, indicating fungicidal effects; + = mycelia growth in subculture, indicating fungistatic effects

Based on these results, two *B. subtilis* strains with strong antifungal activities were subjected to the genomic library construction. Approximately 20,000 transformants each were gleaned. Plasmids of randomly selected transformants were isolated and checked for recombination. Most of the recombinant plasmids had DNA fragment insertion ranging from 4 to 15 kb (fig. 1). The gleaned transformants are being screened for antifungal gene(s).

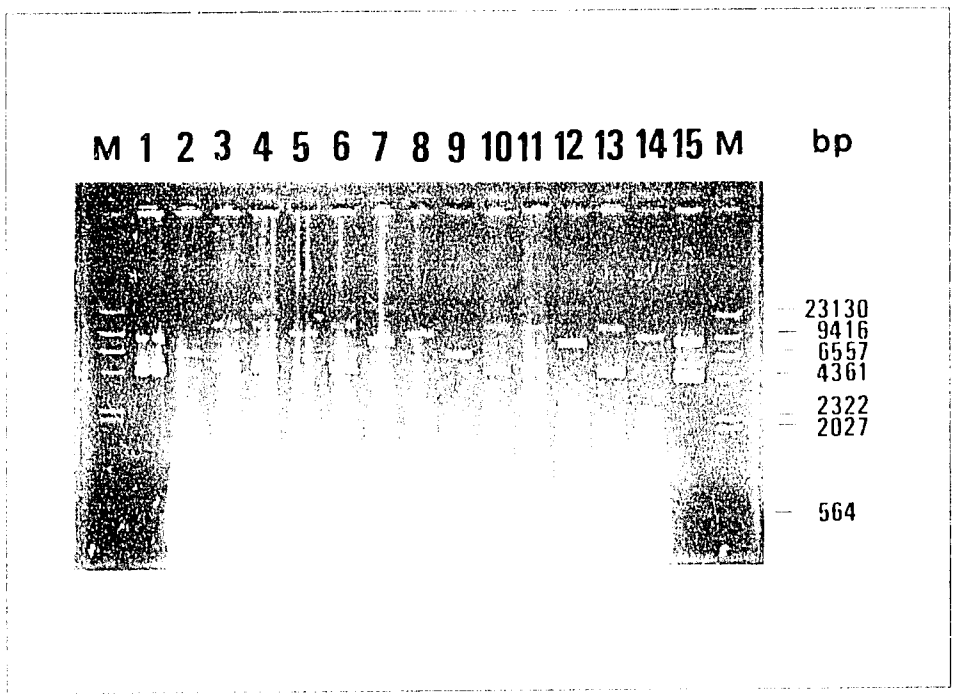


Fig. 1. Gel analysis of recombinant plasmids from the genomic library of *Bacillus subtilis*
M: DNA-*Hind* III digestion. Lanes 1 and 15: plasmid pMK$. Lanes 2-14: recombinant plasmids

Inheritance of NPTII and GUS Genes in Transgenic Tomatoes

Summary

Transformation of tomato was carried out with the transformed *Agrobacterium tumefaciens* strain containing the chimeric NPTII and GUS genes by the method of cocultivation. Both the expression of kanamycin resistance and Southern blot analysis confirmed that transformed tomato plants contained the introduced NPTII and GUS genes. Transformed tomato plants appeared normal phenotypically and produced fruit with viable seeds. The first generation (R_1) plants derived from self-pollinated regenerated plants (R_0) were scored for kanamycin resistance and presence of GUS gene by Southern blot analysis. Segregation ratio of R_1 progeny deviated from the Mendelian ratio (3:1), which might be because R_0 plants were chimerically transformed and the introduced foreign genes may have been lost during meiosis. Thus, these two foreign genes in tomato introduced by the *Agrobacterium*-mediated method were inheritable, but their inheritance did not follow the Mendelian ratio in the R_1 progeny. Whether or not the inheritance of transgenic tomatoes follow the Mendelian ratio needs more investigation in advanced generations.

Introduction

Tomato (*Lycopersicon esculentum*) is a valuable species for studying plant transformation. Many have reported that gene transformation in tomato can successfully express foreign genes and develop stable hereditary cultivars in progeny. Mendelian inheritance of foreign genes in petunia and tobacco has been documented. In the past, we have successfully developed gene transformation systems to transfer foreign genes into tomato and pepper. The objective of this project was to use transgenic tomato obtained via the *Agrobacterium*-mediated method as a model system to study the inheritance of foreign genes in transgenic plants.

Materials and Methods

Plant transformation

Tomato varieties L 4783 and Tainan Selection No.2 were used to obtain transgenic plants via *Agrobacterium*-mediated transformation system. Explants from the cotyledon were inoculated with transformed *Agrobacterium tumefaciens* LBA4404, harboring pBI121 containing NPTII and GUS genes, and selected on kanamycin containing medium. Regenerated tomato plants were specified as R_0 plants (i.e., immediate generation) and self-pollinated to obtain R_1 plants (i.e., the first generation).

Progeny analysis

Seeds (R_1) harvested from self-pollination of R_0 plants were germinated and scored on 1/2 MS medium containing 100 mg/ml kanamycin at 25°C for selecting the presence of NPTII gene.

Southern blot analyses

Genomic DNA was extracted from 5 g of fresh leaf tissue from greenhouse-grown R_0 and R_1 plants. Eight micrograms of each of the resulting DNA samples was digested with *Eco*RI and *Hin*dIII, and electrophoresed in 0.8% agarose gel, and then transferred to Hybond-N membrane (Amersham) by neutralization blotting procedure. A 3 kb GUS fragment from pBI121 was labeled with DIG-dUTP as probe hybridizing against the membrane to examine the presence of GUS gene in R_0 and R_1 plants.

Results and Discussion

The regenerated plantlets which survived the kanamycin selection media were transplanted to the soil. The immediate regenerated plants, specified as R_0, appeared phenotypically normal and produced fruit with viable seeds. Southern blot analysis revealed that R_0 plants contained the inserted 3 kb fragment of the chimeric GUS gene (fig. 2).

To characterize the integration and inheritance of foreign DNA in tomato genome, we analyzed R_1 progeny derived from self-pollinated R_0 lines. Whether or not R_1 seeds survived the kanamycin containing medium served as an indication of the presence of NPTII gene in R_1 progeny. All the segregation ratio of R_1 lines scored strongly deviated from the 3:1 (resistant/susceptible) Mendelian ratio. This is because R_0 plants were chimerically transformed, consisting of both transformed and nontransformed cells, and resulted in a high proportion of nontransformed R_1 progeny than expected. Moreover, the aberrant segregation ratios in R_1 plants suggested that R_0 plants were heterozygous for the transformed trait. It was probable that foreign DNA integrated into the genome of the R_0 plants were partially lost during meiosis. However, R_1 progeny derived from R_0 A47 line segregated for kanamycin resistant/susceptible in a 1:1.6 ratio, and Southern blot analysis of the kanamycin resistant seedlings confirmed that R_1 progeny contained the GUS gene (fig. 3).

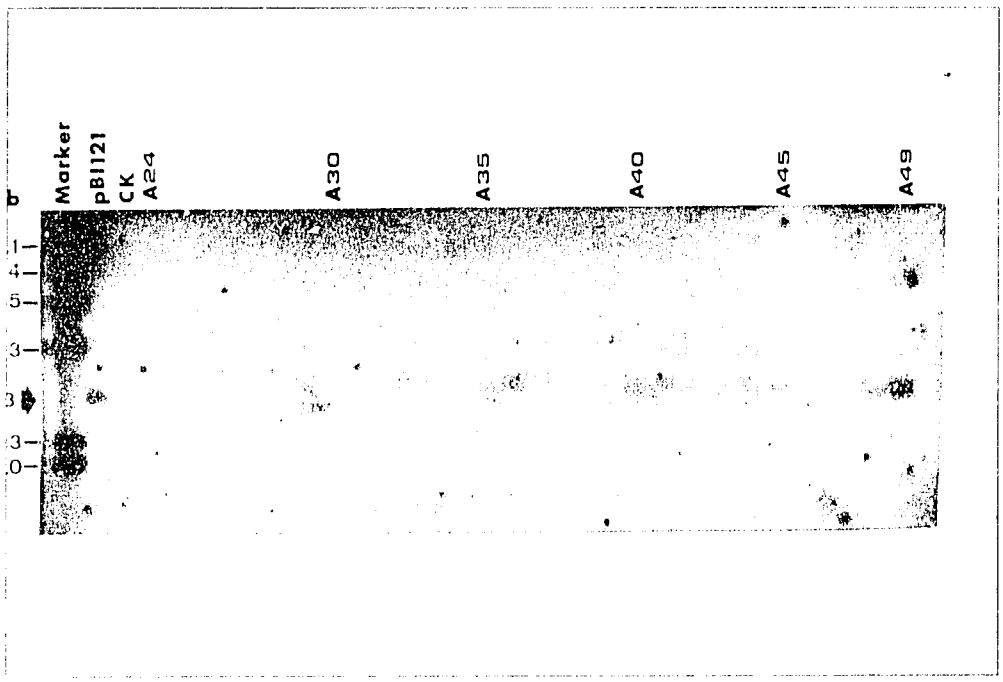


Fig. 2. Southern analysis of R_0 tomato plants DNA samples (8 mg) were digested with *Eco*RI and *Hin*dIII
Lane Marker was the *Hin*dIII digested λDNA. Lane pBI121 was the 3 kb GUS fragment. Lane CK was a nontransformed tomato. Lane A24 to Lane A49 were R_0 regenerated tomato plants

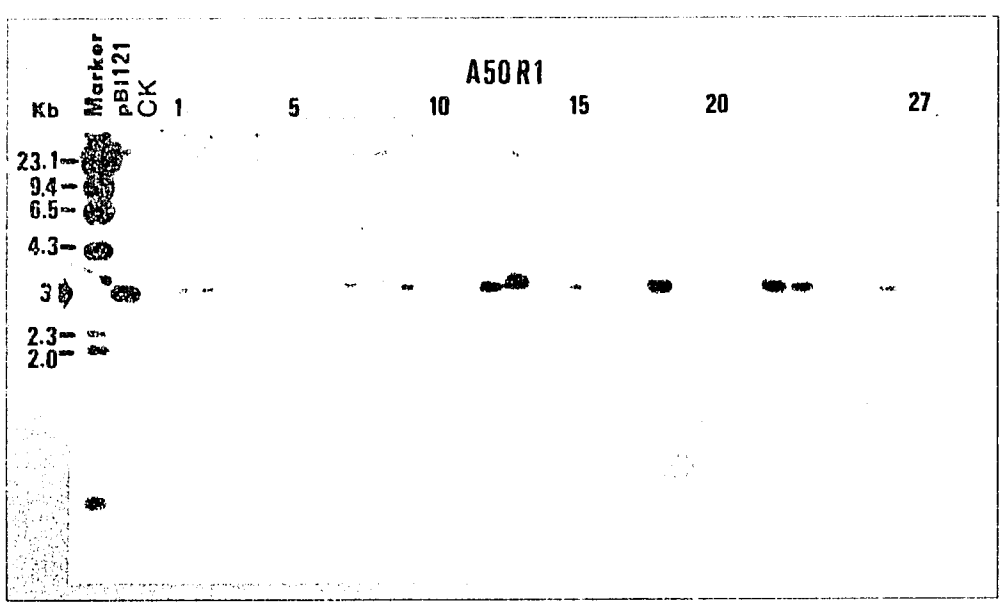


Fig. 3. Southern analysis of R_1 progeny derived from R_0 A50 lines DNA samples (8 mg) were digested with *Eco*RI and *Hin*dIII
Lane Marker was the *Hin*dIII digested λDNA. Lane pBI121 was the 3 kb GUS fragment. Lane CK was a nontransformed tomato. Lanes A50R_1 (1 to 27) contained DNA from self-pollinated R_0 plants

We concluded that foreign DNA introduced into tomato plants via *Agrobacterium*-mediated transformation system is inheritable, but its inheritance did not follow the Mendelian ratio at the R_1 progeny stage. Work is still in progress to investigate the expression and inheritance of the two foreign genes in the advanced generation.

Identification of Genetic Markers Linked to Heat Tolerance in Tomato by RAPDs

Summary

Random amplified polymorphic DNA (RAPD) provides a rapid identification of genetic makers linked to important genes, which could facilitate conventional breeding. The objective of this study was to identify RAPD markers associated with heat tolerance in tomato. Ninety-eight F_2 segregating individuals derived from a single cross between CL 5915-206 (heat-tolerant in fruit-set) and L 4422 (heat-sensitive) were obtained and scored for fruit-set, fruit weight, and seed yield under greenhouse conditions in the summer. The distribution of average fruit weight of F_2 segregating population was strongly skewed towards small size, characteristic of L 4422, whereas, there was no obvious conclusion about seed yield due to low amount of seed production of both parent lines. A total of 640 random primers are being screened for an RAPD marker that co-segregates with high fruit-set rate (i.e., heat-tolerant trait). Several loci segregating in F_2 individuals were identified; however, these loci are not co-segregating with the high fruit-set heat-tolerant trait. Since fruit-set is a polygene trait, the possibility of these identified loci being linked to minor genes cannot be ruled out. Identification of RAPD markers associated with major gene(s) of high fruit-set heat-tolerant trait is still in progress.

Materials and Methods

Plant materials

One hundred F_2 segregating individuals derived from a single cross between CL 5915-206 (heat-tolerant inbred line) and L 4422 (heat-sensitive accession, PI 390716) were obtained by the Tomato Breeding Unit at AVRDC. Ten seeds of each parental line and 100 F_2 seeds were planted in the greenhouse in February 1994, and scored for fruit-set, fruit weight, and seed yield from May to June of the same year. Eight clusters of each plant were scored. Average temperature was 18/9°C in February and 39/24°C in June in 1994. Two F_2 individuals and one L 4422 plant died because of high tempeature.

DNA extraction and RAPD

Five grams of leaf tissue was sampled from 4-month-old individuals plants, and extracted for genomic DNA with modified CTAB method. RAPD reactions were performed in 25 µl volume containing 2.5 µl 10X buffer, 2.5 mM MgCl2, 2.5 mM dNTPs, approximately 0.2 µM primer, 0.625 unit *Taq* DNA polymerase (Perkin Elmer Cetus) and 25 ng of plant genomic DNA from each individual. Of the 640 primers tested, 140 were from Operon kits A, I, M, T, U, V, and W (20 primers per kit) and 500 from UBC set 1, 2, 3, 6, and 7. Reaction mixtures were overlayed with 1 drop mineral oil. Amplifications were performed in an Ericomp Thermal Cycler for three cycles (1 min at 94°C,

1 min at 36°C, and 2 min at 72°C), followed by 40 cycles (30 sec at 94°C, 30 sec at 36°C, 1 min at 72°C) and further extension at 72°C for 10 min. PCR products were analyzed by electrophoresis in 1.6% agarose gels run at 100 V in TBE buffer for approximately 4 h. Gels were stained in ethidium bromide solution and photographed under UV light.

Results and Discussion

Heat reduced fruit-setting more severely in L 4422 line. CL 5915-206, a heat-tolerant line in terms of fruit-set, yielded 19.9 fruits out of 58.3 flowers per plant; and L 4422, a heat-sensitive line, yielded 2.8 fruits out of 79.2 flowers per plant. The F_1 population tended to be heat-tolerant dominant (according to the Tomato Breeding Unit), and the distribution of fruit-setting of the F_2 population was skewed towards low fruit-set rate (fig. 4).

Nine F_2 individuals had higher fruit-set rate than CL 5915-206 plants (fig. 4). Transgressive segregation, which is the presence of some dominant genes from L 4422, explains the high fruit-set. The distribution of average fruit weight of F_2 segregating population was strongly skewed towards small size, characteristic of L 4422 (fig. 4). Fruit weight was determined by multiple genes, and the gene action was additive. The skewness suggested that small fruit size was dominant. There was no obvious conclusion about seed yield due to the low amount of seeds obtained from both parental lines (fig. 4).

A total of 640 random primers are being screened for an RAPD marker that co-segregates with high fruit-set rate (i.e., heat-tolerant trait). Several loci segregation in F_2 individuals were identified; however, these loci are not co-segregating with high fruit-set heat-tolerant trait (fig. 5). Since fruit-set is a polygenic trait, the possibility of these identified loci being linked to minor genes cannot be ruled out. Identification of RAPD markers associated with major gene(s) of high fruit-set heat-tolerant trait is still ongoing.

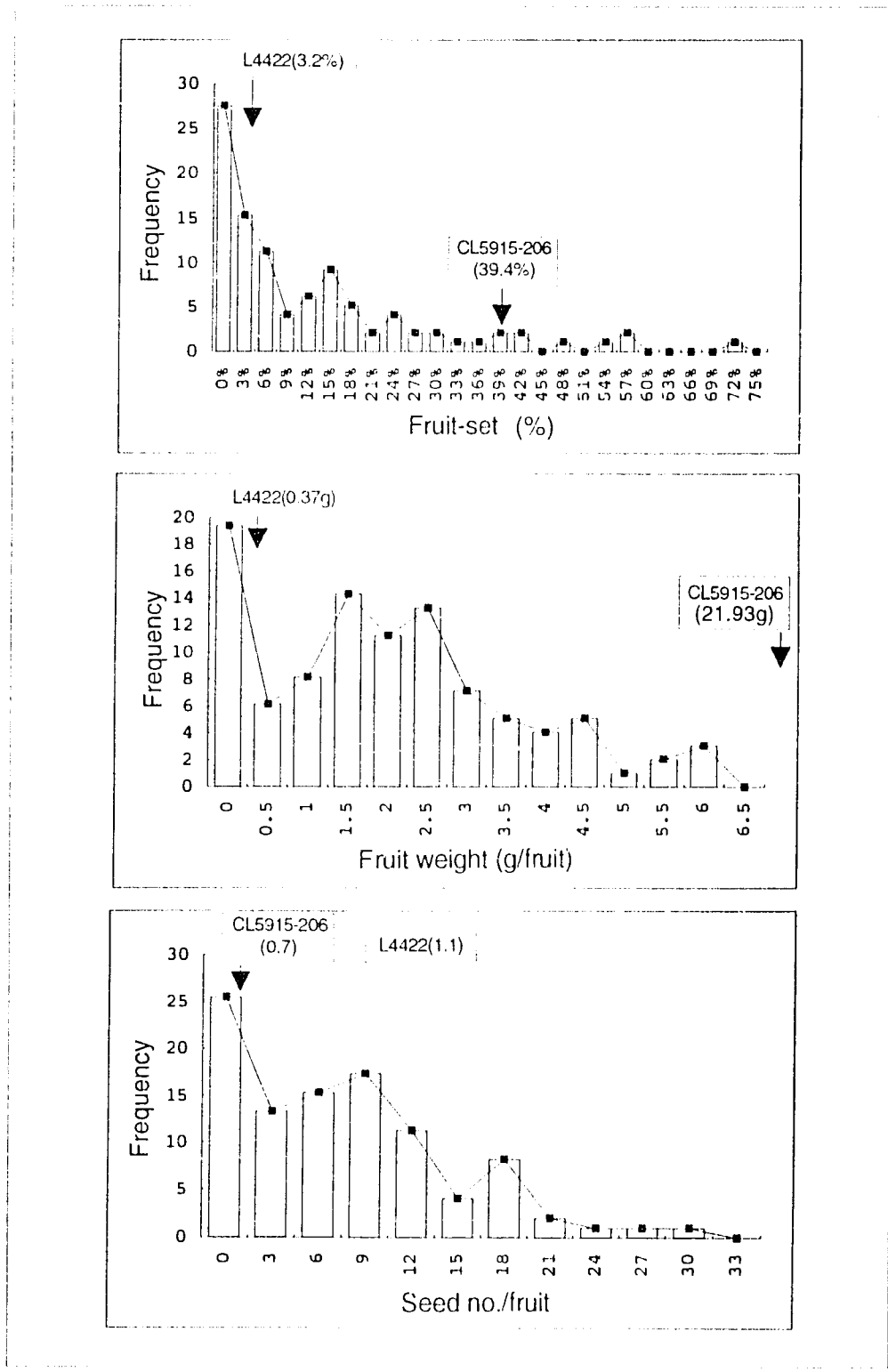


Fig. 4. Frequency distribution for fruit-set (top), fruit size (middle), and seed number (bottom) of F_2 lines of a cross of heat-tolerant CL 5915-206 and heat-sensitive L 4422 tomatoes

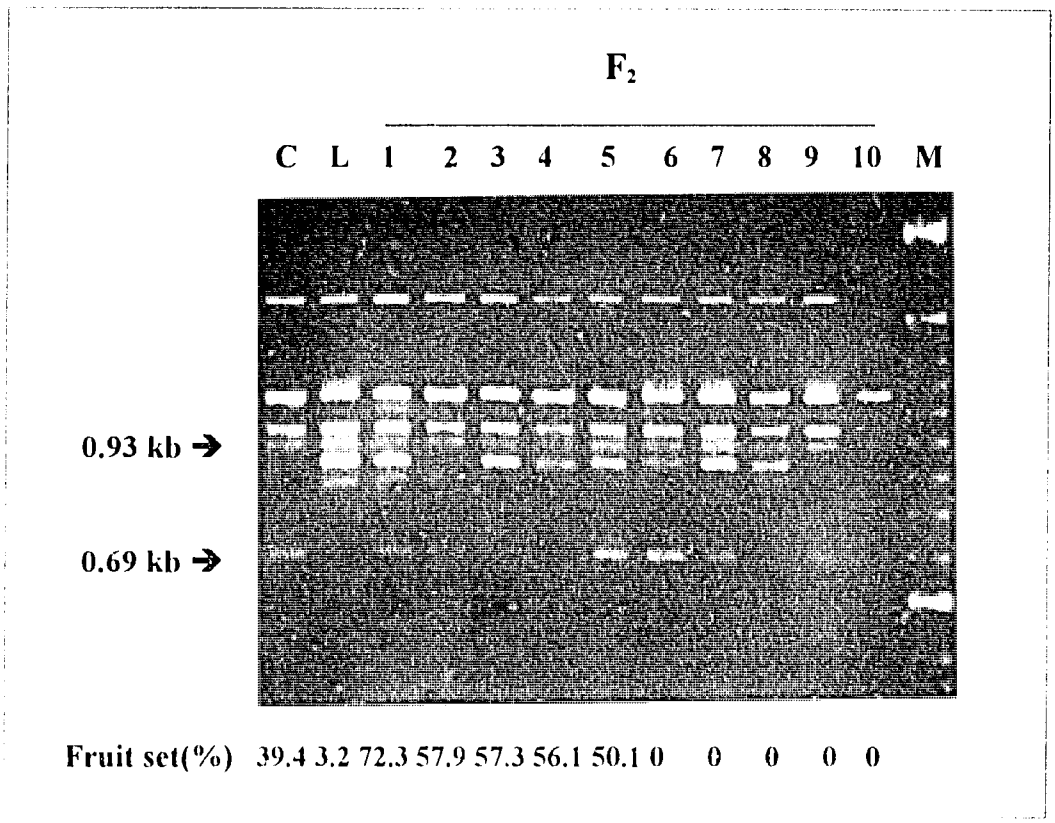


Fig. 5. Electrophoresis profile of RAPD products generated by UBC primer 173 with DNA template from CL 5915-206, L 4422, and F_2 individual #1-10

The arrows indicate the 0.84 and 1.20 kb RAPD bands which detected polymorphorism between CL 5915-206 and L 4422. These two polymorphisms were segregating in the F_2 population derived from CL 5915-206xL 4422, but not associated with fruit-set trait (M = 100 bp DNA ladder, C = CL 5915-206, and L = 4422)

Genetic Resources

Status of the AVRDC Germplasm Collection

As of 31 December 1994, the total number of registered accessions in the AVRDC collection was 41,868 (table 1). This represents an increase of 2539 over that reported last year. Of this, 1969 belongs to the principal crops of AVRDC and 570 to other crops of regional importance. The eggplant collection increased by 1243 accessions with the addition of accessions from the USDA collection. The collection includes four species reported resistant to shoot and fruit borer: *S. integrifolium*, *S. nigrum*, *S. sisymbrifolium*, and *S. xanthocarpum*.

Table 2 shows a summary of the 1994 acquisitions of various crop species.

Table 1. The AVRDC germplasm collection, 1994

Crop		No. of accessions
Principal crops		
	Soybean	13,618
	Tomato	6,806
	Pepper	6,629
	Mungbean	5,769
	Brassica	1,466
	Eggplant	1,807
	Allium	518
	Subtotal	36,613
Other crops		
	Yard-long bean and cowpea	1,608
	Phaseolus bean (including lima)	548
	Black gram	452
	Luffa	280
	Cucumis	268
	Abelmoschus	212
	Pea	211
	Cucurbita	197
	Lablab bean	173
	Benincasa	127
	Others	1,179
	Subtotal	5,255
	Total	41,868

Table 2. Summary of 1994 acquisitions

Genus	No. of accessions	Genus	No. of accessions
Abelmoschus	13	*Ipomoea*	13
Allium	124	*Lablab*	12
Amaranthus	45	*Lagenaria*	58
Amphicarpaea	1	*Lycopersicon*	108
Apium	6	*Luffa*	38
Basella	1	*Momordica*	21
Benincasa	14	*Nicandra*	1
Brassica	41	*Ocimum*	4
Brunfelsia	1	*Pachyrrhizus*	19
Cajanus	15	*Phaseolus*	110
Canavalia	3	*Physalis*	1
Capsicum	266	*Pisum*	4
Citrullus	5	*Psophocarpus*	43
Clitoria	4	*Raphanus*	5
Cucumis	63	*Sesamum*	4
Cucurbita	53	*Solanum*	1253
Daucus	13	*Trichosanthes*	2
Glycine	15	*Vigna*	66
		Total	2445

Multiplication and Characterization of the Germplasm Collection

The multiplication and characterization of new acquisitions and the regeneration of accessions with low viability or which are low in quantity is a continuing activity of GRSU.

In 1994, three locations were involved in multiplication and characterization: the headquarters in Taiwan; the Philippines in collaboration with the National Plant Genetic Resources Laboratory, University of the Philippines Los Baños (UPLB); and Thailand in collaboration with the AVRDC-Asian Regional Center and Kasetsart University. Prioritization in multiplication was based on the needs of the center researchers and the condition of the seeds in storage.

The number of accessions planted for multiplication in 1994 are shown in table 3.

Table 3. Number of germplasm accessions multiplied in 1994

Crop species	Site			Total
	Taiwan	Philippines	Thailand	
Soybean	2,000			2,000
Pepper	236	400	361	997
Tomato	35		40	75
Eggplant	86		222	308
Allium	36			36
Brassica	27			27
Others		680	54	734
Total	2,420	1,080	677	4,177

At headquarters the multiplication of one accession each of five crop species (pepper, wild tomato, eggplant, *Allium*, and *Brassica*) in one net cage with honeybees as pollinators was continued.

Intensive multiplication of pepper was continued. In 1994, a total of 997 accessions were multiplied: 236 accessions at GRSU, 361 at AVRDC-ARC, and 400 in the Philippines bringing the total number of accessions multiplied and characterized to 4143 accessions or 63% of the total in the collection.

In soybeans, the last 2 years have been devoted to the regeneration of accessions that have been in storage for more than 8 years and have very low viability. Multiplication was done both at headquarters in Taiwan and AVRDC-ARC, Thailand. A set of the accessions multiplied in Thailand was kept there to serve as part of the active collection which eventually will all be transferred to AVRDC-ARC. Under the supervision of Dr. Yeong-Ho Lee, seconded scientist from the Rural Development Administration of Korea, a total of 2000 accessions of soybean were multiplied and characterized. Two plantings were done, one in March and another in September. Characterization was based on 26 traits.

Eleven virus-cleaned garlic accessions have been turned over by the Virology Unit to GRSU. One to ten plantlets of each accession were maintained in multiplication and conservation media. The multiplication medium used was B5+0.5 mg/L NAA + 2mg/L 2-iP and the conservation medium was MS+30g/L sucrose +6.5 g/L agar +0.5 mg/L NAA + 2% mannitol. In this conservation medium, cultures can be maintained for 6 months. The cultures were maintained at 25 °C, 8 h dark and 16 h light (4000 lux). Bulblets formed in vitro can be used for distribution and as starting material for the multiplication cycle. One to seven plantlets of six accessions have been potted out in preparation for turn-over to the breeding group.

Distribution of Vegetable Germplasm

Table 4 shows the countries that received AVRDC germplasm including breeding lines. Thailand received the biggest number of samples followed by Taiwan and the Philippines. The large number of samples sent to Taiwan and the Philippines is the result of joint projects on multiplication of vegetable germplasm with the National Plant Genetic Resources Center, Taiwan Agricultural Research Institute, and the National Plant Genetic Resources Laboratory, Philippines. Table 5 lists the major institutions that received AVRDC germplasm in 1994.

Tables 6 and 7 show the utilization of the germplasm collection by the center scientists. Most of the materials were used in screening for resistance/tolerance to biotic and abiotic stresses (anthracnose, bacterial wilt, black leaf mold, blight, viruses, pod borer, flooding, and heat). Others went directly to yield trials and evaluation as tropical leafy vegetable. Rootstock selection and grafting experiments have been done using wild species, usually *Solanum torvum*. The Plant Physiology Unit requested materials for use in inoculation and transformation experiments. Genetic polymorphism based on RFLP/RAPD and protein electrophoresis was studied in some populations. There were also studies on effect of planting dates, daylength, and temperature. Newly introduced garlic were sent to the virology group for virus elimination. Known sources of desirable traits were used by breeders in their hybridization programs.

Table 4. External distribution of AVRDC germplasm and breeding lines in 1994

	No. of samples[a]									
Country	A	B	C	G	I	L	O	S	V	Total
Thailand	0	35	112	2,066	0	970	10	0	4,298	7,491
Taiwan	0	44	240	794	0	1,410	131	0	3,475	6,094
Philippines	3	83	496	61	0	217	1,010	38	6	1,914
Nigeria	0	0	0	1,000	0	22	0	0	0	1,022
Indonesia	0	0	710	7	0	50	0	0	0	767
Korea	0	0	68	412	0	5	95	0	140	720
India	9	1	321	118	0	188	10	20	0	667
Tanzania	29	80	192	42	0	174	115	32	0	664
USA	2	12	174	0	2	269	11	2	6	478
Vietnam	0	2	59	297	0	18	5	0	47	428
Others[b]	102	591	985	767	34	2,241	149	154	5	5,028
Total (89)	145	848	3,357	5,564	36	5,564	1,536	246	7,977	25,273

[a] A - *Allium*; B - *Brassica*; C - *Capsicum*; G - *Glycine*; I - *Ipomoea*; L - *Lycopersicon*; O - Others; S - *Solanum*; and V - *Vigna*

[b] Includes 79 countries and territories: Angola, Antigua, Argentina, Australia, Austria, Bangladesh, Belgium, Belize, Bhutan, Botswana, Brazil, Burkina Faso, Cambodia, Canada, Cape Verde, China, Colombia, Costa Rica, Cote D'Ivoire, Czechoslovakia, Dominican Rep., Egypt, El Salvador, Ethiopia, Fiji, France, Gibraltar, Grand Cayman, Ghana, Grenada, Guam, Guyana, Honduras, Hong Kong, Iran, Israel, Italy, Japan, Jordan, Kenya, Kuwait, Laos, Lebanon, Liberia, Malawi, Malaysia, Mauritius, Mongolia, Mozambique, Namibia, Nepal, Netherlands, New Caledonia, New Zealand, Nicaragua, Pakistan, Panama, Papua New Guinea, Portugal, Saudi Arabia, Seychelles, Sierra Leone, Singapore, Slovenia, South Africa, Sri Lanka, St. Lucia, Sudan, Suriname, Swaziland, Tchad, Turkey, Tuvalu, Uganda, United Kingdom, Venezuela, Zaire, Zambia, and Zimbabwe

Table 5. Recipients of AVRDC vegetable germplasm in 1994

Angola
Popular Television of Angola

Antiqua
Caribbean Agricultural Research and Development Institute

Argentina
Universidad de Buenos Aires
Universidad Nacional de la Plata
Universidad Nacional del Rosario

Australia
Quarantine and Export Center
University of Western Sydney

Austria
Landeskammer Fur Land - Und Forstwirtschaft Steiermark

Bangladesh
AVRDC/USAID/Bangladesh Project
Mennonite Central Committee (MCC)
USAID/FAO

Belgium
Phytotechnie des Regions Intertropicales

Belize
Agricultural Technical Mission of ROC to Belize

Bhutan
Department of Agriculture
NPPC

Brazil
EMBRAPA - CNPH
Empresa Brasileira de Pesquisa Agropecuaria - Embrapa
Excola Superior de Agricultura de Lavras
Universidade de Brasilia

Burkina Faso
INERA - Station de Farako-ba

Canada
Agriculture Canada
Bureau de Renseignements Agricoles
University of Guelph

Table 5. continued.

China
Chekiang Provincial Agricultural Sciences
Chinese Academy of Agricultural Science
Hainan Academy of Agricultural Sciences
Jiangsu Academy of Agricultural Sciences
Kansu Provincial Agricultural Sciences
Ninpou Academy of Agricultural Sciences
Shanghai Academy of Agricultural Sciences
Zhenjiang Agricultural College

Colombia
Instituto Linguistico de Verano

Costa Rica
Asociacion Anai
Escuele de Agricultura de la Region Tropical Humede
Universidad de Costa Rica

Czechoslovakia
Reseach Institute for Crop Production

Dominican Republic
Japan International Cooperation Agency

Egypt
Minufiya University
Suez Canal University

Ethiopia
Alemaya University of Agriculture
Mekelle College of Dryland Agriculture
Melkassa Research Center
Nazareth Research Center

Fiji
Agricultural Technical Mission of ROC to Fiji
Sigatoka Research Station

Ghana
Bubinja Tomato Irrigation Project
Crop Research Institute
Oil Palm Research Institute
University of Ghana

Grenada
Agricultural Technical Mission of the ROC

Guam
University of Guam

Guyana
National Agricultural Research Institute

Honduras
Central American Bank for Economic Integration
Zamorano Escuela Agricola Panamericana

Hong Kong
University of Hong Kong

India
Annamalai University
Central Agricultural Research Institute
Dr. Y.S. Parmar University of Horticulture and Forestry
G.B. Pant University of Agriculture and Technology
Harrisons Vanderhave Ltd
Haryana Agricultural University
Himachal Pradesh Krishi Vishvavidyalaya
Indian Institute of Horticultural Research
Indian Council of Agricultural Research
Indian Grassland and Fodder Research Institute
Kerala Agricultural University
Nath Seed Ltd.
National Bureau of Plant Genetic Resources
National Research Center for Spices
Proagro Seed Company Ltd.
Punjab Agricultural University
Punjab Krishi Vidyapeeth
Sakthi Soyas Limited
Tamil Nadu Agricultural University
Vikki's Agrotech Ltd.
Zuari Agro Chemicals Ltd.

Indonesia
Bogor Agricultural University
Central Research Institute for Horticulture
Gadjah Mada University
Lembang Horticultural Research Institute
Universitas Advent Indonesia
University of Brawijaya

Table 5. continued.

Iran
Agricultural and Natural Resources Research Organization

Israel
Bar-Ilan University

Italy
Istituto di Orticoltura e Floricoltura
Lodagri
Seed Exchange and Information Center, FAO

Japan
Chiba University
JIRCAS Okinawa Subtropical Station
Kagome Research Institute
Kunisada Plant Breeding Station
National Agriculture Research Center

Jordan
National Center for Agricultural Research and Technology Transfer

Korea
Agricultural Biotechnology Institute
Crop Experiment Station
Horticultural Experiment Station
International Technical Cooperation Center

Laos
Hatdokkeo Agricultural Station

Lebanon
American University of Beirut

Malaysia
MARDI
Pengurus Perkhidmatan dan Pembangunan Pertanian
Pusat Penyelidikan Pertanian, Semong
Rural Development Corporation
Universiti Pertanian Malaysia

Mauritius
Mauritius Sugar Industry Research Institute
The University of Mauritius

Mongolia
Vegetable Research Association in Mongolia

Nepal
FAO, GCP/NEP/043/SWI
Tribhuvan University
International Center for Integrated Mountain Development
Lumle Regional Agricultural Research Center
Vegetable Development Division

Netherlands
Enza Zaden B.V.
Leen de Mos B.V.
Rijk Zwaan
Wageningen Agricultural University

New Caledonia
Centre de Cooperation Internationale en Recherche Agronomique pour le Development

New Zealand
New Zealand Institute for Crop and Food Research Limited

Nicaragua
Agricultural Technical Mission of the ROC

Nigeria
Ahmadu Bello University
International Institute of Tropical Agriculture

Pakistan
Industrial Management and Special Services
National Agricultural Research Center
Pakistan Agricultural Research Council
USAID - Pakistan
Village Qaldara (Dargai Pattak)

Panama
El Valle de Anton Cocle

Papua New Guinea
Agriculture Science Department
Erap Research and Development Center

Table 5. continued.

Philippines
Agribusiness System Assistance Program
Asian Development Bank
AVRDC - Philippines Outreach Program
Baguio National Crop Research and Development Center
Bureau of Plant Industry
International Rice Research Institute
Los Baños National Crop Research and Development Center
National Plant Genetic Resources Laboratory
University of the Philippines Los Baños

Portugal
Universidade Tecnica de Lisboa

Sierra Leone
Rice Research Station

Singapore
Eastreco (Pte.) Ltd.
IPGRI Regional Office for Asia, the Pacific and Oceania

South Africa
MayFord Seeds

Sri Lanka
Department of Agriuclture
Eastern University
Field Crops Research and Development Institute
Karandagolla Estate
Regional Agricultural Research and Development Center

St. Lucia
Ministry of Agriculture and Lands
The Agricultural Technical Mission of the ROC

Sudan
Agricultural Research Corporation

Suriname
University of Suriname

Swaziland
The Agricultural Technical Mission of the ROC

Taiwan, ROC
Academia Sinica
Hengchun - Pingtun Farmer's Association
Kaohsiung District Agricultural Improvement Station
Known-You Seed Company
National Chung Hsing University
National Plant Genetic Resources Center
National Taiwan University
Nong-sen Seed Company
Tainan District Agricultural Improvement Station
Taiwan Agricultural Chemicals and Toxic Substances Research Institute
Taiwan Agricultural Research Institute

Tanzania
AVRDC-Africa Regional Program
CMML/RSTI
Iringa Municipal Council

Tchad
Office National de Development Rural

Thailand
AVRDC Asian Regional Center
Chiang Mai University
Department of Agriculture
East West Seed Co., Ltd.
Kasetsart University
Khon Kaen University
King Mongkut's Institute of Technology Ladkrabang
Northeast Regional Office of Agriculture

Turkey
Gaziosmanpasa Universitesi

Table 5. continued.

USA
Beltsville Agricultural Research Center
College of Tropical Agriculture and Human Resources
Double Harvest
Echo
Harris Moran Seed Company
Iowa State University
Louisiana State University
New Mexico Research Station
Petoseed Co.
Sakata Seed America, Inc.
Texas Tech University
The Pennsylvania State University
University of California, Berkeley
University of California, Davis
University of Guam
University of Hawaii at Hilo
University of Hawaii at Manoa
USDA, ARS
Water Isle Botanical Garden
World Food Tech Services

Uganda
Namulonge Horticultural Farmers Group
Uganda School for the Deaf
VOCA - Uganda

United Kingdom
Natural Resources Institute
Nickerson Seeds Limited
Rothamsted Experiment Station
University of Edinburgh
University of Manchester
University of Wales

Venezuela
Fundacion Servicio para el Agricultor Estacion Experimental de Cagua

Vietnam
Can Tho University
Institute of Agricultural Science for South Vietnam
Legumes Research and Development Center
Seed Company of Ho Chi Minh City
Vietnam Agricultural Science Institute

Zaire
Institut National Pour L'etude et la Recherche Agronomique

Zambia
Zambia Seed Co., Ltd.

Zimbabwe
Certseed International (PNT)

Table 6. Internal seed distribution of germplasm in 1994

Department	A[a]	B	C	G	L	O	S	V	Total
Entomology	0	0	0	0	0	0	0	1,279	1,279
Pathology	2	2	220	0	455	0	0	0	679
Plant Breeding	0	1	29	350	4	0	6	0	390
Country Program	6	59	0	0	0	99	109	0	273
Home Garden	0	0	67	0	11	0	33	0	111
Physiology	1	0	6	0	17	0	4	0	28
Crop Management	0	0	0	0	3	0	8	0	11
Analytical Laboratory	0	0	0	0	0	0	0	1	1
Total	9	62	322	350	490	99	160	1,280	2,772

[a] A - *Allium*; B - *Brassica*; C - *Capsicum*; G - *Glycine*; L - *Lycopersicon*; O- Others; S - *Solanum*; and V - *Vigna*

Table 7. Utilization of AVRDC germplasm by center scientists in 1994

Crop species	Purpose
Allium	Evaluation as tropical leafy vegetable; effect of planting month, temperature, daylength, and flooding; study on stemphylium blight associated yield loss; virus elimination
Crucifer	Evaluation as tropical leafy vegetable; international yield trial
Eggplant	Yield comparison; screening for bacterial wilt resistance, grafting compatibility on peppers and flooding tolerance; elite trials
Pepper	Screening for resistance to PMMV and other viruses; studies on anthracnose, grafting, inoculation, and transformation; greenhouse studies
Soybean	Evaluation trials
Tomato	Screening for resistance to late blight, bacterial wilt, black leaf mold, *A. rhizogene* infection, flood and heat tolerance; RFLP/RAPD; DNA polymorphic assay; heat shock proteins; selection for tomato rootstocks; testing for TLCV; compatibility tolerance of grafting materials with peppers; hybridization
Mungbean	Screening for podborer resistance
Others (*Spinacia, Chrysanthemum, Amaranthus, Cleome, Apium, Lactuca, Ocimum Coriandrum, Basella*, kangkong, and yard-long bean)	Evaluation as tropical leafy vegetable; observation trials on nonprincipal crop

Development of a Mungbean Core Collection

Summary

Procedures for generating relatively similar groups and for nominating accessions to the core collection were developed. Using Ward's minimum variance and average linkage method, cluster analysis was done on each country collection. The number of clusters to be used in nominating accessions to the core was determined using four criteria suggested in the SAS/STAT guide. For each cluster, means of quantitative traits were used to describe an "average accession." The accession closest to the average accession was nominated to the core. Accessions with qualitative traits not covered in the first nomination and accessions with special or rare traits were further nominated. Country cores including an average of 5% of the total were formed.

Introduction

Germplasm collections have grown markedly in size and genebanks now face problems of resource constraints in the evaluation, seed distribution, regeneration, and routine maintenance of such large holdings. AVRDC is no exception. Its holdings now total more than 40,000 accessions. Since the center has accepted responsibility for the global base collection of mungbean, it has increased its mungbean collection to more than 5000 accessions. After multiplication and characterization, separation of variants within accessions has resulted in 8347 entries.

Principles have been formulated for restraining the size of collections while endeavoring to improve the contents in terms of representaive variability. This gave rise to the concept of a core collection. A core collection is a condensed yet representative assembly of accessions which would represent, with minimum redundancy, the genetic diversity of a crop species or its wild relatives (Frankel 1984)[1]. The purpose of the core collection is to provide efficient access to the whole collection since its components represent and cover the major kinds of diversity present.

Preliminary studies have been done to establish a procedure that can be followed in the development of a mungbean core collection based on the available morphoagronomic characterization data at AVRDC (AVRDC 1993)[2]

This study, done in collaboration with Dr. A.L.N. Gironella and Ms. M.P. Cababasay of UPLB, presents the procedure that was finally developed.

Materials and Methods

Three passport information (accession number, name, and country of origin) and 33 descriptor information for each of the mungbean accessions were obtained.

The following preliminary analysis on all accession entries were done: frequency distribution of accession entries by country, computation of basic statistics, computation of correlation coefficients of 22 quantitative (ratio) traits, and coding of quantitative (ratio) traits. The main analyses done by country of origin were: cluster analysis of accession entries using standardized quantitative (ratio) traits, graphical representation of the clustering sequence, determination of number of clusters based on cluster analysis, description of the clusters obtained, and selection of nominated accessions from each cluster.

Computer hardware and software support consisted of an IBM PC-compatible desktop computer with the following configuration: 80486 DX-25, 66 MHz, 406 MB hard disk, 640 K base memory, 23,552 K extended memory, 256 K cache memory, co-processor installed. The software used was Statistical Analysis System (SAS), ver. 6.03 with the following modules: SAS/Base and SAS/STAT.

A hierarchical cluster analysis was chosen with a tree or dendrogram as output. Two measures of similarity or resemblance were used: Ward's minimum-variance distance and average linkage distance.

Four criteria, suggested in the SAS/STAT guide were used to determine the number of clusters to be used in nominating accessions to the core: semi-partial R^2(5%), cubic clustering criterion (peak value), pseudo-F^2 (peak value), and pseudo-t^2 (low value).

[1] Frankel, O.H. 1984. Genetic perspectives of germplasm conservation. In Arber, W., Illmensee, K., Peacock, W.J., and Starlinger, P. (eds.) Genetic Manipulation and Impact on Man and Society. Cambridge University Press, U.K.

[2] AVRDC. 1993. AVRDC 1992 Progress Report. AVRDC, Shanhua, Tainan, Taiwan.

For each cluster identified from cluster analysis, means of quantitative traits of all accessions forming the cluster were computed to obtain what was designated as the "average accession." The distance of each accession in the cluster from the "average accession" was then computed. The accession closest to the "average accesssion" was nominated to the core. To cover all detectable variation, accessions with qualitative traits not covered in the first nomination were further nominated. Accessions with "special" or "rare traits" were further included.

Results and Discussion

From the frequency distribution, the top three of the 49 countries contributing to the collection are: India (53.5%), Iran (12.6%), and Philippines (5.2%). The three most variable traits and their CVs were pods per plant (49.2%), yield per plot (43.5%) and number of clusters/plant (41.1%). The three least variable traits and their CVs were starch content (5.0%), protein content (6.3%), and number of seeds/pod (8.9%).

To reduce redundancy in traits due to high associations, the contingency and correlation coefficients were computed and only one of two traits with high association was included in the cluster analysis. To minimize loss of accession observation traits with high frequency of unrecorded data were also not included in the cluster analysis. Thus, the following traits were excluded: hypocotyl color (spring), plant height at maturity (spring and autumn), date to maturity (spring and autumn), pod length (spring), pod pubescence color (spring), and pod pubescence length (spring).

Using Ward's clustering technique, the 22 quantitative traits were each grouped to lower their level of measurement from ratio to ordinal. This allows the qualitative description of each classes.

To illustrate the rest of the procedure, the analysis for one country (Indonesia) was chosen. Table 8 shows the output of Ward's minimum variance clustering technique. Based on this, Ward's average linkage, the dendrogram and the four criteria for determining the optimum number of clusters, seven clusters were used to nominate accessions to the country core for Indonesia.

The quantitative traits of the members of each cluster was then printed out and their basic statistics computed. From there an "average accession" was described. The accession within the cluster closest to the average was chosen and nominated to the core. Then the qualitative traits of the members of the cluster were printed out. The traits of the accessions first nominated to the core were then examined to make sure that all the qualitative traits are represented. In cases where a qualitative trait was found not included in the nominated accessions, additional accessions were included. In table 9, the first seven accessions representing the seven clusters were included in the first nomination for the Indonesian country core. Another accession from cluster 4 was added to include the ratings 2 and 4 for pod color at maturity and lodging, respectively. These were not included in the first seven nominations but were present in the collection.

Conclusions and Recommendations

Using a hierarchical clustering technique, accessions in a collection that has been characterized can be separated into homogeneous groups from which representatives can be systematically chosen for nomination to a core. The process is reiterative and a computerized procedure can probably be developed. The core collection should not be considered as fixed but dynamic. It should be modified and refined as additional characterization and evaluation data become available.

Table 8. Ward's mininum variance cluster analysis for accessions from Indonesia

NCL	Clusters joined		FREQ	SPRSQ	RSQ	ERSQ	CCC	PSF	PST2
45	V02059B-G	V02060B-G	2	0.002	1.00	-	-	12.6	-
44	CL45	V02862B-BR	3	0.002	1.00	-	-	11.0	1.3
43	V02255A-G	V03490A-G	2	0.003	0.99	-	-	10.1	-
42	V01473B-G	V01474B-G	2	0.003	0.99	-	-	9.4	-
41	V02052B-G	V02058B-G	2	0.004	0.99	-	-	8.8	-
40	V01434B-G	V01472B-G	2	0.004	0.98	-	-	8.4	-
39	V02048B-BR	V02048B-G	2	0.004	0.98	-	-	8.1	-
38	V01344A-G	V01434A-G	2	0.004	0.97	-	-	8.0	-
37	V02049B-G	V02050B-G	2	0.004	0.97	-	-	7.8	-
36	V01498B-G	V01522B-Y	2	0.005	0.96	-	-	7.6	-
35	V01438B-G	CL42	3	0.006	0.96	-	-	7.4	1.7
34	V01458B-G	V01495A-G	2	0.006	0.95	-	-	7.3	-
33	V01432B-G	V01500B-G	2	0.006	0.95	-	-	7.2	-
32	V01465B-G	V02057B-G	2	0.006	0.94	-	-	7.0	-
31	CL37	V02828B-G	3	0.007	0.93	-	-	7.0	1.5
30	CL38	V01472B-BR	3	0.007	0.93	-	-	6.9	1.7
2	CL44	V02862B-G	4	0.008	0.92	-	-	6.8	3.7
28	CL32	CL41	4	0.008	0.91	-	-	6.7	1.7
27	CL31	V02053B-G	4	0.009	0.90	-	-	6.6	1.6
26	V01437A-G	V01511B-Y	2	0.009	0.89	-	-	6.5	-
25	CL40	V01465A-G	3	0.009	0.88	-	-	6.5	2.4
24	CL33	CL36	4	0.010	0.87	-	-	6.5	1.7
23	V03210B-G	V03211A-G	2	0.010	0.86	-	-	6.5	-
22	CL30	CL25	6	0.010	0.85	-	-	6.6	1.6
21	V01526B-Y	V02061B-G	2	0.011	0.84	-	-	6.6	-
20	V01474A-G	V02055B-G	2	0.011	0.83	-	-	6.7	-
19	CL34	CL39	4	0.012	0.82	-	-	6.7	2.3
18	CL26	V01471B-G	3	0.012	0.80	-	-	6.8	1.4
17	CL28	V01504B-Y	5	0.014	0.79	-	-	6.9	2.2
16	CL35	V02056B-G	4	0.017	0.77	-	-	6.9	3.9
15	CL24	V01494B-G	5	0.018	0.76	-	-	6.9	2.6
14	CL20	V02049A-G	3	0.019	0.74	-	-	6.9	1.7
13	CL27	CL29	8	0.019	0.72	-	-	7.0	3.6
12	CL16	CL13	12	0.021	0.70	-	-	7.1	2.7
11	V03210B-BR	CL23	3	0.021	0.68	-	-	7.3	2.1
10	CL19	CL11	7	0.025	0.65	-	-	7.5	2.4
9	CL22	V01517B-Y	7	0.027	0.62	0.66	-2.18	7.7	3.9
8	CL15	CL17	10	0.028	0.60	0.64	-2.17	8.0	3.2
7	CL8	CL21	12	0.030	0.57	0.61	-1.90	8.5	2.7
6	CL12	CL14	15	0.038	0.53	0.57	-2.00	9.0	3.8
5	CL9	CL18	10	0.048	0.48	0.53	-2.07	9.5	4.6
4	CL6	CL43	17	0.052	0.43	0.48	-1.87	10.5	4.6
3	CL5	CL10	17	0.066	0.36	0.41	-1.51	12.2	4.7
2	CL7	CL4	29	0.135	0.23	0.28	-1.52	12.9	10.1
1	CL3	CL2	46	0.226	0.00	0.00	0.00	-	12.9

NCL = no. of clusters; FREQ = frequency; SPRSQ = semipartial R^2; RSQ = R^2; ERSQ = expected R^2; CCC = cubic clustering criterion; PSF = pseudo F; PST2 = pseudo t^2

Table 9. Nominations to core collection: Indonesia

Cluster no.	1	2	3	4	5	6	7	4
Accession entry	V1474	V2255	V2057	V1517	V3210	V1471	V1474	V1434
Accession ID[a]	B-G	A-G	B-G	B-Y	B-BR	B-G	A-G	A-G
leaf length (cm)	5.2	5.7	5.1	5.2	3.9	4.4	5.7	5.0
leaf width (cm)	1.7	2.1	2.0	1.4	1.6	1.3	1.9	1.4
plant height flowering (cm)	30	19	38	50	45	67	27	53
days to flowering	35	41	48	44	48	48	34	44
no. of clusters	7	4	5	14	6	18	7	14
pods/plant	19	11	19	19	21	39	24	27
pod length (cm)	8.8	10.7	10.5	6.4	9.9	7.3	10.7	7.9
seeds/pod	12	10	12	13	14	12	13	13
seed weight (g)	45.4	81.0	52.0	30.8	33.9	22.1	67	32
% protein	22.1	22.8	23.7	26.7	25.4	24.6	22.3	22.6
% fiber	4.0	3.0	3.8	4.1	2.8	4.1	3.4	4.3
% starch	45.4	48.8	47.2	40.0	42.3	44.7	45.4	43.6
yield/plot (g)	179.4	97.5	229.4	57.8	115.5	265.7	210.3	220.3
hypocotyl color	2	2	2	1	2	2	1	2
growth habit	1	1	1	1	1	1	1	1
lodging	2	1	1	5[b]	2	3[b]	1	4[b]
basal petiole color	2	2	2	2	2	2	1[b]	2
leaf pubescence no.	1	1	3[b]	1	1	1	2	1
primary leaf shape	3	3	3	4	4	4	3	4
leaf pubescence	1	1	1	1	1	1	1	1
raceme position	2	3[b]	2	2	2	2	2	2
wings petal color	y^{3b}	y^2	y^2	y^2	y^2	y^2	y^{1b}	y^2
calyx color	2	2	2	2	2	2	1*	2
pod color maturity	4	1	5	5	5	4	3	2[b]
pod pubescence no.	3	2	1[b]	2	3	3	3	3
pod pub length	2	1	1	3	1	1	2	1
seedcoat color	G	G	G	Y[b]	BR[b]	G	G	G
seed luster	B	A	B	B	B	B	A	A

[a] Seed luster and seedcoat color used to further identify the accession
[b] Indicates 'special' or 'rare' trait

Diversity in Seed Proteins in *Capsicum annuum*

Summary

A total of 208 accessions of *Capsicum annuum* from 46 different countries were analyzed, and their genetic diversity measured based on SDS-PAGE of their seed proteins. All bands observed in the previous study were noted except for the rare band 7. Seven additional banding patterns were observed. As in the previous study, this population of *C. annuum* was monomorphic for bands 2, 8 and 9. Polymorphism for bands 4, 5, and 6 which was not observed before was observed this time.

Specific bands predominated in some regions. Diversity in terms of banding pattern was ranked as Central America > Western and Central Europe > Southeast Asia = Balkan States = Africa > North America = East Asia = South Asia = Southwest Asia > South America.

Similarity index values were ranked as Southwest Asia > Western and Central Europe > South America > West Indies > East Asia > Africa > North America > Central America > South and Southeast Asia > Balkan States. Central America and South and Southeast Asia exhibited the lowest similarity index value.

Accessions with inserted stigma showed the highest similarity followed by those with the same level stigma and then by those with exerted stigma indicating that breeding structure influences diversity in seed proteins. The band patterns revealed that the population of selfers was genetically homogeneous, exhibiting only one kind of banding pattern. This suggested that breeding behavior may influence diversity in seed proteins.

Majority of the variants of the same accession exhibited the same banding pattern showing that they were genetically similar at least for seed proteins.

Seed protein banding pattern was associated with morphological diversity.

Introduction

Previous to this study, seed protein banding pattern using SDS-PAGE was done on different species of *Capsicum* (AVRDC 1994[3]; Cruz et al.[4]). It was found that the procedure can be used as a rapid means to confirm species identity of accessions, to characterize genetic diversity in a population and to determine species relationship. This additional study was done on a bigger number of *C. annuum* to see if all possible bands have been detected in the first study, to compare seed protein banding pattern of selected varieties of *C. annuum* of different geographic origin, to study seed protein diversity in accessions that differ in flower morphology and to see if variants of the same accession also differ in seed protein.

Materials and Methods

A total of 208 accessions fom 46 different countries were used in this study. The procedure followed is the same as that reported in a previous study (AVRDC 1994).

Results and Discussion

Band patterns resolved by SDS-PAGE

Based on the electrophoretic data gathered, seven bands were recognized (fig 1). Comparing the bands with those previously reported (AVRDC 1994), similarities were noted. For consistency, the bands identified as the same as those previously reported (AVRDC 1994) were given the same designations. These bands were 2, 3, 4, 5, 6, 8, and 9. Band 1 which was observed mostly in *C.*

[3] AVRDC. 1994. AVRDC 1993 Progress Report. Shanhua, Tainan, Taiwan.

[4] Cruz, V.M.V., Engle, L.M., Shieh, J.C. and Laude, R.P. 1994. Diversity in seed protein in the white-flowered *Capsicums*. Proceedings of the 7th International Congress of the Society for the Advancement of Breeding Researches in Asia and Oceania (SABRAO) and International Symposium of World Agriculture Association (WSAA). p. 621-629.

baccatum and *C. chacoense* and only in 1 out of 87 accessions of *C. annuum*, were not observed in this study. Neither was band 7 which was observed before only in two accessions. All bands observed in the previous study were noted except for the rare band 7. Band 1 which was predominant in *C. baccatum* and *C. chacoense* were not observed in this group of *C. annuum*. However, seven additional banding patterns were observed. As in the previous study, this population of *C. annuum* was monomorphic for bands 2, 8 and 9. Polymorphism for bands 4, 5, and 6 which was not observed before was observed this time.

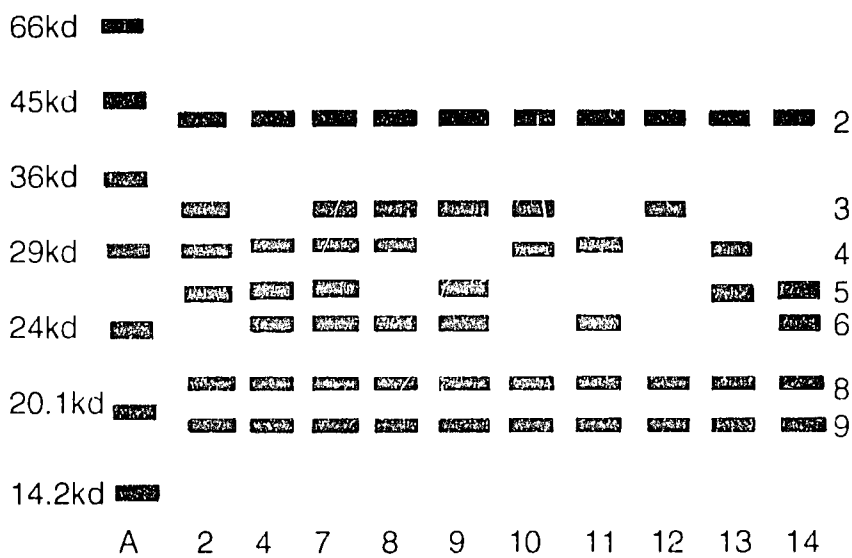


Fig. 1. Diagrammatic representation of the seed protein banding patterns observed in different varieties of *C. annuum* (A is Dalton Mark VII)

Table 10 shows the banding patterns observed and their frequencies. The most frequent banding pattern that was observed was banding pattern 8 which occurred in 51% or more than half of the total number of accessions. There were some banding patterns which rarely appeared, and these were banding pattern 14 which was observed in a single accession from India and banding pattern 2 in two accessions (one from Belize and another from the Philippines). The other banding patterns were fairly distributed throughout the other remaining accessions.

Among the seven band members, bands 2, 8, and 9 were seen in all of the accessions. Therefore, *C. annuum* was monomorphic for each of these three bands. Bands 3, 4, 5, and 6 appeared at different frequencies (table 11). Bands 4 and 6 were the most frequent of the variable bands. Band 3 was observed in 76% and band 5 in only 26% of the accessions. Band 1 was predominant in a different species *C. baccatum* (AVRDC 1994; Cruz 1994). Band 7 was a very rare band observed only in 2% of the accessions previously studied.

Table 10. Number and percent[a] of accessions exhibiting a specific banding pattern in *C. annuum*

Origin	No. of accessions	Banding pattern 2	4	7	8	9	10	11	12	13	14
South America	7		2 (28)	3 (43)	2 (28)						
Central America and West Indies	55	1 (2)	2 (4)	3 (5)	17 (31)	8 (14)		11 (20)	12 (22)	1 (2)	
North America	14				7 (50)	3 (21)		3 (21)	1 (7)		
East Asia	16		5 (31)	5 (31)	8 (3)			3 (18)			
Southeast Asia	11	1 (9)			6 (54)	1 (9)	2 (18)	1 (50)			
South Asia	14		3 (21)		9 (64)	1 (7)					1 (7)
Southwest Asia	11		1 (9)		8 (73)		1 (9)	1 (9)			
Balkan States	8			1 (12)	1 (12)		3 (38)	1 (12)		2 (25)	
West and Central Europe	52		4 (8)	1 (2)	40 (77)			2 (4)	2 (4)	3 (6)	
Africa	18		1 (6)		12 (67)	2 (11)	2 (11)		1 6)		
Unknown	2				2 (100)						
Total	208	2	18	13	107	14	5	24	17	7	1
(%)		(1.0)	(8.6)	(6.2)	(51.4)	(6.7)	(2.4)	(11.5)	(8.2)	(3.4)	(0.5)

[a] Figures in parenthesis are in percent

Geographic distribution of banding patterns

The most number of banding patterns (8) was observed in the Central American region (table 10). Diversity in terms of banding pattern was ranked as Central America > Western and Central Europe > Southeast Asia = Balkan States = Africa > North America = East Asia = South Asia = Southwest Asia > South America.

In many of the regions, a specific band predominated (table 11). However, in Central America and the West Indies, the four variable bands occurred in almost the same frequency (42-71%). In West and Central Europe, the Balkan States and North America, band 4 predominated, in South America band 5, in East Asia, Africa and South Asia band 6, in Southeast Asia bands 3 and 4, and in Southwest Asia bands 4 and 6. It is suggested that Central America is the center of diversity for seed proteins. Secondary centers of diversity for seed proteins exist in West and Central Europe, the Balkan States, Southeast Asia, and Africa.

Table 11. Geographic distribution of variable seed protein bands in *C. annuum*

Origin	No. of accessions	Band number 3	4	5	6
South America	7	5	5	7	5
		(71)[a]	(71)	(100)	(71)
Central America and W. Indies	55	35	35	23	39
		(64)	(64)	(42)	(71)
North America	14	11	12	1	11
		(79)	(86)	(7)	(79)
East Asia	16	8	13	13	16
		(50)	(81)	(81)	(100)
Southeast Asia	11	10	10	2	8
		(91)	(91)	(18)	(73)
South Asia	14	10	12	5	14
		(71)	(86)	(36)	(100)
Southwest Asia	11	9	10	1	10
		(82)	(91)	(9)	(91)
Balkan States	8	4	7	3	3
		(50)	(88)	(38)	(38)
Western and Central Europe	52	43	50	8	47
		(83)	(96)	(15)	(90)
Africa	18	14	16	4	17
		(78)	(89)	(22)	(94)
Unknown	2			2	(100)
Total	208	158	176	55	177
(%)		(76)	(85)	(26)	(86)

[a] Figures in parenthesis are in percent

Similarity index

Similarity index values were ranked as Southwest Asia > Western and Central Europe > South America > West Indies > East Asia > Africa > North America > Central America > South and Southeast Asia > Balkan States. Central America and South and Southeast Asia exhibited the lowest similarity index value.

Central America has a higher average compared to South and Southeast Asia and the Balkan States but lower when compared to the Western and Central European region. However, Central America with South and Southeast Asia exhibited the lowest similarity index value of 42.9. This confirmed the high degree of diversity in seed protein that exists in Central America and South and Southeast Asia.

Similarity index of accessions with different flower morphology

The varieties of *C. annuum* with inserted stigma projected a 100% similarity index value. These accessions exhibited a common banding pattern (11). The varieties with the same level stigma and anther had an average similarity index value of 85%. The band patterns that were present in these accessions were banding patterns 4, 7, 8, 9, and 12. On the other hand, the varieties with exerted stigma projected a slightly lower average similarity index value (82.9). The band patterns that were present

in these accessions were banding patterns 4, 5, 8, and 9. This suggested differences in the genetic composition of the three populationss. It may also reflect the degree of genetic homogeneity of the populations. The population of selfers was genetically homogeneous, exhibiting only one kind of banding pattern. Breeding behavior may influence diversity in seed proteins.

Similarity index of variants of the same accession

When an accession is planted, distinct types may be detected. This may be due to mixtures or inherent heterogeneity of the population. At AVRDC, the variants are separated and given sub-accession numbers (the original accession number followed by letters A,B,C...etc.). Majority of the variants of the same accession exhibited the same banding pattern showing that they were genetically similar at least for seed proteins. Out of 20 pairs compared, 17 exhibited 100% similarity index values. However, the remaining variants had similarity index values ranging from 71.4 to 85.7%. The result of this experiment should be correlated with other data such as the morphological characteristics of the plants to see the significance of this observation.

Seed protein banding pattern was associated with morphological diversity.

Conclusions and Recommendations

This study confirms the usefulness of SDS-PAGE on seed protein in the study of genetic diversity in *Capsicum*, confirming identity of species, variation within accessions, and possibly provides additional criteria in the identification of varieties.

Bands and banding patterns are associated with geographic distribution.

Diversity in seed protein varies with geographic distribution and breeding behavior.

It is recommended that seed protein patterns of incoming seeds be taken to provide a reference file that can be used in confirming identity of accessions.

Varietal Effect of Ultra-drying Seeds on the Storage Life of Soybean

Summary

Equilibrium humidity for ultra-drying in 10 soybean varieties differing in seed characteristics was determined using 10 saturated salt solutions at 25°C for 20 days. All seeds reached less than 5% moisture content (MC) at 11% RH. There was no apparent association between seed characteristics and seed MC under equilibrium humidity conditions.

To study the effect of storage, 10 soybean varieties were dried to different MC: 8.5 to 9.0 (OD or ordinary-dried, 5.1 to 5.6 (HD or high-dried) and 4.0% (UD or ultra-dried). Comparing OD and HD, there were differences in percent germination among cultivars and between results of standard germination test (SGT) and the accelerated aging test (AAT). Percent germination was monitored over a period of 24 months of storage at 25°C. Accessions with black seed coats showed high percent germination after 0 and 3 months' storage but low germination (AAT) after 6 and 9 months' storage. In general, peroxide value, total phospholipid content and acid value differed among cultivars, between drying conditions and among storage periods. However, the values were not correlated with percent germination (SGT or AAT).

The effects of silica gel:seed ratio (1:1, 2:1 and 3:1), seed size (26.5 and 11.5 g/100 seeds), kind of container (iron screen and cheese cloth) and seed amount on the drying of seeds were determined. Seed MC was reduced to about 4% from 8.7% after 47 days of drying. Higher ratio of silica gel:seed (3:1 and 2:1) resulted in lower MC than the lower ratio (1:1) until 28 days of drying, but did not show differences after 43 days of drying. Large seeded soybean seeds dried faster than small seeded ones. Kind of container and seed amount per container did not affect percent germination but seed sizes did.

To compare storage life of OD and UD seeds, seven cultivars that differed in 100-seed weight were dried using silica gel at 23±1°C. Percent reduced seed weight was not correlated with 100-seed weight and original seed MC. After 76 days of drying, seed MC reached lower than 3.5%. After 76 days, seed MC was highly correlated with original seed MC (r = 0.961), but not with 100-seed weight.

Introduction

Storage life of seeds depends on moisture content of seed and storage temperature. Low seed MC and low storage temperature increase storage life of seeds. However, maintenance of low seed MC under airtight conditions is more cost-effective than maintenance of low temperature and low humidity storage conditions. Recently, the drying of seeds to very low MC (<5%) has been found to increase longevity. Such ultra-drying technique is especially effective in seeds with high oil content. Therefore, it is possible to employ this method to increase storage life of soybean seeds at low cost. However, this technique has been tested only on a limited number of soybean cultivars. Varietal differences in seed size and seed coat characteristics are known to affect longevity of soybean seeds. These seed characteristics of soybeans may influence the life of ultra-dried seeds in storage and could also be used to devise a strategy for monitoring seed viability in the genebank.

Materials and Methods

Experiment 1

Ten soybean cultivars differing in seed size and seedcoat characteristics were sown for seed multiplication on 22 September 1992 and harvested in December 1992 and January 1993. To determine equilibrium humidity for ultra-drying, seeds of 10 soybean cultivars were equilibrated over 10 saturated salt solutions at 25°C for 20 days. To prepare ultra-dried seeds (<5% MC) for the storage experiment, soybean seeds were dried using silica gel (1:1 gel to seed ratio) at 15°C and a drying chamber (15°C, 15% RH). High-dried (5:1 to 5-6% MC) and ordinary-dried (OD) (8.5 to 9.0% MC) seeds obtained were kept in sealed, laminated aluminum bags and stored at 25°C.

Percent germination using standard germination test, seed vigor using accelerated aging test, and total phospholipid content at 0, 3, 6, and 9 months after storage were determined continuously at 3-month intervals during the 24-month storage period. Before the germination test, HD seeds were premoistened in a sealed desiccator over a saturated solution of $CaCl_2$ for 48 h under 20°C, and then transferred in another one with NH_4Cl saturated solution for 24 h under 20°C.

SGT using rolled towel was conducted according to the ISTA rules (1985) using four replicates of 50 seeds each. Acceleration of aging was conducted under 41°C for 72 h with Precision Scientific's circulating waterbath, model 270. After accelerated aging SGT was conducted. For analysis of peroxide value, 200 mg soybean powder was extracted with 10 ml n-hexane three times. Two-milliliter extracts, 2 ml 30% ammonium thispanate and 2 ml 20 mM $FeCl_3$ (dissolved in HCl) were mixed for 3 min, and absorbance measured at 500 mm. Total phospholipid was determined by the colorimetric method described by Ueda, Hayashi, and Namiki (1986).

The results were analyzed statistically using a PC-SAS package. Seed MC was determined by low drying oven method (ISTA 1985).

Experiment 2

To develop ultra-drying methods using silica gel, the effects of silica gel to seed ratios (1:1, 2:1, and 3:1 in w/w), seed sizes (11.5 and 26.5 g per 100-seed weight), kinds of container (iron screen and cheese cloth), and seed amount per container on the drying of seeds were determined at 23±1°C. For each silica gel to seed ratio, three different desiccators (representing replicates) were used. Relative humidity was checked everyday using a Barigo's precision-hygrometer-supratherm (0-100% RH). When a less than 0.1% change in RH was noted, silica gel was replaced by the same amount of dry silica gel. Seed weight was checked every time silica gel was replaced.

To prepare experimental materials for comparison of storage life between OD and UD seeds, seven cultivars differing in 100-seed weight [which were stored 4 years at short-term storage condition (20°C, 45% RH)] were dried using silica gel.

Results and Discussion

Experiment 1

To determine equilibrium humidity for ultra-drying, soybean seeds were equilibrated over 10 saturated salt solutions at 25°C for 20 days. The results are shown in table 12. All seeds of 10 soybean accessions reached less than 5% seed MC (ultra-dried condition) of 11% RH. At 7% RH, MC of G 11238 (brown seedcoat, cracked surface and 20.6 g/100 seeds) reached 2.4%, but that of G 11121 (brown seedcoat, entire surface, and 19.8 g/100 seeds) was 3.5%. Although much variation among accessions was found, there was no apparent trend between seed characteristics and seed MC under certain relative humidity condition.

With the use of silica gel (silica gel to seed ratio 1:1), seed MC of soybean seeds was reduced from 8.5-9.0% to about 5.5-6.0%. Additional drying in a chamber at 15°C and 15% RH for 2 months reduced it further to only 5.1-5.6%. Therefore, UD seeds could not be obtained in this manner.

Table 12. Seed moisture content of 10 soybean accessions under equilibrium humidity at 25°C for 20 days

Cultivars	Seedcoat characteristics/ Color/Surface	100-seed weight (g)	Equilibrium humidity (%) 7	8	11	22	31	43	52	64	74	81
AGS 191	Yellow/Entire	29.4	3.6	3.9	4.4	5.6	6.5	7.7	9.0	11.3	15.3	19.1
AGS 2	Yellow/Entire	5.9	3.0	3.4	4.3	5.9	6.9	7.9	9.3	11.5	15.6	19.1
G 11120	Black/Entire	14.8	2.8	3.5	4.3	5.7	6.5	7.7	9.0	11.2	15.1	18.0
G 08465	Black/Entire	7.8	3.4	3.6	4.8	6.0	6.8	7.9	9.1	11.0	13.2	15.8
G 11121	Brown/Entire	19.8	3.5	3.8	4.7	5.9	6.9	8.0	9.2	11.4	15.6	19.1
G 8611	Brown/Entire	8.1	2.9	3.3	5.0	6.1	6.8	7.6	9.0	11.1	15.3	18.1
G 8513	Green/Entire	18.1	3.4	4.0	4.5	5.9	6.9	7.8	9.4	11.7	16.0	18.5
G 8586	Green/Entire	11.1	3.0	3.4	4.6	6.0	7.0	7.4	8.9	11.6	15.2	18.7
G 10963	Black/Crack	20.6	3.2	3.7	4.7	6.0	6.9	7.6	9.3	11.9	16.0	19.8
G 11238	Brown/Crack	22.4	2.4	3.2	4.0	5.4	6.4	7.2	8.9	11.4	15.3	18.7
Mean		15.8	3.1	3.6	4.5	5.9	6.8	7.7	9.1	11.4	15.3	18.5

The results of combined analysis of percentage germination of soybean seeds by cultivars, drying conditions, and germination tests during storage are shown in table 13. There were differences in percent germination among cultivars between germination tests observed at 0, 3, 6, and 9 months of storag Also, interactions between cultivar x drying condition, cultivar x germination test, cultivar x drying condition x germination test were observed at 0, 3, 6, and 9-month storage. No constant trend was observed between drying conditions and interaction between drying condition x germination test. In SGT, black seed-coated G 11120 and G 8465 showed high percent germination, and green seed-coated G 8513 and brown and defective seed-coated G 11238 showed low percent germination. Difference between HD and OD was not present in SGT. Also, percent germination decreased from 99.1% at 3-month storage to 98.1% at 9-month storage. In AAT for determination of seed vigor, black seed-coated G 11120 and brown seed-coated G 11121 showed high vigor, but brown seed-coated G 8611 showed very low seed vigor. Black seed-coated G 8465 showed high seed vigor at 0 and 3-month storage. Seed vigor decreased very fast from 6 months' storage. Difference in germination between OD and HD was observed in AAT. Brown seed-coated G 11121 showed the narrowest difference in percent germination between SGT and AAT.

Correlation between 100-seed weight and PG (table 14) was not statistically significant, except in SGT of OD seeds at 3-month storage (r = -0.708*), AAT of OD seeds at 6-month storage (r = 0.670*), and AAT of UD seeds at 0 month storage (r = 0.831**).

Table 13. Combined analysis of percentage germination of soybean seeds by cultivars, drying conditions and germination tests during 0, 3, 6, and 9 months' storage under 25°C

Source of variance		MS by storage period (months)			
	DF	0	3	6	9
Cultivar (a)	9(8)[a]	1,732.23**	27.56**	403.80**	289.54**
Drying condition (b)	1	1,704.34**	5.63	616.23**	6.67
Germination test (c)	1	3,645.57**	140.63**	6,734.03**	7,700.06**
a x b	9(8)	237.95**	18.29**	190.34**	236.89**
a x c	9(8)	1,616.82**	26.74**	410.03**	365.97**
b x c	1	90.32	1.23	664.23**	21.01
a x b x c	9(8)	734.42**	14.67**	181.89**	170.10**
Error	120(108)	91.80	4.29	18.69	17.24

[a] Numbers in the parenthesis are df at 9 months after storage; the df of error at 0 month storage is 118
**Statistically significant at the 1% level

Table 14. Correlation coefficients between 100-seed weight and percentage germination by drying conditions and germination tests during 0, 3, 6, and 9 months storage under 25°C

Drying condition	Germination[a] test	Storage period (months) 0	3	6	9
Ordinary	SGT	-0.370	-0.708*	-0.270	0.047
	AAT	0.349	0.179	0.670*	0.471
Ultra	SGT	-0.378	-0.590	-0.319	-0.358
	AAT	0.831**	0.118	0.425	-0.169

[a] SGT = standard germination test; AAT = accelerated aging test
*, ** Statistically significant at the 5 and 1% level, respectively

The results of combined analysis for peroxide value (POV), total phospholipid content (TPL), and acid value (AV) by cultivars, drying conditions, and storage periods are shown in table 15. There were highly significant differences in POV between storage periods, but none between cultivars and drying conditions. POV at O.D. 500 were 0.68, 0.64, 0.81, 0.79 at 0-, 3-, 6-, and 9-month storage, respectively. TPL differed significantly between cultivars, drying conditions, and storage periods. Mean of TPL for the cultivars ranged from 14.6 to 17.2 mg/g over the storage periods. OD seeds had lower (15.9 mg/g) TPL than ultra-dried seeds (15.7 mg/g). TPL were 15.9, 16.3, 15.6, and 15.4 mg/g at 0-, 3-, 6-, and 9-month storage, respectively.

Table 15. Combined analysis of peroxide value, total phospholipid content, and acid value by cultivars, drying conditions, and storage periods

Source of variance	DF	MS		
		Peroxide value	Total phospholipid	Acid value
Cultivar	9	0.005	3.647**	0.054**
Drying condition	1	0.010	1.038**	0.515**
Storage period	3(1)[a]	0.135**	3.022**	0.896**
Error	65(27)	0.004	0.144	0.013

[a] Numbers in parenthesis are df for the acid value
** Statistically significant at the 1% level

Highly significant differences in AV between cultivars, drying conditions, and storage periods were observed. Mean of AV for the cultivars ranged from 0.35 to 0.72 mg KOH/100 g fat during 6- and 9-month storage. OD seeds had higher (0.70 mg KOH/g fat) AV than ultra-dried seeds (0.48 mg KOH/g fat). AV were 0.74 and 0.44 mg KOH/100 g fat at 6- and 9-month storage, respectively.

Storage life of OD and UD seeds of seven cultivars differing in 100-seed weight (8.1 to 34.9 g) was compared (table 16). The percentage of reduced seed weight by drying was largest in AGS 292 and smallest in GC 84056-1-2-2-1. The percentage of reduced seed weight was not correlated with 100-seed weight (r=0.159) and original seed moisture content (r=0.334). After completion of 76 days of drying, AGS 181 was highest and GC 84056-1-2-2-1 was lowest in seed MC. Also, seed MC after drying was highly correlated with original seed MC (r = 0.961), but not correlated with 100-seed weight (r = 0.556).

Table 16. Results of drying 7 soybean cultivars by silica gel (1:1) in desiccators for 76 days at 23±1°C

Cultivar	100-seed weight (g)	Original seed moisture content (%)	Percent of reduced seed weight	Dried moisture content (%)
IAC 80-4228	8.1	8.0	5.0c	3.3bc
GC 84056-1-2-2-1	9.9	7.9	4.9c	3.1c
AGS 181	15.9	8.5	5.3b	3.4a
GC 87017-40-B-2	20.7	8.0	4.9c	3.2bc
SS 86013-7-4	26.5	8.2	5.0c	3.4ab
G 9053	31.2	8.0	5.0c	3.2c
AGS 292	34.9	8.8	5.7a	3.3bc
Mean	21.0	8.2	5.1	3.3

* Means followed by the same letter are not significantly different by DMRT

Conclusion

The equilibrium humidity for ultra-drying soybean seeds at 25°C is 11% RH. The ultra-dried condition (<5% MC) could not be reached in the usual drying chamber with 15% RH, 15°C.

Percent germination during storage was affected by cultivar, drying condition, and the germination test used.

No correlation was observed between percent germination, and chemical components associated with seed determination during short storage periods.

Effect of Lipoxygenase Deficient Near-isogenic Lines on the Storage Properties of Soybeans

Summary

To determine the effect of lipoxygenase on the storage life of soybean seeds, two lipoxygenase-1 deficient near-isogenic lines and two parental cultivars were dried to about 7% seed moisture content and stored under room temperature and short-term storage (15°C, 45% RH) condition for 12 months. Percent germination (PG) using standard germination test and accelerated aging test and chemical components related to seed deterioration were determined at 2-month intervals during storage. PG of two lipoxygenase-1 deficient near-isogenic lines were intermediate of their two parents in SGT but lower than that of two parents in AAT.

There was no constant trend during storage between room temperature and short-term storages in PG. Peroxide value increased with longer storage period generally but there were no differences among cultivars and between storage conditions. Total phospholipid content decreased with longer storage periods but there were no differences among cultivars. Two lipoxygenase-1 deficient lines were intermediate of two parental cultivars in TPL. TPL was significantly positively correlated with PG (SGT) in general. There was no apparent trend in acid value during storage periods. However, there were differences observed among cultivars, but no differences between storage conditions. Two lipoxygenase-1 deficient near-isogenic lines had lower AV than their two parental cultivars. It is concluded that storage longevity and seed vigor of lipoxygenase-1 deficient near-isogenic lines were not higher than those of one parental cultivar AGS 129, and that TPL was not decreased by lipoxygenase-1 isozyme.

Introduction

Soybean seed is rich in oil that is high in polyunsaturated fatty acids. These unsaturated fatty acids are easily peroxidized under ambient condition. Lipid peroxidation, controlled by lipoxygenases, is suspected to be the main cause of seed deterioration of soybeans. It occurs during natural aging of soybeans, but not during accelerated aging. The rate of lipid peroxidation is affected by lipid composition, oxygen pressure, seed irradiation, antioxidant, and hydration treatments. In soybean seeds, lipid hydroperoxides are converted to volatile compounds, which develop beany flavors in soybean meal. Lipoxygenases normally exist in soybean seeds in three isozyme forms (Shibata et al. 1987 and 1988; Yenofsky et al. 1988). Null mutants have been found for each of the three isozymes (Davies and Nielsen 1987), and near-isogenic lines with deficiency in lipoxygenase-1 have been developed at AVRDC. Since near-isogenic lines likely have low lipid peroxidation, they are expected to stay viable for longer storage periods.

Materials and Methods

Two parental cultivars, PI 408251 (deficient for lipoxygenase-1, donor parent) and AGS 129 (with lipoxygenase-1, recurrent parent), and their backcross progenies, two lipoxygenase-1 deficient near-isogenic lines (GC 88067-10 and GC 88072-5), were sown on 1 March and harvested in June 1993. Harvested seeds were threshed and processed manually and dried to about 7% seed moisture content, packed in aluminum foil bags, and stored under natural room temperature and short-term storage (15°C, 45% RH) conditions. Standard germination test and accelerated aging test for seed vigor were done on stored seeds. Peroxide value, total phospholipid content, and acid value at time of storage and at 2-month intervals for a 12-month storage period were determined.

SGT was conducted according to the ISTA rules (1985) using four replicates of 50 seeds each. Acceleration of aging was conducted under 41°C for 72 h with Precision Scientific's Circulating Water Bath, model 270. After accelerated aging, SGT was done.

For analysis of peroxide value, 200 mg soybean powder was extracted with 10 ml n-hexane three times. Two milliliters each of the extract, 30% ammonium thispanate and 20 mM $FeCl_4$ (dissolved in HCl) were mixed for 3 min, and the absorbance was measured at 500 µm.

TPL values were determined by the colorimetric method described by Ueda, Hayashi, and Namiki (1986). AVs were determined by the AOAC official methods of analysis (1984). The results were analyzed statistically using a PC-SAS package.

Results and Discussion

The standard germination test showed differences in percent germination (PG) among cultivars (table 17). AGS 129 had the highest and PI 408251 the lowest PG; GC 88067-10 and GC 88072-5 were intermediate of both parents during all storage periods. The differences in PG between storage conditions showed no constant trend, but large differences between storage conditions were noted in AGS 129 during the storage period.

In the accelerated aging test, differences in PG were apparent among cultivars (table 18): PI 408251 had the highest, and GC 88067-10 and GC 88072-5 the lowest seed vigor. High PG of PI 408251 in AAT could be due to its black and hard seedcoat which protected it from over-imbibition during accelerated aging. Interactions between cultivar x storage condition, between cultivar x germination test, and between storage condition x germination test and between cultivar x storage condition x germination test were observed (table 19). From the results of SGT and AAT, we can conclude that lipoxygenase-1 deficient near-isogenic lines do not have longer storage life than their parent AGS 129.

Peroxide value increased with longer storage period except at 12-month storage (table 20), but no differences between cultivars and between storage conditions were observed. Correlation coefficient of POV with PG of SGT and AAT was not statistically significant. It can be concluded that lipoxygenase-1 deficient near-isogenic lines do not affect lipid peroxidation.

Total phospholipid content decreased with longer storage period (table 21). Also, differences in TPL were observed among cultivars. AGS 129 had the highest and PI 408251 the lowest TPL; GC 88067-10 and GC 88072-5 were intermediate. There were no differences between storage periods. TPL was significantly positively correlated with PG of SGT except at 2 months' storage, but TPL was not significantly correlated with PG of AAT.

Acid value was not constant during storage (table 22). Differences among cultivars were observed. AGS 129 had the lowest and PI 408251 the highest AV; GC 88067-10 and GC 88072-5 were intermediate. There were no differences between storage periods. AV was not correlated with PG of SGT and AAT except in two cases.

Table 17. Percent germination of soybean seeds by standard germination test[a] with 2 storage conditions

Cultivar	Storage condition[b]	Storage period (months) 0	2	4	6	8	10	12	Mean
PI 408251	R	79.0	20.0	27.7	6.0	66.5	68.5	62.5	41.9
	S	-	36.0	24.0	14.0	80.5	69.5	75.0	49.8
	Mean	79.0b[c]	28.0b	25.9b	10.0c	73.5c	69.0c	68.8b	45.9
AGS 129	R	89.5	35.0	76.0	60.0	97.0	97.0	97.5	77.1
	S	-	66.5	42.1	30.5	86.0	84.5	82.5	65.4
	Mean	89.5ab	50.8a	59.1a	45.3a	91.5a	90.8a	90.0a	71.3
GC 88067-10	R	83.5	14.0	45.0	22.0	87.0	88.0	85.0	56.8
	S	-	53.0	65.0	23.0	85.5	71.0	84.0	63.6
	Mean	83.5ab	33.5b	55.0a	22.5b	86.3b	79.5b	84.5a	60.2
GC 88072-5	R	91.0	35.3	54.3	44.0	90.5	82.5	87.5	65.7
	S	-	37.0	58.8	27.0	88.0	87.0	88.5	64.4
	Mean	91.0a	36.2b	56.6a	35.5a	89.3ab	84.8b	88.0a	65.1
Mean	R	85.8	26.1	50.8	33.0	85.3	84.0	83.1	60.4
	S	-	48.1	47.5	23.6	85.0	78.0	82.5	60.8
	Mean of all	85.8	37.1	49.2	28.3	85.2	81.0	82.8	60.6

[a] Rolled paper towels were laid on the tray in the 0 to 6 months storage test and placed upright in an iron stand in the 8-12 months' storage test

[b] R = room temperature; S = short-term

[c] Means in vertical column followed by the same letters are not statistically significant at the 5% level (DMRT)

Table 18. Percent germination of soybean seeds by accelerated aging test[a] with 2 storage conditions

Cultivar	Storage condition[b]	Storage period (months) 0	2	4	6	8	10	12	Mean
PI 408251	R	53.5	34.5	19.0	12.0	42.0	35.0	36.5	29.8
	S	-	36.5	10.0	15.0	46.0	50.0	42.5	33.3
	Mean	53.5a[c]	35.5a	14.5a	13.5ab	44.0a	42.5a	39.5a	31.6
AGS 129	R	43.5	11.0	10.5	14.5	27.3	36.5	25.5	20.9
	S	-	13.0	22.5	29.5	33.5	44.0	35.0	29.6
	Mean	43.5ab	12.0b	16.5a	22.0a	30.4b	40.3ab	30.3b	25.3
GC 88067-10	R	18.0	16.0	4.0	3.0	12.0	17.0	14.0	11.0
	S	-	13.0	16.0	12.0	39.0	35.0	33.0	24.7
	Mean	18.0b	14.5b	10.0a	7.5b	25.5b	26.0c	23.5c	17.8
GC 88072-5	R	37.0	23.5	11.5	10.5	30.0	24.0	23.0	20.4
	S	-	15.0	12.0	13.0	28.0	36.5	24.0	21.4
	Mean	37.0ab	19.3b	11.8a	11.8ab	29.0b	30.3bc	23.5c	20.9
Mean	R	38.0	21.3	11.3	10.0	27.8	28.1	24.8	20.5
	S	-	19.4	15.1	17.4	36.6	41.4	33.6	27.3
	Mean of all	38.0	20.4	13.2	13.7	32.2	34.8	29.2	23.9

[a] Rolled paper towels were laid on the tray in the 0 to 6 months storage test and placed upright in an iron stand in the 8-12 months' storage test

[b] R = room temperature; S = short-term

[c] Means in vertical column followed by the same letters are not statistically significant at the 5% level (DMRT)

Table 19. Combined analysis of percent germination of soybean seeds by cultivars, storage conditions, and germination tests

Source of variance	DF	Mean squares by storage period (months) 0	2	4	6	8	10	12
Cultivar (a)	3	450	103	900**	1621**	70	475**	131*
Storage condition (b)	1	-	1808**	58	28	293**	210	272**
Germination test (c)	1	18241**	4186**	17152**	3570**	44785**	34225**	46010**
a x b	3	-	524**	343*	231	241**	120	143**
a x c	3	568**	1318**	936**	679**	974**	705**	1072**
b x c	1	-	1805**	272	1208**	328**	1482**	361**
a x b x c	3	-	168	584**	490**	294**	134	227**
Error	48[a]	166	83	116	163	27	68	34

[a] df of error at 0, 2, and 4 months were 24, 41, and 37, respectively

*, ** = statistically significant at the 5 and 1% level, respectively

Table 20. Changes in peroxide values by cultivars and storage conditions

Cultivar	Storage[a] condition	Storage period (months) 0	2	4	6	8	10	12	Mean[b]
PI 408251	R	0.6[c]	0.8	0.7	0.7	0.6	0.8	0.6	0.7
	S	-	0.6	0.6	0.6	0.8	0.9	0.6	0.7
	Mean	0.6	0.7	0.6	0.6	0.7	0.8	0.6	0.7
AGS 129	R	0.6	0.8	0.7	0.6	0.6	0.8	0.5	0.7
	S	-	0.6	0.6	0.7	0.8	0.7	0.6	0.7
	Mean	0.6	0.7	0.7	0.7	0.7	0.8	0.6	0.7
GC 88067-10	R	-	0.6	0.8	0.8	0.7	0.8	0.6	0.7
	S	-	0.6	0.7	0.6	0.8	0.8	0.5	0.7
	Mean	-	0.6	0.7	0.7	0.8	0.8	0.55	0.7
GC 88072-5	R	0.6	0.5	0.5	0.71	0.6	0.8	0.6	0.6
	S	-	0.7	0.6	0.7	0.6	0.8	0.6	0.7
	Mean	0.6	0.6	0.6	0.7	0.6	0.8	0.6	0.6
Mean	R	0.6	0.7	0.7	0.7	0.6	0.8	0.6	0.7
	S	-	0.6	0.6	0.7	0.7	0.8	0.6	0.7
	Mean of all	0.6	0.7b[d]	0.6b	0.7b	0.7b	0.8a	0.6c	0.7
Correlation with SGT[e]			-0.126ns[g]	0.287ns	0.016ns	0.074ns	-0.304ns	-0.182ns	
Correlation with AAT[f]		-0.183ns	-0.340ns	-0.124ns	0.235ns	-0.097ns	-0.560ns		

[a] R = room temperature; S = short-term

[b] Did not include 0 month data

[c] Numbers are peroxide value at O.D. 500

[d] Means in horizontal row followed by the same letters are not statistically significant at the 5% level (DMRT)

[e] Percent germination using standard germination test

[f] Percent germination using accelerated aging test

[g] Not significant at the 5% level

Table 21. Changes in total phospholipid content (mg/g) in soybean cultivars during storage

Cultivar	Storage[a] condition	Storage period (months) 0	2	4	6	8	10	12	Mean[b]
PI 408251	R	16.6[c]	16.3	16.0	16.0	15.9	15.7	14.9	15.8
	S	-	16.3	16.1	16.6	16.4	15.6	14.8	16.0
	Mean	16.6	16.3	16.1	16.3	16.2	15.7	14.9	15.9d[d]
AGS 129	R	17.7	18.1	17.8	17.6	18.4	17.1	16.8	17.6
	S	-	18.0	17.1	17.5	17.7	16.1	16.4	17.1
	Mean	17.7	18.1	17.5	17.6	18.1	16.6	16.6	17.4a
GC 88067-10	R	-	17.1	16.8	17.0	16.7	16.0	16.0	16.6
	S	-	17.3	17.2	17.2	17.1	16.4	16.3	16.9
	Mean	-	17.2	17.0	17.1	16.9	16.2	16.2	16.8c
GC 88072-5	R	17.5	17.8	17.2	17.4	17.2	16.6	16.2	17.1
	S	-	17.7	17.6	16.9	17.6	16.7	16.2	17.1
	Mean	17.5	17.8	17.4	17.2	17.4	16.7	16.2	17.1b
Mean	R	17.2	17.3	16.9	17.0	17.1	16.4	16.0	16.8
	S	-	17.3	17.0	17.1	17.2	16.2	15.9	16.8
	Mean of all	17.2	17.3	17.0	17.0	17.1	16.3	15.9	16.8
Correlation with SGT			0.488ns	0.931**	0.845**	0.869**	0.734*	0.881**	
Correlation with AAT			-0.843**	-0.107ns	0.291ns	-0.361ns	-0.225ns	-0.560ns	

[a] R = room temperature; S = short-term
[b] Did not include 0 month data
[c] Means in vertical column followed by the same letters are not statistically significant at the 5% level (DMRT)
[d] Percent germination using standard germination test
* Percentage germination using accelerated aging test
ns = not significant at the 5% level; *significant at the 5% level; **significant at the 1% level

Table 22. Changes in acid value (KOH/g fat) in soybeans during storage

Cultivar	Storage[a] condition	Storage period (months) 0	2	4	6	8	10	12	Mean[b]
PI 408251	R	0.90[c]	0.64	0.84	0.78	0.89	0.86	1.04	0.84
	S	-	0.63	0.95	0.76	0.99	0.77	1.04	0.86
	Mean	0.90	0.64	0.90	0.77	0.94	0.82	1.04	0.85a[c]
AGS 129	R	-	0.39	0.49	0.49	0.46	0.24	0.45	0.42
	S	-	0.50	0.54	0.50	0.60	0.42	0.49	0.51
	Mean	-	0.45	0.52	0.50	0.53	0.33	0.47	0.47b
GC 88067-10	R	-	0.45	0.60	0.60	0.43	0.38	0.48	0.49
	S	-	0.43	0.36	0.75	1.01	0.36	0.43	0.56
	Mean	-	0.44	0.48	0.68	0.72	0.37	0.46	0.52b
GC 88072-5	R	0.91	0.74	1.01	0.66	0.86	0.70	0.70	0.78
	S	-	0.88	0.56	0.54	0.93	0.65	0.69	0.71
	Mean	0.91	0.81	0.79	0.60	0.90	0.68	0.70	0.74a
Mean	R	0.91	0.56	0.74	0.63	0.66	0.55	0.67	0.63
	S	-	0.61	0.60	0.64	0.88	0.55	0.66	0.66
	Mean of all	0.91	0.59	0.67	0.64	0.77	0.55	0.67	0.65
Correlation with SGT[d]			-0.137ns[f]	-0.629ns	-0.687ns	-0.453ns	-0.633ns	-0.764*[g]	
Correlation with AAT[e]			0.381ns	-0.137ns	-0.350ns	-0.745*	0.197ns	-0.596ns	

[a] R = room temperature; S = short-term
[b] Did not include 0 month data
[c] Means in horizontal row followed by the same letters are not significant at the 5% level (DMRT)
[d] Percent germination using standard germination test
* Percent germination using accelerated aging test
[f] Not significant at the 5% level
[g] Statistically significant at the 5% level

Production Systems Program

Analytical Laboratory

Effects of Cooking on In Vitro Bioavailability of Iron in Mungbean

Summary

The effect of wet-heating on the in vitro bioavailability of iron in mungbean seeds and sprouts was examined. The dialyzability of mungbean seeds and sprouts was enhanced after cooking. L-ascorbic acid, 10 times the concentration of intrinsic iron, was added to meals before cooking. The iron dialyzability was increased by more than 20%. Addition of ascorbic acid and then cooking can enhance iron bioavilability more than using either one of these two treatments alone. Phytic acid, high in beans but low in sprouts, may not be very important to hinder the effect of cooking. The viscosity of bean meals is more than that of sprouts and does not prevent the enhancing effect either. At higher vitamin C levels (30-fold of intrinsic iron), the enhancing effect of cooking is not obvious. The iron dialyzability in the unheated control was, on the contrary, decreased – possibly by the partial oxidation of vitamin C due to heating. The heating process seemed to influence the profile of extrinsic iron in the food matrix; the higher the soluble iron, the higher the dialyzability.

Introduction

Iron deficiency anemia is commonly found in both affluent and economically deprived populations. To prevent this nutritional deficiency disease, an increase in both dietary iron and its availability is necessary. Food processing and iron chemistry are important factors affecting iron bioavailability. The chemistry of iron, particularly its valence, solubility, and types of chelation, influence its absorption. Food processing methods such as baking, canning, drying, and cooking may have different effects on iron bioavailability. Thus, the chemistry optimized processing method should be considered for obtaining the best possible iron bioavailability.

Current methodology for estimating iron bioavailability includes animal bioassay, human assay, and in vitro measurement. The in vitro assay involves simulated gastrointestinal digestion using a commercially available enzyme with subsequent measurement of the soluble iron released by the digestion. It is a simple, rapid and inexpensive method, and useful for food screening and identifying factors which may influence iron availability.

Recent studies at Rutgers State University of New Jersey indicated that in vitro bioavailability of iron in cruciferous vegetables can be enhanced from 5% up to over 20% through cooking. In this study, an attempt was made to confirm this finding in another type of vegetable. Mungbean seed and sprout forms were used as test materials. The effects of cooking on iron bioavailability in mungbean seeds containing extrinsic iron and ascorbic acid are discussed here.

Materials and Methods

Materials

Seeds of mungbean breeding line VC 1628A were used. To obtain sprouts, seeds were first soaked for 6 h (first sprouting stage) before they were placed in the sprouting machine for 4 days. The machine automatically supplied water to sprouting seeds every 6 h to allow them to germinate (second sprouting stage), and sprout completely (third stage).

In vitro determination of iron bioavailability

The simulated digestion method introduced by Miller et al.[1] (1981) with minor modifications by Lee[2] (1994) was used for the in vitro determination of iron bioavailability. The equation used for the calculation of the dialyzability is:

$$\text{Iron dialyzability (\%)} = \frac{\text{mg Fe in dialysate} \times 100\%}{\text{mg Fe in pepsin digest}}$$

Fig. 1 illustrates the details of this assay.

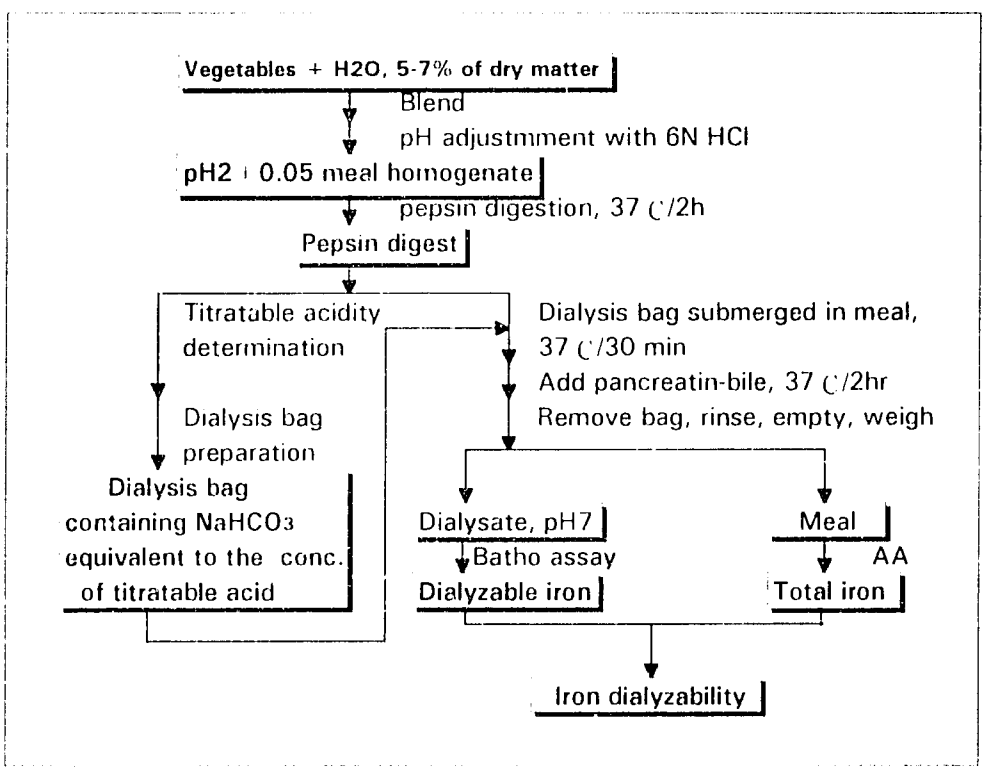


Fig. 1. Schematic diagram of the in vitro method used to determine iron bioavailability

[1] Miller, D.D. et al. 1981. An in vitro method for estimation of iron availability from meals. Am. J. Clin. Nutr. 34:2248-2256.

[2] Lee, T.C. et al. 1994. In vitro iron bioavailability of three raw and processed cruciferous vegetables. J. Food Sci.

Experiments

1. Iron, ascorbic acid and phytic acid content of the three sprouting stages were analyzed by atomic absorption, AOAC, and a method developed by Winters et al.[3] (1984), respectively.
2. The soaked, germinated, and sprouted mungbean were blended in deionized water with and without extrinsic vitamin C and boiled on a hot plate for 15 min with constant strirring. The dialyzability was determined by methods mentioned earlier.
3. The dialyzability of soaked seeds was determined before and after cooking with addition of ascorbic acid (3, 10, and 30 μM/20 g test meal).
4. The dialyzability of soaked seeds was determined before and after cooking with addition of 1, 3 and 7 μM ferric chloride/20 g test meal which contained 10 μM of ascorbic acid.

Results and Discussion

Iron content in seed and sprout was similar (about 2.5 μg). The ascorbic acid content increased gradually during the sprouting process from 0.39 mg/100 seeds to 5.32 mg/100 sprouts, while the phytic acid decreased from 50.95 mg/100 seeds to 1.18 mg/100 sprouts (fig. 2).

The dialyzabilities of cooked meals of the three stages were apparently higher than the raw ones, especially for sprouts (fig. 3). Although water, starch, protein, phytate and ascorbic acid in mungbean changed a lot from seed to sprout, there was only a slight difference in iron dialyzability. Cooking may have the potential to alter the iron profile in the food matrix and promote dialyzability. For cooked sprouts, intrinsic vitamin C is capable of enhancing iron dialyzability. When the amount of vitamin C added was 10 times the iron concentration before cooking, experimental results showed that the iron dialyzability increased from 9.0 to 25.4 and 5.1 to 21.2% for the first two stages (table 1). The dialyzability of sprouts remained at 18% regardless of the vitamin C treatment. This could be because of the high concentration of intrinsic ascorbic acid in the sprout, which vicitated any added benefit of extrinsic ascorbic acid.

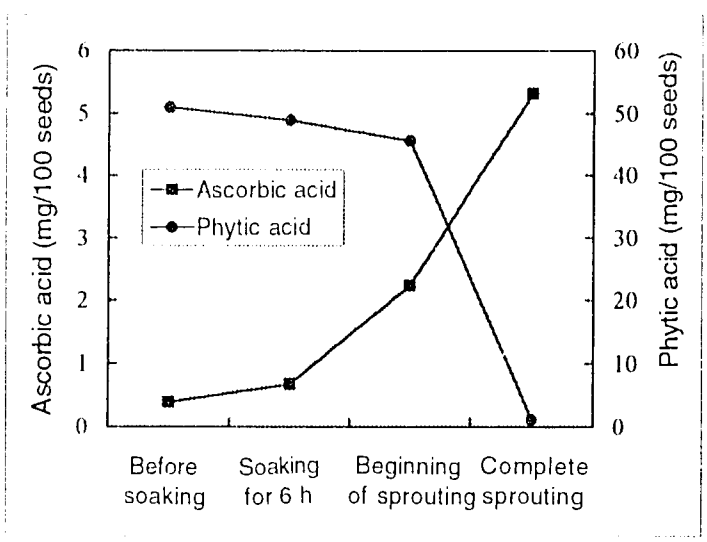


Fig. 2. Changes in ascorbic acid and phytic acid contents in mungbean during sprouting

[3] Winters, D.D. et al. 1984. Phytic acid content of crude, degummed and retail soybean oils and its effect on stability. J. Food Sci. 49(4):1113-1114.

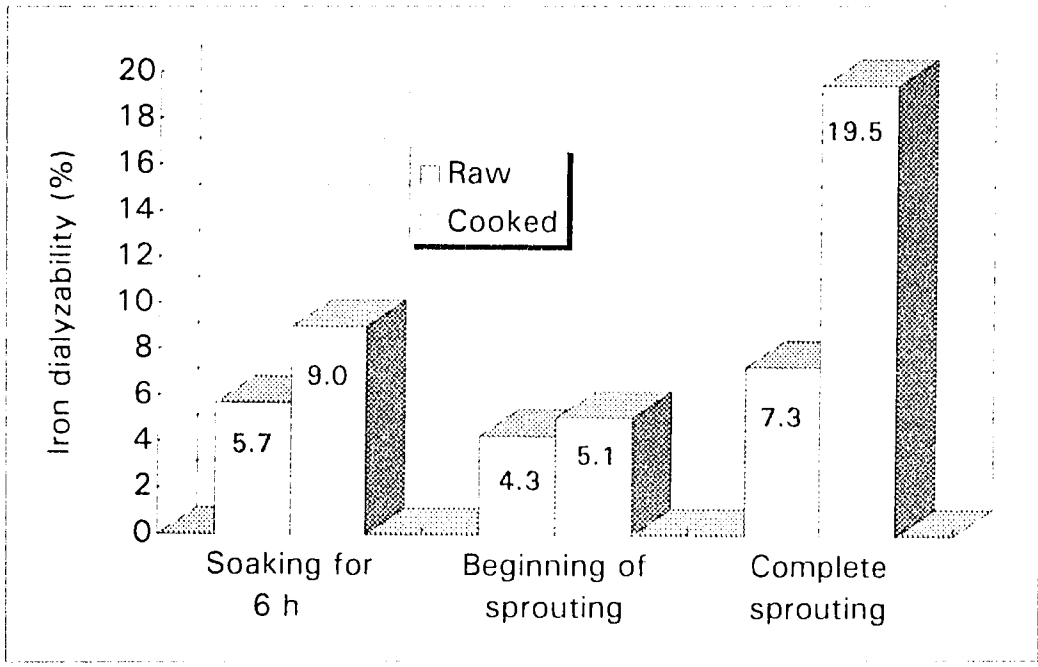


Fig. 3. Effect of cooking on in vitro iron bioavailability of mungbean during sprouting

Table 1. Effect of cooking and vitamin C addition on in vitro bioavailability of iron in mungbean during sprouting

	Iron dialyzability (%)					
	Soaking for 6 h		Beginning of sprouting		Complete sprouting	
	Raw	Cooked	Raw	Cooked	Raw	Cooked
Control	5.7	9.0	4.3	5.1	7.3	19.5
Extrinsic vit. C[b]	13.1	25.4	11.5	21.2	12.6	18.0

[a] Cooking condition: boiling for 15 min

[b] The amount of extrinsic vitamin C was 10 times the iron concentration in each sample

Addition of energy in the form of heat has been shown in several studies to increase iron bioavailability. Not all the heating enhances iron absorption; in some cases, heating was inhibitory. In this study, dialyzability increased due to the addition of ascorbic acid to beans even before cooking. In addition, high intrinsic starch content (over 70% in dry weight) did not prevent the enhancing effect of added vitamin C and heating. Phytic acid, normally an inhibitor, did not also seem to affect the enhancement of dialyzability.

The enhancing effects of cooking on iron dialyzability with extrinsic ascorbic acid and iron in mungbean meals were also investigated. L-ascorbic acid, in molar ratios of 3, 10, and 30, was added to mungbean meals before cooking. The results are shown in fig. 4. According to the previous data, cooking and addition of vitamin C could enhance dialyzability, and the enhancing effects can be amplified with both treatments. At higher vitamin C levels (over 30x) dialyzability was saturated (data not shown), and the enhancing effect of added vitamin C was greater than that of cooking. The dialyzability was, on the contrary, decreased possibly by the partial oxidation of ascorbic acid due to heating.

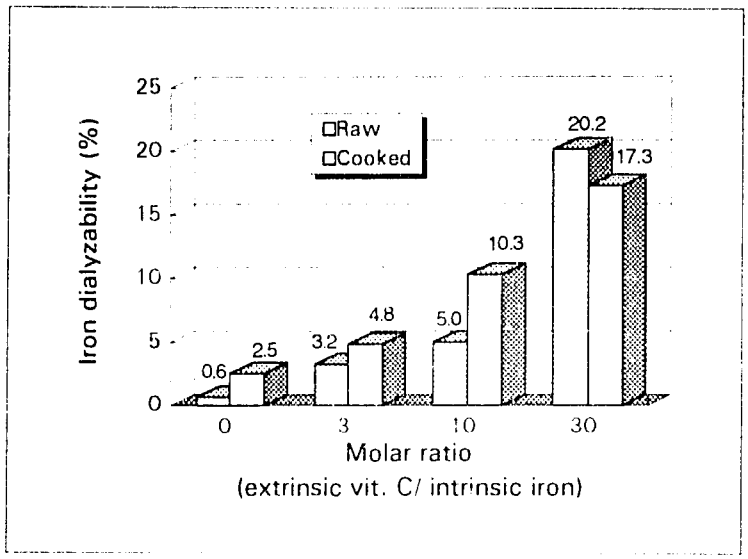


Fig. 4. Effect of cooking on in vitro bioavailability of iron in mungbean seeds with added vitamin C

Ferric chloride, 1, 3, and 7 μM, was added to test meals of mungbean seeds. In addition, 10 μM/ 20 g meal vitamin C, which represents 10 times the concentration of intrinsic iron, was also added. The variation of iron dialyzability is plotted in fig. 5. The percentage of dialyzable iron after cooking was more than before cooking. Heating seemed to influence the extrinsic iron profile. The difference between the values of raw and cooked meals, which varied from 10% to 2%, could be due to the addition of vitamin C at different molar ratios to iron (10:1, 5:1, 2.5:1, and 1.25:1).

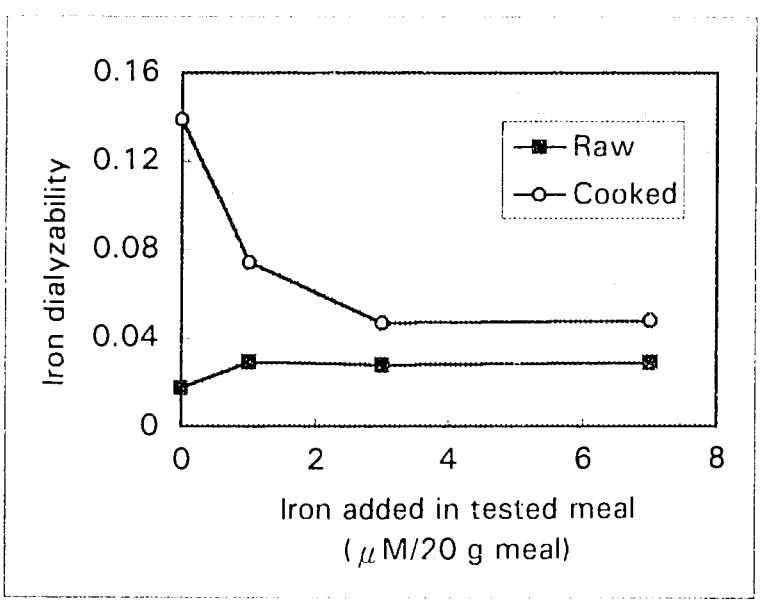


Fig. 5. Effect of cooking on in vitro bioavailability of extrinsic iron ($FeCl_3$) in mungbean meal with added ascorbic acid (10 μM/20 g meal)

Quality Assessment of Soybean by Near Infrared Reflectance Spectroscopy

Summary

An assessment system for processing quality of soybean was developed. This near infrared reflectance spectroscopy (NIRS)-based system is able to detect major quality indicators of soybean, such as tannin content, degree of lipid oxidation, degree of damage to cell membrane, harvest season, water absorption rate, and major compositions. Simulation studies demonstrated that this system is able to sort and identify soybean samples not suitable for tofu making. Validation results also suggested that this model could be useful as a mass-screening technique for quality evaluation of breeding materials.

Introduction

Many processed soyproducts are popular in the world, especially in Asia. Different processing procedures usually require specific seed properties. Processing quality, high protein recovery, and gel yield have been considered as desirable properties for making soymilk and tofu. Experimental results indicate that properties such as tannin content, water absorption, and degree of lipid oxidation and cell membrane damage affect processing quality of soybean.

This study tried to develop evaluation models for assessing the processing quality of soybean.

Materials and Methods

Sixty soybean varieties supplied by the soybean breeder were analyzed for tannin and protein contents; calibration curves of tannin and protein contents were further developed by near infrared reflectance spectroscopy. The vanillin-HCl method was used to determine tannin content in soybean seeds. Tannin content was measured as having an absorbance value of 500 nm. The micro-Kjeldahl method was used to determine the total nitrogen of soybean seed. Protein content was determined by multiplying nitrogen content by 6.25. Multiple regression analysis was used to develop the calibration curve of NIRS.

Twenty soybean varieties harvested in autumn were stored in the laboratory at room temperature for 1 year and used to investigate storage effect on processing quality. The protein recovery of soybean milk was expressed as the percentage of protein in milk per total protein in soybean seed. The tofu-like gels were prepared by heating 30 ml soybean milk and coagulant (2 mg of $CaSO_4 \cdot 2H_2O$) in the centrifuge tube. Tofu gel was developed by the centrifuge at 4000 rpm for 10 min. The weight retained after discarding the supernatant was expressed as gel yield. The principal component analysis was used to classify the processing quality of stored sample by NIRS.

The same 20 varieties harvested in three different seasons were used to study the effect of seasonal variation on processing quality. The canonical discriminant analysis (CDA) was used to group soybean samples from the three different seasons by NIRS.

An NIR System model 6500 was used in this study. The reflectance spectra, ranging from 400 to 2500 nm, were used for analysis.

Results and Discussion

Evaluation of tannin and protein contents of soybean by NIRS

Tannin is one of the major factors affecting the processing quality of soybean materials. Soybeans with high tannin content have poor protein recovery in soybean milk. Calibration curves of tannin content of soybean were developed in 1993, and showed accuracy with a high coefficient of regression determination ($R^2 = 0.89$) and validation value (prediction of standard error, SEP = 0.64).

Evaluation of protein content of soybean by NIRS

Thirty samples were selected for calibration and validation. The statistical results showed that the determination coefficient of regression (R^2) for calibration and prediction of standard error (SEP) was 0.99 and 0.21, respectively. These results showed that calibration curves of protein accurately predicted the protein content of soybean samples.

Evaluation of water absorption rate of soybean by NIRS

The presence of hard-to-cook seeds reduces processing quality due to lack of water uptake. Up to now, there is no method available to determine whether the hard-to-cook property is a varietal characteristic or results from long-term storage. The water absorption rate (WAR) of seeds was used to determine the hard-to-cook property of soybean. The NIRS technique with CDA was applied to group the samples with low WAR.

Sixty samples were classified into three groups: H (high), M (medium), and L (low) according to WAR. Reflectance of 16 wavelengths at NIR regions were selected for CDA analysis. The statistical results showed that 0.95 total accumulated variance of two synthesis variables were developed from CDA. One-third of groups H and M overlapped and obviously separated from group L (fig. 6). Twenty samples were used for validation. The results showed that this model is able to classify soybean with low WAR.

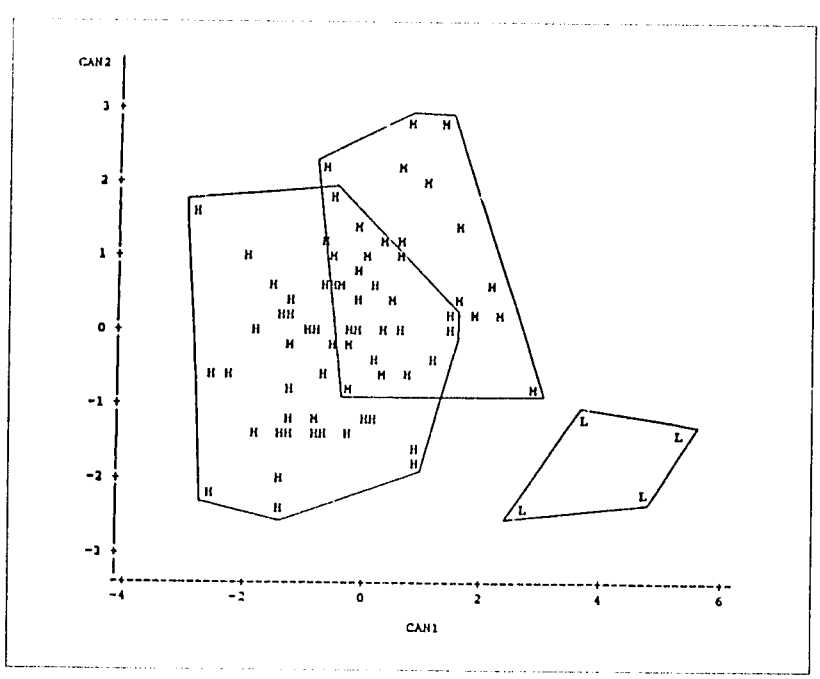


Fig. 6. Canonical discriminant analysis of water absorption rate of soybean

Evaluation of processing quality of stored soybean

Storage decreases not only the germination rate of soybean but also its processing quality. Poor processing quality of soybean seeds could result from the oxidation of lipid and the damage to the biological membrane. Principal component analysis (PCA) was applied to evaluate the degree of lipid oxidation in soybean seeds. Three varieties of soybean were stored at 30°C and 80% RH for 1 year. Reflectance of 16 wavelengths at NIR which are related to the lipid and phospholipid component were selected as variables for PCA. Fig. 7 shows that the representation of the two first PCA variables in two groups were quite different (left group: stored for less than 4 months; right group: stored for over 4 months) based on the storage time.

The other 20 samples, which were stored at room temperature for several years and had low germination rate, were selected for validation classification. Samples with poor processing quality, serious lipid oxidation, and cell membrane damage were classified into right group.

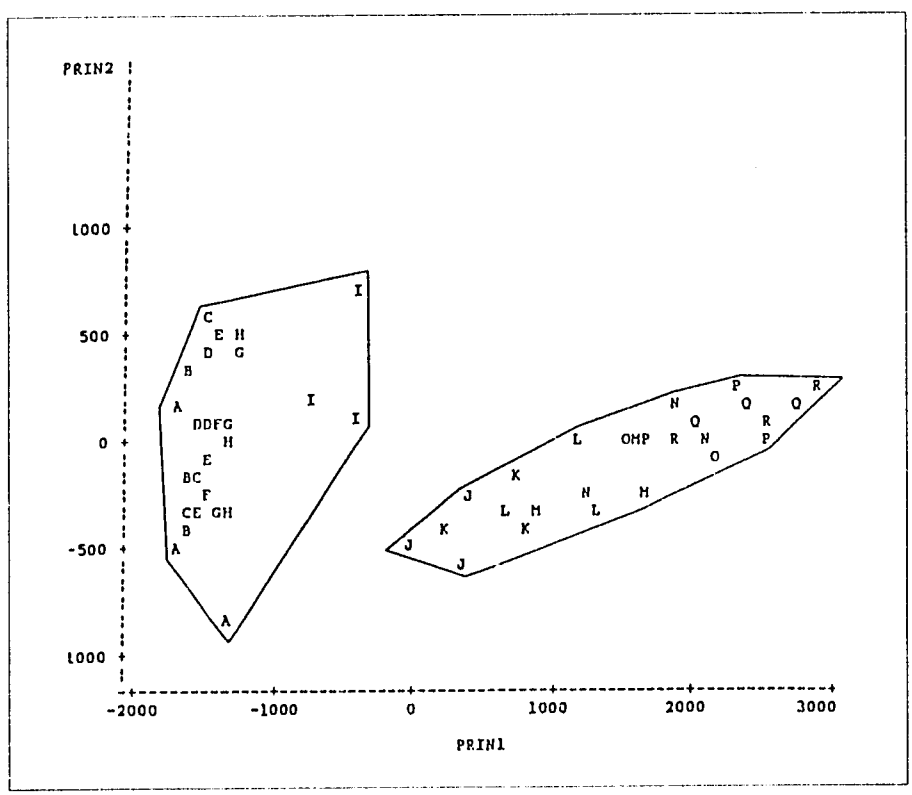


Fig. 7. Principal components of soybean during storage
Representation of the first two variables (1st and 2nd component)
A — R: storage time

Evaluation of processing quality of soybean harvested in three seasons

Soybean seeds harvested in three seasons (spring, summer, and autumn) varied in processing quality. Seeds of 20 varieties harvested in autumn showed superior quality over those in the other two seasons. The CDA with NIRS was applied to group soybean seeds from different seasons. Reflectance of 16 wavelengths at visible and NIR regions were selected as variables for CDA. A distinct separation of raw materials by harvesting seasons was achieved (0.95 total accumulated variance) (fig. 8). Results of the validation of the other 20 samples correctly classified seed properties into seasons.

Evaluation model for soybean quality

Five evaluation models for soybean quality were developed. Fig. 9 shows the procedure used for evaluating characteristics of unknown samples. High tannin content, low WAR, serious lipid oxidation, and membrane damage adversely affect coagulation of tofu gel. These properties should not be included. Soybean with high protein content and harvested in autumn has high protein recovery in soybean milk and high tofu yield. These are two desirable properties for tofu making and should, therefore, be selected. These evaluation models for soybean quality could be adopted as protocols for mass screening.

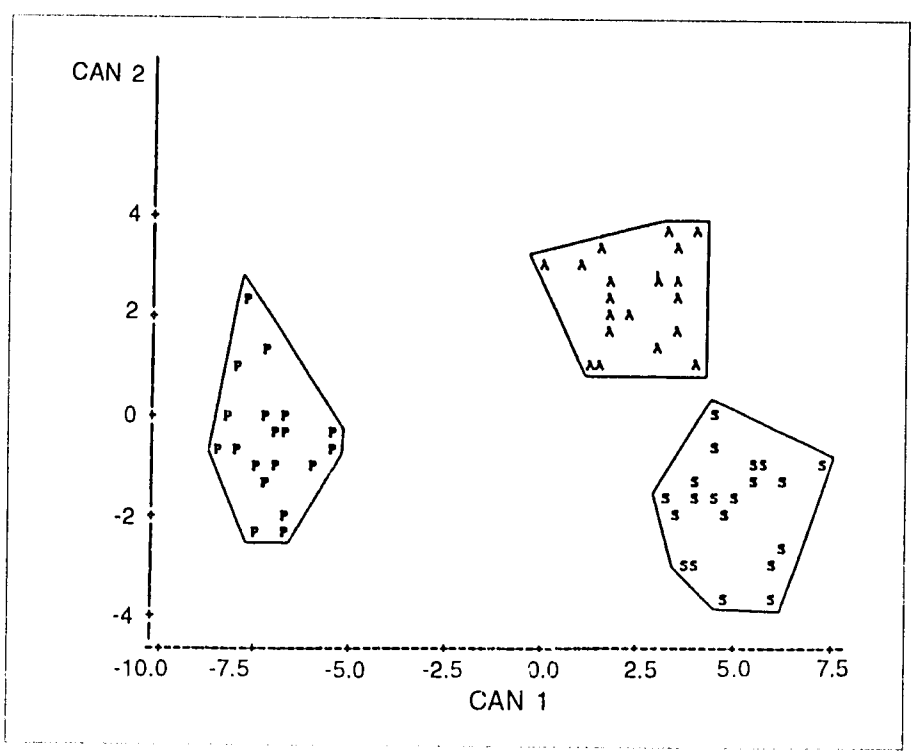


Fig. 8. Canonical discriminant analysis of soybean harvested in three seasons
Representation of the two discriminant variables; P = spring; A = autumn; S = summer

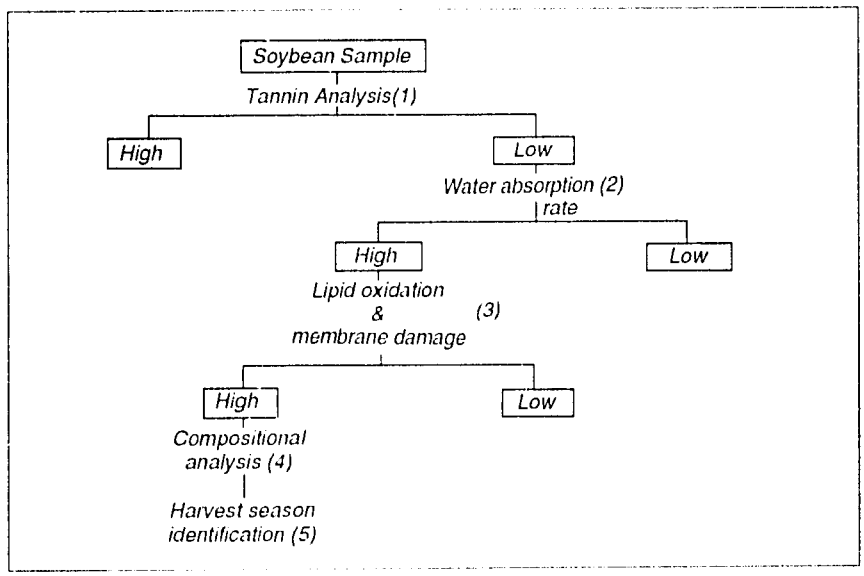


Fig. 9. Proposed assessment system of soybean processing quality by NIRS
1 = Multiple regression equation 4 = Multiple regression equation
2 = CDA model 5 = CDA model
3 = PCA model

Improving the Efficiency of Rotary Dehumidifier for Vegetable Dehydration

Summary

The rotary dehumidifier has been used to prepare samples of dehydrated vegetables for NIRS. It minimizes changes in composition and reduces the drying time while dehydrating vegetables under low temperature and low humidity, compared with the conventional method. Two-stage temperature dehydration (90°C, and 45°C, 10% RH) was adopted for shortening drying time. To reduce energy consumption for drying, a solar energy system was installed. The rotary dehumidifier with solar energy system saves 3.08 kw/h. Cabbage and mustard were dried in this dryer at two-stage temperatures (90°C, and 45°C, 10% RH). The quality of dehydrated vegetables was not significantly different between two- and one-stage temperature dehydration.

Introduction

Using a rotary dehumidifier for vegetable dehydration could shorten the drying time and improve the quality of dehydrated vegetables. It has potential application in the dehydration of leafy vegetables as ingredients of instant foods. The rotary dehumidifier operation needs improvement to reduce energy consumption and dehydration time.

At the initial stage during dehydration, the surface of the sample remains saturated with liquid water. Removing water from within the samples occurs at a rate as great as the rate of evaporation from the surface. Drying takes place by removing water vapor from the saturated surface through a stagnant air film into the main stream of the drying air. Drying rate is dependent on the rate of heat transfer

toward the drying surface. The rate of mass transfer balances the rate of heat transfer, and so the temperature of the drying surface remains constant. This minimizes damage to vegetable quality.

This study attempted to use a solar energy system in place of an electric heater in the rotary dehumidifier and to adopt two-stage drying temperatures for vegetable dehydration.

Materials and Methods

Dryer

The rotary dehumidifier connected to a solar energy system is shown in fig. 10. It was designed and constructed in cooperation with the Energy and Resources Laboratory, Industrial Technology Research Institute, Hsinchu, Taiwan. The energy consumption of a rotary dehumidifier with and without solar energy system was measured and compared. The dryer removes moisture from the samples with its rotary action. The moisture is dried with hot air (about 90-100°C). The relative humidity (RH) can be reduced to 10% at 45°C using this instrument.

Sample preparation of leafy vegetables

Cabbage and mustard were harvested in summer. The vegetables were cut into 10 mm^2 pieces and dehydrated at 90, 80, 70, and 45°C, 10% RH.

Quality analysis

The moisture content was determined by drying at 105°C in an oven by AOAC (Association of Official Analytical Chemists) method. The vegetable samples were extracted with water and the water extract was measured at 420 nm for the degree of browning. The 85% acetone was used to extract chlorophyll, and the extracts were measured at 660 and 642.5 nm. The absorbances of these two wavelengths were converted into total chlorophyll.

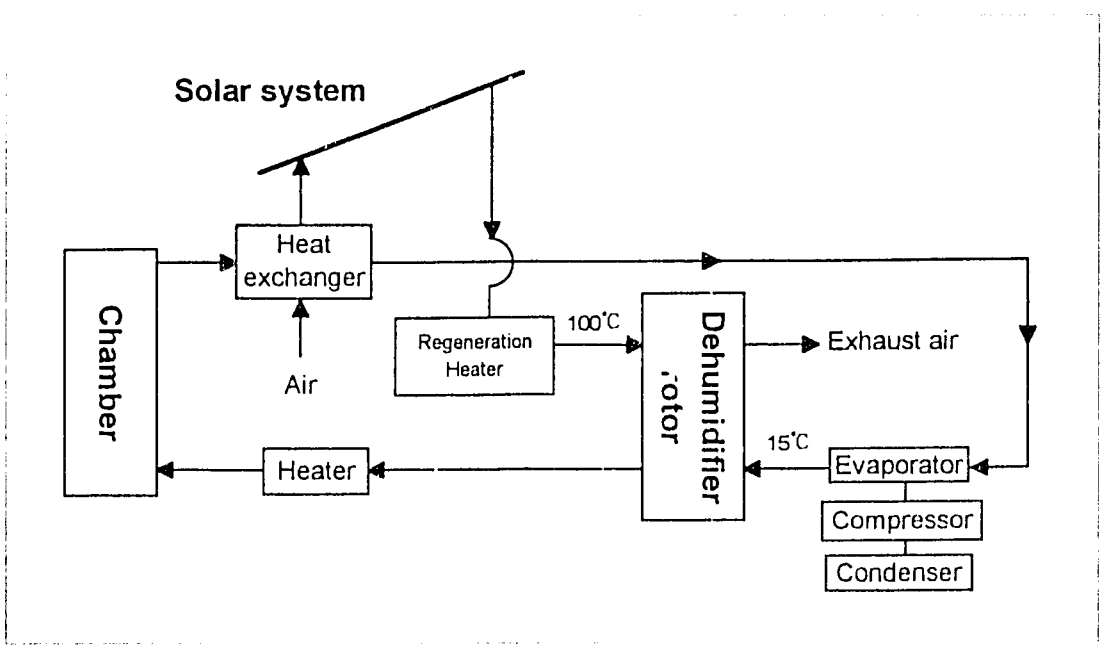


Fig. 10. Dehydration system of the rotary dehumidifier with solar regeneration

Results and Discussion

Energy consumption of rotary dehumidifier

The energy consumption using electricity to run the rotary dehumidifier was about 6.3 kw/h. To decrease the energy consumption, a solar energy system was connected to the rotary dehumidifier to substitute for the heater. Experimental results showed that with the solar energy system attachment, the electricity consumption was only 3.2 kw/h. This resulted in a savings of 3.08 kw/h of electricity during one typical drying operation.

Dehydration condition

Dehydration rates of cabbage and mustard dried at three temperatures (90, 70, and 45°C, 10% RH) are shown in fig. 11. Constant-rate stage of both cabbage and mustard was achieved at the moisture content level of 400-700 g H_2O/100 g dry matter. These results are for both vegetable samples.

Browning of cabbage and changes in chlorophyll of mustard during dehydration under various temperatures were compared (fig. 12). There was no significant difference in browning of cabbage within the 30% moisture content treatment under three-temperature dehydration. The chlorophyll of mustard degraded rapidly. The chlorophyll loss (below 15%) in mustard showed no significant difference between dehydrated and fresh samples. Thus, 90°C could be adopted as the temperature of the constant-rate stage for vegetable dehydration.

Dehydration by two-stage temperature

There was a significant difference in the dehydration time and energy consumption of drying cabbage using two-stage temperatures (90°C and 45°C, 10% RH) and one-stage temperature (45°C, 10% RH) (table 2).

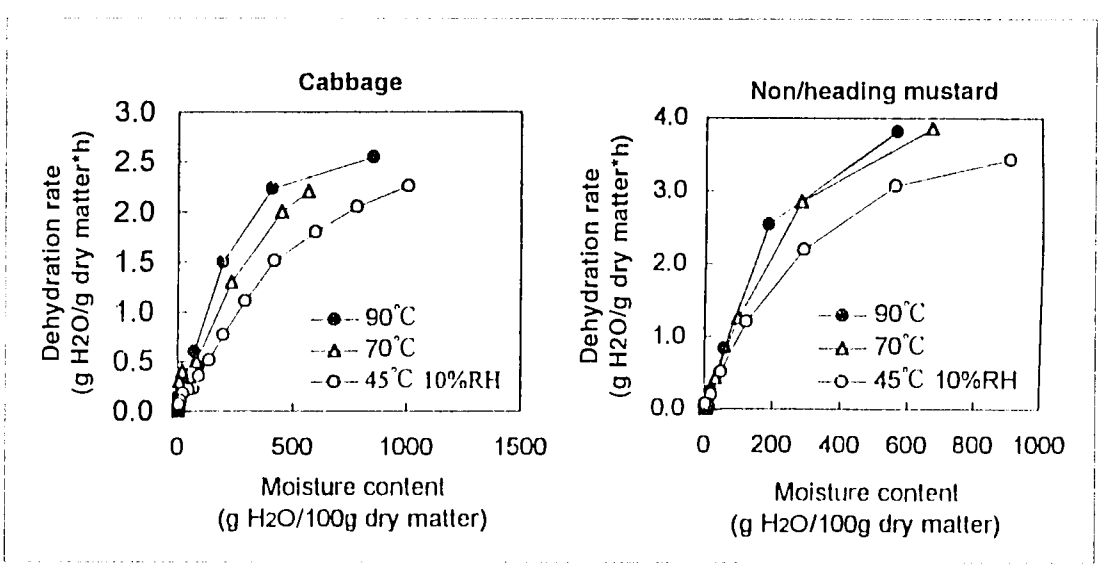


Fig. 11. Dehydration rates of cabbage and mustard under various dehydration conditions

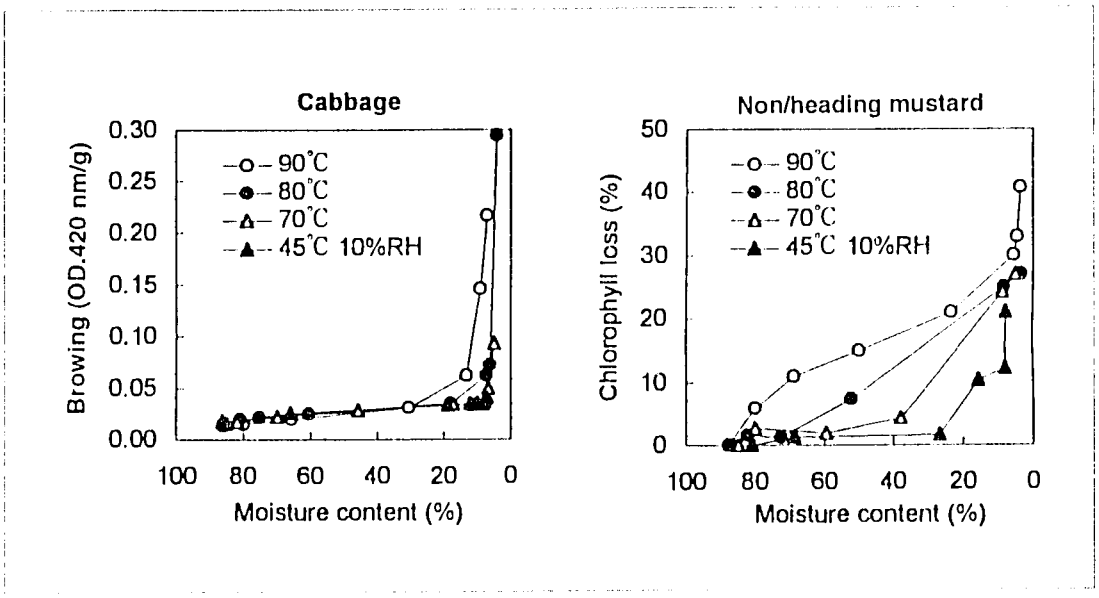


Fig. 12. Quality changes in cabbage and nonheading mustard under various dehydration conditions

Table 2. Quality of dehydrated cabbage and nonheading mustard, and energy consumption by various dehydration conditions

Vegetable	Dehydration condition						Energy
	Temperature (°C)	RH (%)	Time (h)	Moisture (%)	Browning (O.D./g)*	Chlorophyll (mg/100 g)	consumed (kw)
Cabbage	45	10	11	8.1	0.041 a	-	20.3
	90 & 45	10	9	6.0	0.047 a	-	16.7
Nonheading	45	10	6	6.4	-	184.9 a	12.3
mustard	90 & 45	10	5	6.0	-	170.3 a	10.1

* $O.D._{420}$ nm/g

The energy consumed in dehydrating cabbage was reduced to 16.7 kw by drying in two-stage temperatures for 9 h; it was 20.3 kw by one-stage drying for 11 h. The energy consumption for mustard drying was reduced to 10.1 kw/h by drying in two-stage temperatures for 5 h; one-stage temperature required 12.3 kw/h.

The degree of browning of cabbage dried by two- and one-stage temperature was not significantly different. Similar results were observed in chlorophyll loss for dehydrating mustard — no significant difference was found between the two dehydration conditions.

Vegetable samples dehydrated by two-stage temperatures not only save on energy but also reduce drying time with minimum quality loss. This technique could be applied in the vegetable dehydration industry.

Crop Management and Cropping Systems

Home Gardens

Summary

The productivity of three types of gardens — monoculture, intercrop, and aquatic — was compared over 2 years. The two latter types performed significantly better than the monoculture plots, and some aquatic species are potentially highly productive under waterlogged and even shaded conditions.

Introduction

In the past, AVRDC developed stereotype gardens designed to provide a significant proportion of the micronutrient requirements for a family of six in the tropics. This led to the development of a simple database, albeit incomplete, from which may be extracted information on expected yields and nutritional value of the harvested produce, in relation to the four seasons characteristic of Southern Taiwan.

Over the past 3 years, however, research focus at AVRDC has been towards the development of gardens and production practices for specific purposes, primarily for production without pesticides, for dryland and wetland or flooded conditions. The justification for working on these arose from feedback with respect to gardens in development projects which suffered from (a) pest and disease attack, (b) drought, and (c) seasonal deficit in vegetable supply during hot-wet periods. If the production and consumption of vitamin-A rich vegetables is to supplant or substitute for the use of encapsulated forms of vitamin A, the supply of vegetables must be maintained year-round. Aside from the biological studies on home gardens reported here, socioeconomic analyses of ongoing development projects in Asia and Central America are being undertaken and reported under the Socioeconomics Section of the Production Systems Program report.

Materials and Methods

Three types of gardens were set up in May 1992: (1) monoculture control, where the 4.5 x 4 m plot was split into three 1.5-m-wide (furrow to furrow) x 4-m-long beds, with each bed split into four equal plots, and each containing one crop at any time; (2) intercrop, using the same design as the monoculture control, with the modification that each of the 12 plots in one garden contained two or more species; (3) aquatic garden, comprising three 4 x 1.1-m raised beds, (50 cm from trough to top), each separated by a 1.1-m wide flooded ditch, with a range of intercropped species on the flat bed surface and a single aquatic vegetable species in each ditch.

Over 35 species of vegetables were grown in the gardens, and, wherever possible, the same species were sown or transplanted simultaneously in each garden. Each garden type was replicated twice and all produce were weighed after harvest. No pesticides were used; pest control was by means of mechanical pricking out, yellow and blue sticky traps, and an olfactory sticky trap [6:3:1 sugar (molasses): vinegar: wine; + glue (tanglefoot)]. Garden wastes were composted and returned to gardens, and nutrient removal in consumed produce was replaced by use of inorganic fertilizer at the annual rate of 240-320 kg N, 310-415 kg P_2O_5 and 310-415 kg K_2O/ha, plus 165-180 t/ha compost.

Results and Discussion

Data are presented on a yearly production basis for each garden, comparing the output of the 24 or 25 species common to three of the garden types and the total output of the garden, which included species not common to each garden (fig. 1). In the first year (1992-93), production on the raised beds in the aquatic garden exceeded that of the intercrop garden; both had higher yields than the monocrop garden. In the following year, 1993-94, when rainfall and flooding were markedly less, total production in the intercrop garden exceeded that of the aquatic garden; again both had greater yields than the monocrop garden.

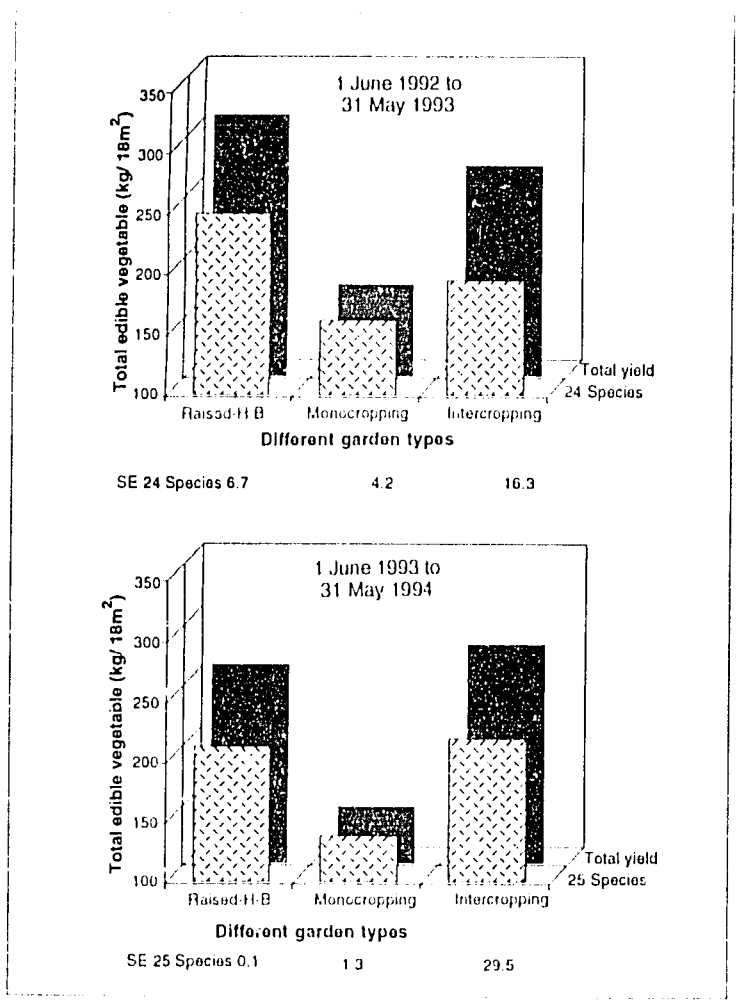


Fig. 1. Gross harvestable yields from three garden types in 2 years

Comparison of yields within each species across the three garden types is time-consuming and will soon be done using nearest-neighbor analyses to account for both positive and negative interference between adjacent plots (of monocrop) and, additionally, between species within each plot (intercrop and aquatic gardens).

The annual output ranged from 8.1 to 16.4 kg/m^2 across gardens; the higher value matching favorably that of Gershon (1988)[1]. Yields of some aquatic species in the aquatic garden (table 1) were considerably lower than those of the upland species whether expressed in a growing day or per year basis. This was particularly true for water caltrops which floats on the water surface, and was shaded by the raised beds and plants growing on the raised beds. Yields of water chestnut and lotus seed were also low, but those of species for which the aboveground parts, e.g., water cress, water convolvulus, water bamboo, and taro, comprise most of the harvest were high. This is particularly so for corms of taro which have significantly less water content than the others.

Erect and perhaps shade-tolerant aquatic species have great potential in home garden plots, especially during the wet season.

Table 1. Harvested yields of aquatic vegetables in the aquatic garden expressed on a per unit area and per unit area-time basis in 1992-93 and 1993-94 (means of two replicate values)

	1992-93		1993-94	
	Yield (kg/m^2)	Yield/growing day (g/m^2)	Yield (kg/m^2)	Yield/growing day (g/m^2)
Water caltrops *Trapa natans*	0.49	4.0	0.71	3.9
Water bamboo *Zizania latifolia*	2.00	16.5	3.56	16.9
Water convolvulus *Ipomoea aquatica*	2.71	18.1	11.52	31.6
Lotus (seed) *Nelumbo nucilera*	0.01	-	0.21	1.7
Taro (stem + leaf)	1.35	9.0	2.26	12.6
Colocasia esculenta (corm)	2.63	17.5	2.06	11.4
Water chestnut *Elocharis dulcis*	-	-	0.28	1.2
Watercress (winter) *Nasturtium officinale*	-	-	7.20	40.0

[1] Gershon, J. 1988. Gardening Nutritious Vegetables. AVRDC, Tainan, Taiwan, ROC. p. 6-39.

Simple Hydroponics

Introduction

Small noncirculating hydroponic units may prove their worth for resource-poor households as a means to produce vegetables important in the diet. Previous research has indicated the feasibility of substituting cheap wooden boxes for polystyrene containers, alternatives to the smoked rice hull media (e.g., offcuts from the garment industry), and the use of fermented pig effluent as the liquid base for the system.

The current experiments were set up to determine (1) how long a container with media and solution could be continuously used to produce leafy vegetables, without replacing the media nor emptying surplus solution after each crop, and (2) the comparative performance of alternative sources of chemicals for the nutrient solution.

Materials and Methods

Both trials were conducted at AVRDC under simple hermetic white net (1 mm mesh) protection with a plastic sheet roof. Meteorological data are presented in the Appendix. No pesticides were used; sticky yellow traps favored the capture of some insect pest; others were removed by hand.

In mid-August 1992, six hydroponic boxes (54 x 34 x 25 cm), each containing 32.5 l of AVRDC hydroponic solution, were set up, each containing one of the following crops: chiang-chiang (nonheading Chinese cabbage), lettuce, rape, pai-tsai (two varieties), and kale. Following harvest of the aboveground parts, the remaining solution was topped to 32.5 l, and seeds of kale were sown in the old media. Following harvest of kale, chiang-chiang was sown and harvested as were subsequent crops (fig. 2).

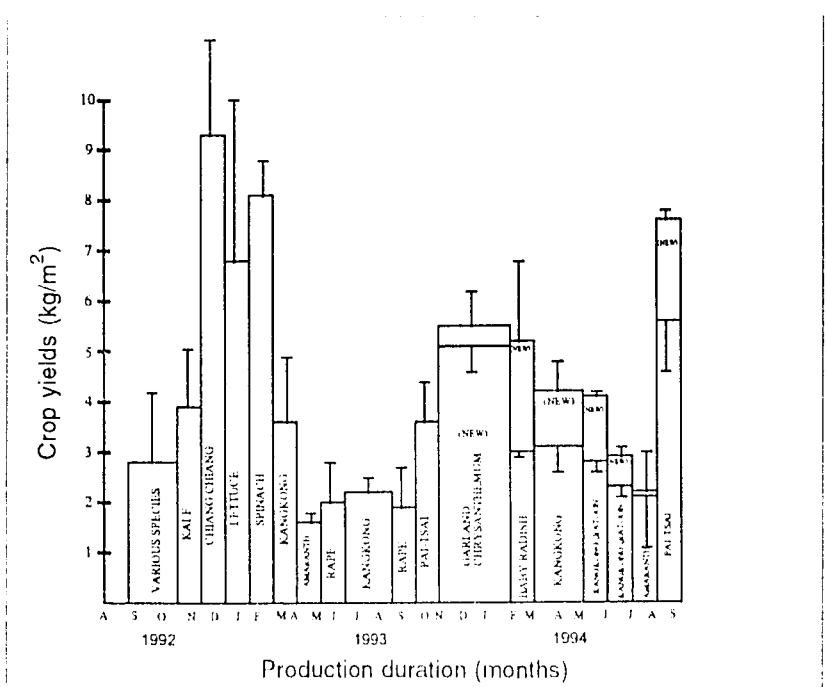


Fig. 2. Production of vegetables over a 2-year period without change of media nor solution Last 9 months compared change of media and solution (NEW) after each harvest

Starting in November 1993, three of the six boxes were given fresh solutions with each sowing, i.e., the unused solution, the rice hull media, and roots were disposed of. The experiment was terminated in mid-September 1994.

In the second experiment, the performance of four hydroponic nutrient mixes were compared to that of the AVRDC standard solution prepared by the Taiwan Fertilizer Company. One hydroponic box containing 32.5 l of solution was used for each source of nutrients (table 2) and sown to the following six crops from March to May 1994: mustard, pai-tsai, baby radish, kangkong, pai-tsai, and mustard.

Table 2. Available nutrients in various sources of hydroponic chemicals

A	Available nutrients (ppm)					
Treatment	NO_3-N	NH_4-N	P	K	Ca	Mg
1 [a]	116	7.5	25.7	138	172	29.7
2	100	9.3	52.8	196	80	13.9
3	100	4.6	47.6	138	102	23.0
4	139	9.3	36.1	336	47	68.5
5	142	9.5	36.1	336	47	68.5

B	Microelement constituents					
Source	Element (ppm)					
	Fe	Mn	Zn	Cu	B	Mo
AVRDC	3.0	0.5	0.05	0.02	0.5	0.01
Yeou-Fa	2.0	0.72	0.09	0.04	1.2	0.01

[a] 1 Taiwan Fertilizer Company - liquid solution
2 Laboratory reagents - based upon uptake in winter, AVRDC
3 Laboratory reagents - based upon uptake in summer, AVRDC
4 Component fertilizer (Yeou Fa Chemical Co., Ltd.)
5 Solid compound fertilizer (Yeou Fa Chemical Co., Ltd.)

Results and Discussion

Yields from the continuous hydroponics experiment showed marked seasonal variation, with greater yields during the winter, irrespective of crop species. The continued production without changing solution and media led to a 10-30% reduction in yield potential 1 year after starting the experiment, although yield of the same species grown at the same time in continuous production during the second year (e.g., kangkong) matched that of the first year. Evidently, some savings in the reuse of the remaining solution and the media is possible — it is not necessary to throw away the remaining solution at the end of each short-season crop nor to dispose of the seedling media. This would, therefore, also lead to a saving of time. The previous crop roots around external PE pots were not removed with the exception of between early June to later September, when seed germination rate was poor. Pythium was recorded in one amaranth and lettuce sowing, causing failure of the former.

Evidently, the AVRDC standard solution was the best for all crops (table 3), with the modified winter solution a close second. The two commercial sources (Yeou Fa Chemical Co., Ltd.) had erratic performance and need further testing.

Table 3. Comparison of vegetable yields (gram per hydroponic box) between AVRDC standard solution and other nutrient solutions in a noncirculating system at AVRDC grown consecutively from March to May 1994

Treatment	Mustard	Pai-tsai	Baby radish	Kangkong	Pai-tsai	Mustard
1	594	357	631	777	1068	844
2	502	340	647	472	691	649
3	420	320	601	829	879	711
4	410	315	693	321	378	194
5	399	288	665	629	711	723

The approximate costs per full container of solution were as follows for the commercial sources of nutrient: AVRDC standard — NT$5.40; Yeou Fa component — NT$3.78; Yeou Fa compound — NT$4.32 (US$1 = NT$26.00).

Grafting

Introduction

In 1993, grafting F_1 fresh market tomato onto tomato or eggplant rootstocks tolerant to waterlogging was shown to extend the option for growing tomato under tropical Taiwan hot-wet summer conditions.

This year, these studies were extended to

a) validate intergenotypic variability for the tolerant trait

b) screen further eggplant accessions

c) search for the physiological basis for the trait

d) quantify performance of grafted lines in farmers' fields

e) identify waterlog-tolerant lines with added tolerance to bacterial wilt

f) compare performance of grafted plants during the cool season

Results of these experiments are reported here.

Materials and Methods

Most field trials were run during the summer at AVRDC (see Appendix for meteorological data) and on farmers' fields in Min Shong and Chung Pu in Chaiyi county.

For experiments 1 and 2 (validation of previous years' results and screening of new rootstock accessions, respectively) eggplant rootstocks were sown on 18 February, and tomato scions (FMTT 22 in experiment 1, and FMTT 22 and cherry tomato AVRDC Line 268 in experiment 2) on 28 February. Grafting was done between 21 and 24 March, and seedlings transplanted to the field on 1 April. Plots in two blocks, including the nongrafted control, consisted of two rows of eight plants in experiment 1 (6 m^2), and six grafted, three natural, and three self-grafted plants in experiment 2 (4 m^2). The field was artificially flooded for 4 days starting 54 days after transplanting (DAT), and then left to drain. It also rained [illegible].2 mm during the flood period. At 60 DAT, a sample of one plant was taken for separation into component fresh weights, and the oxygen consumption of fresh roots was also recorded. On 10 June (71 DAT), three soil cores per plot were removed 15 cm from the base of the

rootstocks, and root length in the 10-20 and 20-30 cm horizons was estimated using the technique of Newman (1966)[2]. Tomatoes were harvested on three occasions only (67, 76, and 83 DAT); infection with tomato leaf curl virus prematurely curtailed canopy growth.

On-farm trials (experiments 3 and 4) were transplanted on 26 April at Min Shong and Chung Pu, respectively. Eggplant rootstocks were sown on 10 March, tomato scions on 28 March, and grafting of cherry tomato took place 11-13 April while grafting of fresh market tomato was on 16 April. Plots in experiment 3a (screening of 17 eggplant rootstocks) compared two-row beds with 12 plants per row spaced at 2.0 m between and 0.9 m within rows. Experiment 3b (comparison, under plastic shelter or in the open, of two outstanding eggplant rootstocks selected from 1993) was comprised of two-row beds with 16 plants per row spaced as for experiment 3a. Two blocks were used in both experiments 3a and 3b. Heavy rain (85 mm) fell at 52 DAT and a further 164 mm over a 3-day period at 70 DAT. The experiment was terminated 104 DAT due to typhoon Katherine. A one-plant sample from each plot was obtained for dry weight determination.

Experiment 4, set up in Chung Pu in a bacterial wilt infested field, was not replicated in blocks. Plots were 6.0 m^2 containing 6-10 plants per plot. Control plots distributed throughout the experimental area had a total of 50 plants for FMTT 22 and AVRDC 268 cherry tomato.

In experiment 5, run in containers with a surface area of 0.36 m^2 and volume of 0.087 m^3, 117 lines (10 lines each with six plants per container) sown on 7 October 1993, were exposed to *Pseudomonas solanacerum* strain PSS97 on 4 November. Soil temperature was maintained above 20°C through the use of polyethylene sheet. Surviving plants were then subjected to complete flooding for 2 weeks and evaluated for mortality after another 16 days.

During late summer 1993, a further trial was run in which fresh market FMTT 22 tomato was grafted to two eggplant rootstocks, TS 98 and TS 126; selfgrafted; or not grafted at all. Superimposed on these treatments within each rootstock as the main plot, was pruning of the main stem to one or two stems. Sowing took place on 5 August 1993, grafting 7 days later, and transplanting after a further 3-5 days. Plots, replicated three times, consisted of one double-row bed, 1.5 m wide with within-row spacing of 0.5 m giving a plot size of 4.5 m^2. Bordered areas were harvested for yield and fruit quality determination.

In all field experiments, fertilizer and water management were optimal within the limits of the natural environment. Tomatotone was not applied in AVRDC experiments; only at the Min Shong field.

Results

Experiment 1

Weight and number of tomato fruit in the first sample was greater in the nongrafted control of either the fresh market or cherry line than in the grafted plants (table 4) — due mainly to the larger size of seedling at the time of transplanting (growth of grafted plants lapsed for a short while following grafting).

Partly due to the flush of fruit in the grafted plants and also to inhibited fruit growth following flooding, tomato yield in the second harvest was inferior in the control plots (table 4). For the third harvest, no tomato was harvested from the control plots due to plant mortality; however, further yield of the grafted plants was limited by top yellow leaf curl virus. Therefore, cumulative yields of grafted plants did not differ from those of control plants.

[2] Newman, E.J. 1966. A method of estimating the total length of a root sample. J. Appl. Ecol. 3: 139-145.

Table 4. Fruit yield (weight and number) of grafted fresh market and cherry tomato on three sample dates as influenced by rootstocks

Treatment	67 DAT				76 DAT				83 DAT			
	Fruit no. (per m²)		Fruit weight (g/m²)		Fruit no. (per m²)		Fruit weight (g/m²)		Fruit no. (per m²)		Fruit weight (g/m²)	
	FMTT 22	268	FMTT 22	268	FMTT 22	268	FMTT 22	268	FMTT 22	268	FMTT 22	268
TS 28	3.4	12.1	205	151	4.0	17.1	288	214	2.8	5.4	183	53
TS 50	1.9	13.5	139	158	2.9	19.3	188	253	2.0	5.3	125	52
TS 56	2.5	17.6	148	199	3.0	17.5	225	215	2.4	2.9	141	28
TS 63	2.3	19.6	148	151	3.9	27.6	270	288	1.9	6.3	101	66
TS 88	1.6	12.4	107	156	3.7	14.6	284	195	2.0	5.1	132	64
TS 91	3.0	10.4	169	143	3.3	15.1	182	195	1.9	3.6	72	38
TS 98	2.3	17.1	132	192	3.4	18.5	224	270	2.6	2.9	187	33
TS 111	1.3	11.5	76	156	2.4	17.5	178	247	1.9	2.8	112	38
Control	4.1	30.4	238	335	2.5	10.3	62	75	0	0	0	0
SED (16 df) treatment mean	1.29		32.2		ns		51.6		0.67		15.46	
SDE (16 df) treatment within a tomato type	1.83		ns		ns		ns		0.93		21.87	

Data on plant morphology and fresh weight collected 60 DAT essentially confirmed the first harvest data, with taller control plants, more trusses (clusters) and fruit, less leaf weight (wilted leaves), and shorter maximum length of roots. No interaction between rootstock and tomato type was evident. Root weight per plant was considerably less in the control than grafted plants, both for fresh market and cherry types, but root respiration of control plants per unit root fresh weight exceeded that of grafted plants.

Data on root length at the two soil depths collected 71 DAT showed a marked difference in root length per unit soil volume at 10-20 cm depth (0.274 cm/cm^3) vs. 20-30 cm (0.094). Root length at 10-20 cm differed across rootstocks, but the variation between rootstocks was not consistent for tomato type (data not presented). For cherry tomato scions, roots of TS 91 rootstocks were more developed than those of the control cherry tomato. All fresh market tomato rootstocks showed improved rooting over the control FMTT 22. Root development of rootstock TS 88 was superior to most other rootstocks with FMTT 22 scions at 20-30 cm depth, but at that depth no differences were evident among rootstocks supporting the cherry tomato line 268.

Experiment 2

A few of the 19 tested lines (e.g., Thin-P, Pusa-P, PI 381155, Fu-C-L and Duen-H) exceeded the fruit yield of the nongrafted FMTT 22, largely as a result of late yield after flooding, and will be studied next year.

Experiment 3a

Following heavy rains (184 mm) 70-72 DAT, plants of the nongrafted cherry tomato line wilted and leaves died. Those of grafted treatments remained healthy. Controls of control FMTT 22 also wilted but recovered, but grafted FMTT 22 scions did not show signs of wilting. The greater canopy of the cherry vs. the fresh market tomato may have created greater transpiration demand on the control rootstock causing mortality.

As for experiment 1, natural (nongrafted) plants gave greatest early yield (180 g/plant vs. 14-90 g/plant for grafted cherry tomato), but after four harvests and just prior to the typhoon yields reached 500-1055 g/plant, with control yield at 850 g/plant (fig. 3). Quite evidently, both fruit number and yield at the third and fourth harvests were considerably less in control than grafted plants (fig. 3), illustrative of the debilitating effect of the heavy rains. Typhoon Katherine damaged all plots in early August, hence, no further harvests were possible. Analysis of a one-plant sample removed from each plot on 8 August (104 DAT) revealed the weak root system and aboveground canopy of control plants, compared to most other grafted lines. A similar response was found for FMTT 22 and among lines TS 11, 63, 379, and Fold-H-B which were consistently superior in all measured attributes.

Observation of the root systems of the sampled plants 104 DAT showed root-knot nematode in the control plants of both cherry and fresh market tomato, and in the following rootstocks: Chu-S-C, Fu-C-L, Thin-P, Uttara TS 56A, TS 63, TS 11, TS 91, TS 111, and TS 88. The remainder showed no nematode attack (Fold-H-B, Pusa-P, Islam-P, Taso-H, Torvum, TS 379, and Duen-H).

Experiment 3b

Although, due to farmer insistence for ease of management, no valid statistical analyses of the main effects of rainshelters and tomato type (cherry or fresh market) could be made, rain shelter improved tomato yields by > 20% (811 vs. 664 g/plant) as shown by greater fruit number.

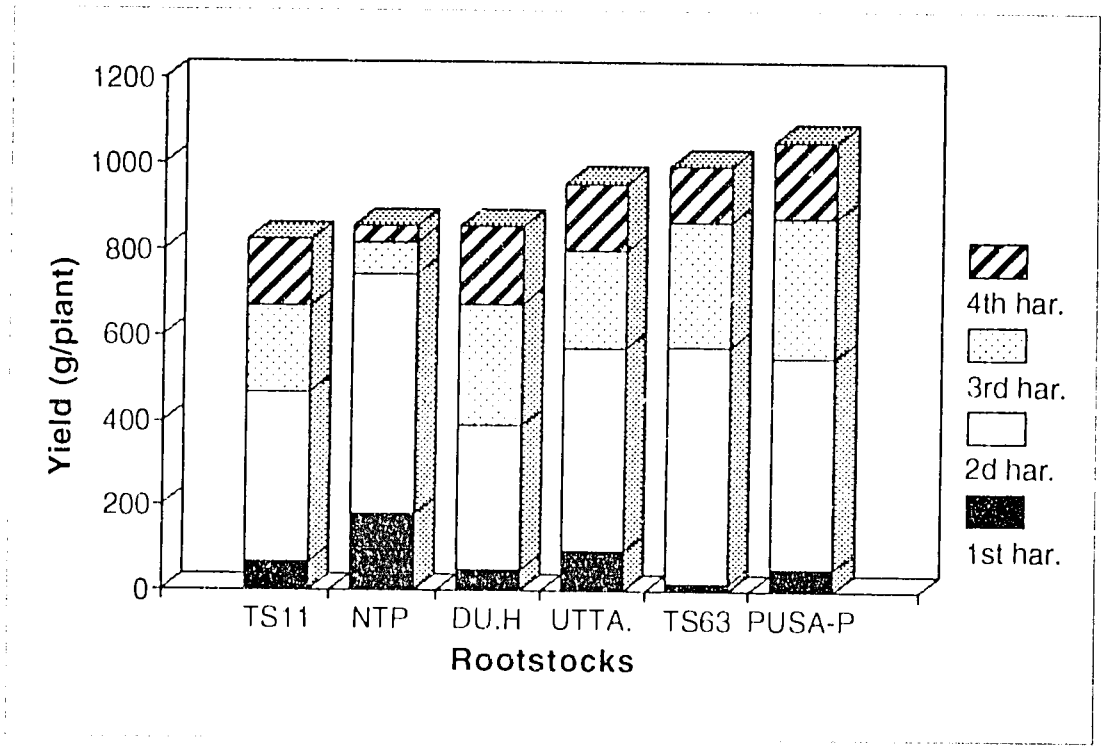


Fig. 3. Tomato yield of cherry var. Santa in experiment 3 at Ming Shong (NTP = natural nongrafted var. Santa)

For FMTT 22, the major effect of rainshelters was to permit a good second harvest (fig. 4) after the heavy rainfall 70-72 DAT, largely attributable to more harvested fruit (fig. 5). The benefit of grafting over the control FMTT 22 was not evident in the open, while under rainshelter yield of the nongrafted control significantly exceeded that of the grafted lines (fig. 4).

As for fresh market tomato, there was a tendency for the rainshelter to improve later harvests of cherry tomato, as did grafting in the treatment without rainshelters (fig. 6). However, the interaction again was not significant.

Plant dry weight samples of cherry tomato plants taken after typhoon Katherine illustrate the continued benefits to grafted plants. Most attributes (table 5) differed significantly between the control and grafted lines, but there was no evidence that the advantage of grafting was greater under nonsheltered conditions. The same was so for fresh market tomato.

Experiment 4

Survival data are presented in table 6. No control (i.e., nongrafted) plants of either the fresh market or cherry tomato survived bacterial wilt attack and waterlogged soil conditions, but some grafted lines, in particular TS 50, TS 56A, Duen-H, and Uttara, showed near or complete survival.

Experiment 5

Of the 117 lines grown in small containers, 34 did not show wilt symptoms 2 weeks after inoculation. Sixteen days following a 14-day flood period, the number of lines not showing wilting symptom (due to flood and/or bacterial wilt) was reduced to 13 (data not presented).

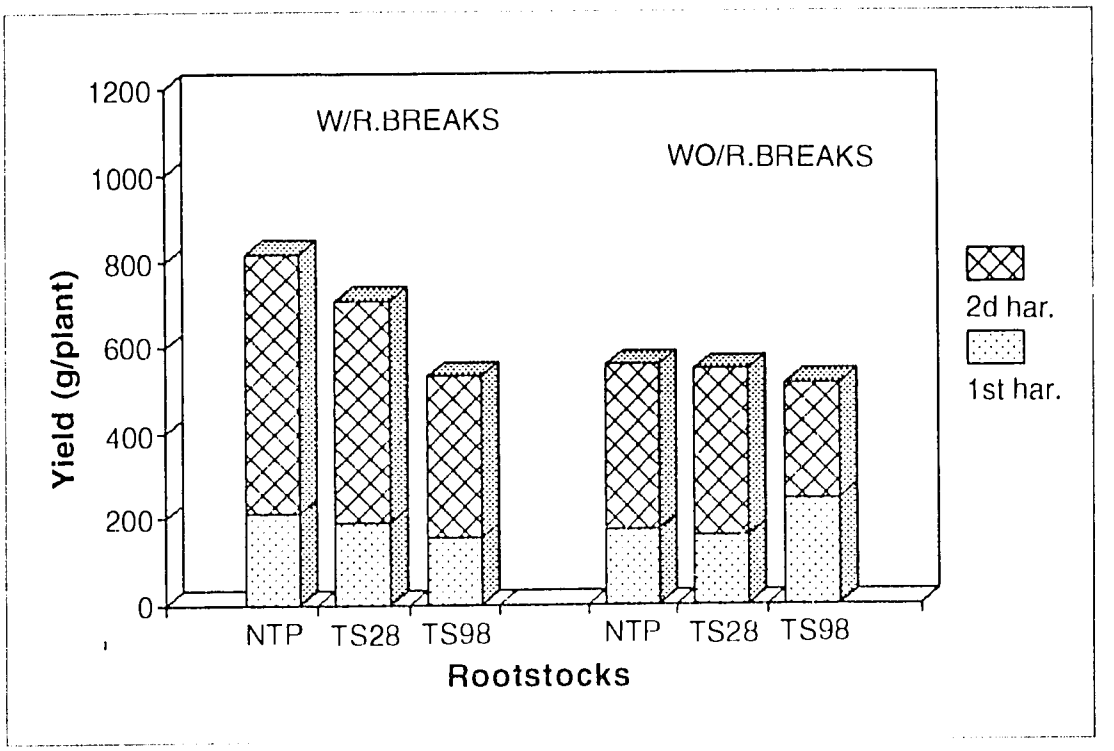


Fig. 4. Fresh market tomato (var. FMTT 22) yields as influenced by rainshelter and grafting onto eggplant rootstocks in Ming Shong (NTP = natural nongrafted var. FMTT 22)

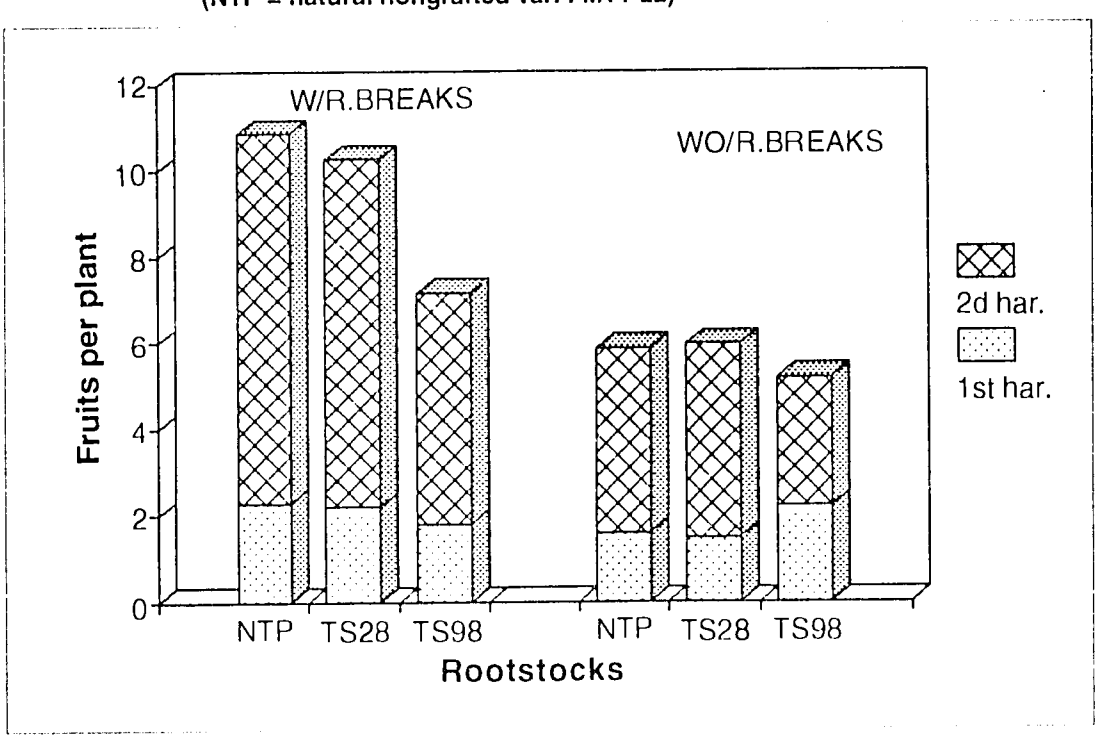


Fig. 5. Fresh market tomato (var. FMTT 22) fruit numbers as influenced by rainshelter and grafting onto eggplant rootstocks in Ming Shong (NTP = natural nongrafted var. FMTT 22)

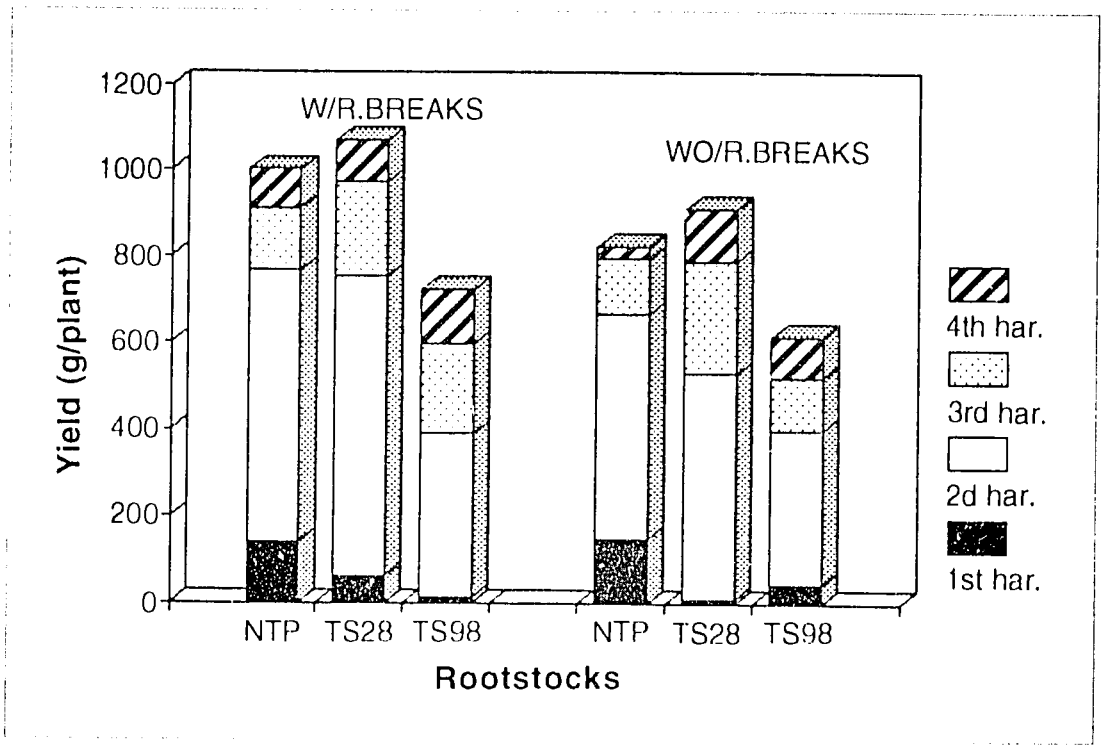


Fig. 6. Cherry tomato (var. Santa) fruit yield as influenced by rainshelter and grafting onto eggplant rootstocks in Ming Shong (NTP = natural nongrafted var. Santa)

Table 5. Plant attributes of nongrafted var. Santa cherry tomato and fresh market FMTT 22 tomato scion samples 104 DAT (mean values with and without rainshelter) in experiment 3b

	Cherry tomato				Fresh market tomato
Treatment	Plant height (cm)	Stem dry weight (g/plant)	Root dry weight (g/plant)	Branch no./plant	stem + root dwt (g/plant)
TS 28 rootstock	209	398	23.1	11.25	169
TS 98 rootstock	208	372	17.5	10.50	160
Nongrafted	170	185	4.4	9.75	95
SED (4 df)	13.8	61.0	4.7	ns	26.4 (P = 0.10)

Table 6. Plant survival of grafted and control tomatoes in farmers' field in Chung Pu (Chiayi) in experiment 4

Rootstock	Scion	No. grafted plants	Survival plants
Chu-S-C	FMTT 22	6	1
Duen-H		9	6
Fold-H-B		6	0
Pusa-P		6	0
Fu-C-L		6	4
Islam-P		6	3
PI 38115		6	0
Thin-P		6	2
TS 11		6	4
TS 28		6	5
TS 485		6	2
TS 50		6	6
TS 56A		6	4
TS 63		6	0
TS 88		10	6
Uttara		6	6
Control		50	0
Fu-C-L	CHT 268	6	0
TS 28		6	2
TS 50		6	1
TS 56A		6	5
TS 88		6	0
Uttara		6	4
Control		50	0

Experiment 6

While grafting to eggplant rootstocks led to a reduction in marketable fruit yield (table 7), self-grafting resulted in a significant yield increase above all other treatments. Pruning to two stems per plant also led to greater yield; more so for nongrafted controls than either for self-grafted controls or tomato grafted onto eggplant (table 7). Rootstocks influenced both Brix and pH of fruits, but not color nor acidity. Brix values were greater for scions grafted to eggplant and pH was lowest in those treatments (table 7).

Discussion

Experiments this year did not demonstrate the yield advantage of grafted plants due to two major reasons: either the typhoon or tomato top leaf curl virus curtailed the growth of grafted plants beyond the time when yield advantages of grafting would become evident, and nongrafted plants had greater earlier yields, but, due to wilting under naturally or artificially induced flooding, later yield (both weight and number) of controls was inferior to that of grafted plants.

Table 7. Effect of grafting (onto self or to eggplant rootstocks) and main-stem pruning on FMTT 22 tomato yield, yield components, and fruit quality in experiment 6

	Grafting	Pruning	Marketable fruit yield (t/ha)	Marketable fruit no./ m^2	Total yield (t/ha)
A	Nongrafted		61.9	64.8	77.7
	Graft-TS-98		32.3	36.8	43.5
	Graft-TS-126		35.4	41.6	48.3
	Self-graft		73.5	72.3	90.8
	SED (14 df)	4.94	5.73	6.42	
B	Nongrafted	Single	53.8	55.9	69.5
	Nongrafted	Double	71.4	72.2	89.7
	Eggplant	Single	31.2	35.6	42.3
	Eggplant	Double	36.5	42.7	49.6
	SED (12 df)		2.61	n.s.	3.84
C	Nongrafted	Single	53.8	55.9	69.5
	Nongrafted	Double	71.4	72.2	89.7
	Self-grafted	Single	66.6	63.6	81.6
	Self-grafted	Double	70.9	73.6	86.2
	SED (12 df)		4.74		4.80
			pH	°Brix	
D	Nongrafted		4.12	4.42	
	Eggplant graft		4.08	4.80	
	Self-grafted		4.09	4.44	
	SED (20 df)		0.014	0.142	

Efforts to improve management of the virus and to reduce damage due to typhoons are called for. Tolerance to bacterial wilt found among rootstocks is currently being capitalized upon by Taiwanese and Filipino farmers. Tomato production is now taking place in areas usually considered unsuitable for tomato, either because of endemic bacterial wilt or because of the need to rotate tomato with other crops. Current experiments are quantifying the interaction between canopy size and tolerance to waterlogging, for a greater canopy per rootstock represents significant savings for farmers who spend time and/or money producing grafted seedlings.

Starter Solution Studies

Summary

Two field experiments with mechanical transplanting and application of starter solution to Chinese cabbage were run in 1994. Starter solution treatments were superimposed on varying basal N rates and plug sizes. Seedlings from larger plug-size plantings were better able to take early advantage of the starter solution, as they were to basal N fertilizer. Head yield was favored by starter solution at each basal N rate, and starter solution substituted for 40 kg basal N/ha.

Introduction

Previous studies (AVRDC 1991-93) have highlighted the response of vegetable crops to applications at transplanting of starter solution containing N and/or P. Crops with high early growth rates (e.g., Chinese cabbage, eggplant) responded well to N, even under conditions where nutrient availability is high. Current experiments were undertaken to determine the feasibility of and response to mechanical application of starter solution in Chinese cabbage.

Materials and Methods

Two studies were conducted at AVRDC, one in spring and the other in autumn 1994. The experimental design was identical for both seasons, comprising a factorial structure in three blocks. Treatments were comprised of two seedling plug sizes (21 and 55 cc), three rates of basal N application (0, 40, and 80 kg/ha), and with or without application of starter solution (240 mg N, 52 mg P, 98 mg K/plant vs. water only). Plots consisted of single 1.5-m-wide and 10-m-long beds containing two rows of Chinese cabbage plants spaced 0.4 m within the row. Fifty milliliters of solution or water (control) were applied to the side of each seedling at transplanting with an Ellis (Verona, Wisconsin, USA) model 6000 mechanical transplanter. To avoid possible carry-over effects, all control plots were transplanted first, followed by the plots with starter solution.

The surface 30 cm soil contained 12 kg N/ha. All plots received a basal application of 35 kg/ha P and 41 kg/ha K and side-dressings at 15 and 29 days after transplanting (DAT) each of 60 kg N/ha and 41 or 49 kg K/ha. Other standard management practices were used. Data on crop cover were collected two or three times during each experiment using a grid methodology. Harvest of the spring experiment was not possible due to a severe infestation of ringspot virus. Two harvests were taken in the autumn experiment, half of each plot 47 DAT and the other half 52 DAT, and were separated into total aboveground and head yields. A subsample of four plants per plot in the autumn was removed 15 DAT to measure fresh and dry weight of above- and belowground parts, and their N, P, and K contents.

Results and Discussion

Spring experiment

No significant effect of starter solution was apparent for crop cover measurement, although at the earliest date (16 DAT) the greater plug size led to a significantly greater, yet insignificant in absolute terms, cover: 16.3 vs. 13.1%, SED (22 df) = 0.7. Development of the ringspot virus inevitably obscured meaningful treatment effects.

Autumn experiment

Application of starter solution led to sizeable increases in above-and belowground dry weight 15 DAT, in the same order of magnitude as the full vs. no basal fertilizer application (table 8). Larger plug size also led to a similar effect. The interaction between plug size and starter solution suggests that larger seedlings (i.e., greater root volume) were better able to take advantage of the starter solution (table 8). Similarly, smaller seedlings were unable to effectively use the greater basal application of N (table 8). Main effects of all three treatments on crop cover were significant, with starter solution, larger plug size, and more basal N application favoring crop cover (table 9). No interactions for crop cover were evident.

Table 8. Aboveground dry weight of Chinese cabbage (g/plant) at 15 DAT as influenced by plug size, starter solution, and basal N application, autumn 1994

		Starter		Mean	Basal N (kg/ha)		
		with	without		0	40	80
Plug size	21	3.06	2.39	2.73	2.35	2.94	2.88
(cc)	55	6.26	3.70	4.98	3.98	4.81	6.15
Mean		4.66	3.04		3.12	3.88	4.52
SED (22 df) between means			0.209			0.256	
SED (22 df) between values within a table			0.295			0.361	

Table 9. Main effects of plug size, starter solution, and basal N application on crop cover (%) of Chinese cabbage, autumn 1994

		Days after transplanting		
Treatment		15	22	29
Plug size	21	9.7	28.5	43.8
(cc)	55	16.6	37.4	50.6
SED (22 df)		0.72	1.10	0.84
Starter solution with	14.3	35.5	50.0	
without	11.1	30.4	44.4	
SED (22 df)		0.72	1.10	0.84
Basal N	0	11.4	30.7	44.8
(kg/ha)	40	13.7	33.5	47.5
	80	14.2	34.7	49.3
SED (22 df)		0.89	1.35	1.03

N concentration 2 weeks after transplanting was greater in plants not receiving starter solution [3.695 vs 3.336%, SED (22 df) = 0.132]; it was unaffected by plug size and basal N rates. Essentially, P and K contents did not differ across treatments, with the exception of a greater P concentration in the seedlings from large plugs [0.601 vs. 0.571%, SED (22 df) = 0.013]. Despite the lower concentration of N in seedlings with starter solution, their greater dry weights offset this such that they had a 40% greater N content per seedling (155 vs. 112 mg/plant).

Significant interactions between starter solution and basal N rate were evident for head yield (table 10), with yields where starter solution substituted for basal N equaling those without starter but with 40 or 80 kg/ha basal N. Benefits of starter solution on total fresh weight were apparently still accruing up to the last 5 days of crop growth, since, over the span of the two harvests, the starter treatment gained a further 7.5 t/ha total weight vs. 4.2 t/ha in the control (table 10). Head yields from the larger plug-size plantings were significantly greater than those of the smaller size [31.3 vs 27.2 t/ha, SED (24 df) = 0.80]. Although larger young plants 15 DAT were disproportionately favored by starter solution (table 9), head yield was not, indicating that starter solution was equally effective for small- or large-sized plug plantings.

Table 10. Chinese cabbage head and total yields (t/ha) as affected by starter solution, basal N rate, and harvest date, autumn 1994

	Head yield			Total yield	
	Basal N application (kg/ha)			Harvest	
	0	40	80	47 DAT	52 DAT
Starter solution with	29.5	31.9	32.5	49.0	56.4
without	22.3	30.6	28.8	45.3	49.1
SED (24 df) between means within a table		1.38		2.77	

The application of about 8 kg/ha of starter solution to Chinese cabbage by way of a mechanical transplanter can obviate the need for 40 kg N basal application, thus, saving the potential leaching of N (see Nitrogen nutrition management in vegetable cultivation and the environment under Environmental Physiology Section). Starter solution can also raise the yield potential at each basal N application rate tested (table 10).

Effects of Seedling Media Type and Module Size on Field Performance of Chinese Cabbage and Tomato Under Suboptimal Field Conditions

Introduction

While previous studies have indicated that total yields of tomato, Chinese cabbage, and chili pepper were unaffected by variation in seedling size at transplanting, whether the variation was due to different media or module types (summarized in AVRDC 1992 Progress Report, p. 333-340), the same may not hold true for suboptimal field conditions which limit full expression of yield potential. Experiments were, therefore, conducted to quantify the response of various-sized seedlings to suboptimal irrigation and nitrogen fertilization.

Materials and Methods

Two experiments were carried out at AVRDC, one with tomato transplanted on 10 September 1993 and the other with Chinese cabbage transplanted on 6 September 1994. In both experiments three media types (AVRDC standard, Dutch import, and a Fison mix) and three plug sizes were compared factorally. Composition of the media was as follows: AVRDC - soil:sugarcane compost:sand:rice hulls in 5:3:1:1 volume proportion; Dutch import - 20% peatmoss, 80% black peat by volume, and 8 kg lime (10% Mgo) plus 1.5 kg 12-14-24 amendment/1 m^3; Fison (# 2 Basic) - Canadian sphagnum moss with perlite, gypsum, dolomitic lime, and wetting agent added.

For tomato, 288, 128, and 72 plugs per tray provided 6.4, 21.0, and 60.0 cc root volume per plant, whereas for Chinese cabbage 288, 128, and 70 plugs per tray provide 6.4, 21.0, and 55.0 cc root volume. A sample of 10 plants per treatment was removed at transplanting to estimate seedling dry weight.

For tomato, plots measured 4 x 1.5 m with 20 plants per plot (in double rows on a 10-cm raised bed). Two blocks consisting of either full irrigation and N fertilizer, or alternate date irrigations only (i.e., half the irrigation amount) with 1/3 N fertilizer side-dressing. Fruit from border plants were harvested 10 times from 23 November 1993 to 2 February 1994. Light interception using a LI-COR LI-1000 radiometer was measured on three occasions: 39, 66, and 94 DAT.

For Chinese cabbage, plots measured 5.6 x 1.5 m with each plot (containing one of the media x plug size combinations) split randomly into halves containing 16 plants and receiving either full (210:35:49 kg N, P, K kg/ha) or 1/3 the application rate. Plots were grouped into two blocks, and within each block main plots were treated with either full or restricted irrigation. The full irrigation schedule provided irrigation at transplanting, 3 days later, then every week thereafter, whereas restricted irrigation was provided at transplanting, 3 and 10 days thereafter, and finally 24 DAT. Crop cover data using a framed grid were collected 21, 30, and 37 DAT. Gravimetric soil water data were collected 24 and 38 DAT, canopy and air temperature 21 DAT, and yield data 38 and 44 DAT.

Results

Tomato

The seedling plug size resulted in anticipated variation in seedling size at transplanting (table 11) but differences between media were less than expected based upon previous years' data. Variation in seedling size at transplanting did not result in differences between treatments in light interception, nor in yield or its components. While light interception was significantly less for the suboptimal field treatment 39 DAT, this was not translated into a significant reduction in tomato yield. Total and marketable yields were greater for the larger plug (and therefore seedling) size, but differences were not significant. Cumulative yields show surprisingly that the benefits of large seedlings (i.e., from the large seedling root volume) were not evident at early harvests, in contrast to previous years' results (data not presented). However, the influence of the poorest medium (Fison) on cumulative yield was notable from the first harvest onwards, but did not reach statistical significance (data not presented).

Chinese cabbage

As for tomato, media and in particular plug size affected seedling sizes at transplanting (table 12). These main effects of media and plug size were manifest throughout the growth cycle, expressed as crop cover and in the yields of early and late harvests (table 13). The effect of the suboptimal fertilizer treatment was also evident throughout the crop growth cycle and at harvest (table 13), but reduced irrigation had no effect. Significant interactions were evident between media and plug size at the first

Table 11. Effect of media and plug size on tomato seedling sample dry weight (mg/plant) at transplanting

	Plug volume (cc)		
Media	60.0	21.0	6.4
AVRDC	45.2	21.8	20.7
Dutch import	42.2	39.8	26.9
Fison	55.5	32.9	28.0

Table 12. Effect of media and plug size on Chinese cabbage seedling sample dry weight (g/plant) at transplanting

	Plug volume (cc)		
Media	55.0	21.0	6.4
AVRDC	0.381	0.201	0.063
Dutch import	0.587	0.209	0.102
Fison	0.119	0.064	0.035

Table 13. Main effect of irrigation, media, plug size, and fertilizer regime on crop cover (%) and yield (t/ha) of Chinese cabbage

				Harvest			
	Crop cover			38 DAT		44 DAT	
Treatment	21 DAT	30 DAT	37 DAT	Total	Head	Total	Head
Full irrigation	24.5	41.0	44.4	13.5	7.3	17.4	10.5
Reduced irrigation	22.4	38.4	42.0	11.3	5.7	15.6	9.3
SED (1 df)	ns	ns	ns	ns	ns	ns	ns
Standard AVRDC media	24.2	40.4	43.3	12.8	6.8	16.9	10.1
Dutch import	27.6	42.6	45.1	13.3	6.9	17.6	10.4
Fison	18.6	36.1	41.1	11.1	5.9	15.2	9.1
SED (16 df)	0.90	1.34	1.51	0.38	0.25	0.50	0.34
Plug volume 6.4 cc	17.0	36.0	40.8	10.7	5.6	14.8	8.8
Plug volume 21.0 cc	23.0	40.1	42.6	12.2	6.4	16.1	9.5
Plug volume 55.0 cc	30.5	43.1	46.1	14.3	7.6	18.7	11.3
SED (16 df)	0.90	1.34	1.51	0.38	0.25	0.50	0.34
Full fertilizer	25.7	43.4	47.5	14.0	7.4	18.3	11.0
Reduced fertilizer	21.3	36.0	38.8	10.8	5.6	14.8	8.8
SED (18 df)	0.62	0.91	0.88	0.27	0.22	0.38	0.28

crop cover sample; crop cover was poorer in the Fison mix relative to the other mixes in the medium root volume treatment (data not presented). Important meaningful interactions were evident between irrigation and plug size, fertilizer management and irrigation, and between plug size and fertilizer. Limited irrigation restricted final crop cover and early total and head yield of the full fertilizer treatment, but by the later harvest the yield of the treatment with limited irrigation yet full fertilizer was close to that of the full irrigation-full fertilizer (fig. 7). Limited irrigation also restricted yield of the largest plug-size treatment at early harvest, and of the medium plug-size treatment at later harvest (fig. 8). Finally, differences between fertilizer treatments were least for early crop cover of small vs. large plug sizes and for early total yield, but not for later crop cover nor later yield (data not presented).

No significant differences between treatments were noted between canopy temperature, expressed either as a depression below air temperature or as the quotient of the former and saturation vapor pressure deficit. Soil moisture content differed significantly between plug-size treatment on both sample occasions, with the larger plug treatments creating greater transpirational demand resulting in less soil moisture. As expected, soil moisture content was considerably less in the alternate event irrigation treatment.

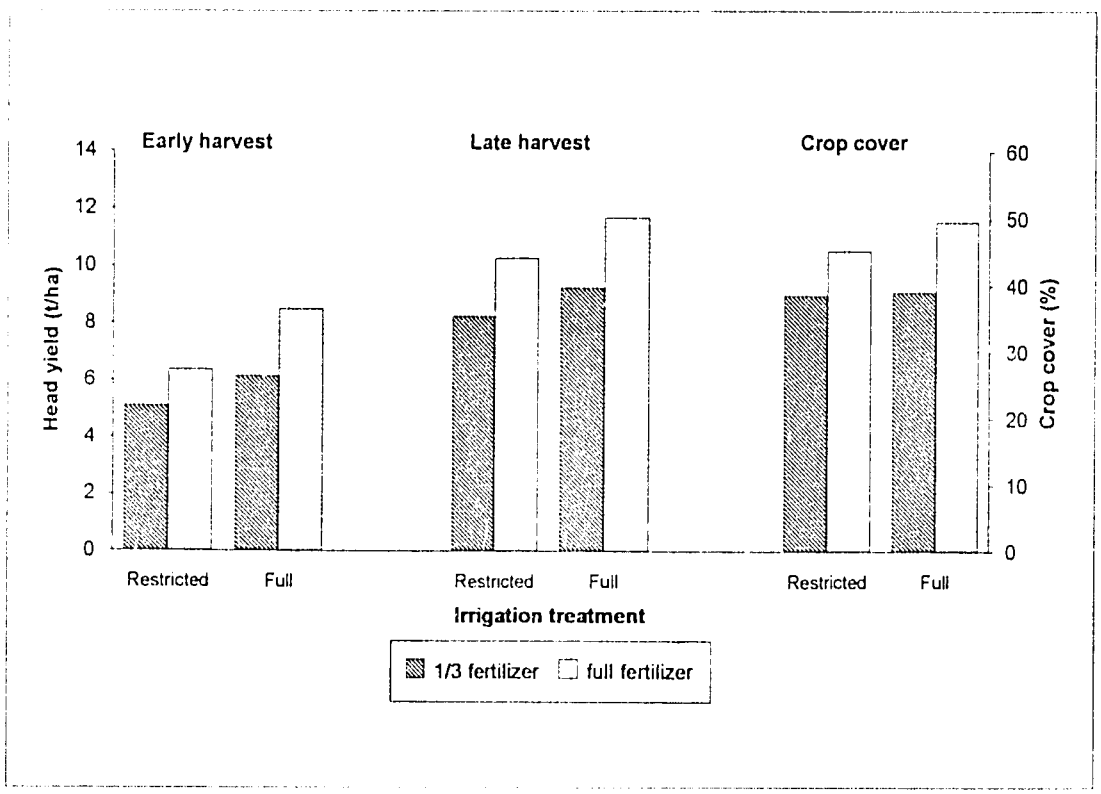


Fig. 7. Interaction effects of irrigation and fertilizer rates on head yields and final crop cover of Chinese cabbage

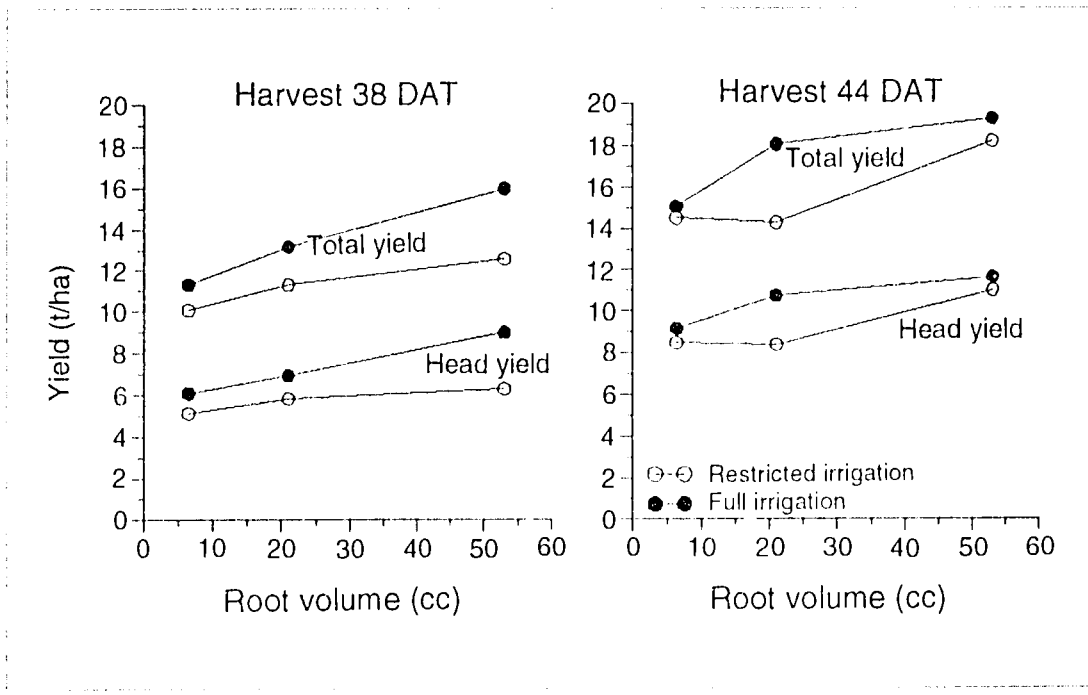


Fig. 8. Total and head yield of Chinese cabbage at two harvest dates, as influenced by root volume of transplants; all field irrigation practice

Discussion

The influence of media was less evident than the influence of plug size on crop cover and yield, particularly for Chinese cabbage (table 13). Similarly, the effect of reduced irrigation after transplanting was less marked than the effect of reduced fertilizer application. Seedlings from small plug sizes never caught up with those of the larger plug size in final yield (table 13). Reduced fertilizer constrained early growth and yield of large root-volume seedlings, but that of small root-volume seedlings were already limited, whether fertilizer was in full or short supply. Later crop cover and yield were equally restricted by reduced fertilizer in all root-volume treatments.

Limited irrigation did not differentially reduce early growth of small vs. large volume seedlings, even though larger seedling volume created a greater demand for soil moisture. The buildup in water stress was evident for the largest plug size treatment at 38 DAT harvest (fig. 8), but by 44 DAT the yield of restricted irrigation treatment in that plug size matched that of the full irrigation treatment. By 44 DAT, the effect of reduced irrigation was most severely expressed in the medium plug-size treatment, perhaps because the benefit of full irrigation was manifest for that plug size by that time. While the results from the tomato experiment shed little light on the differential behavior of seedling production treatments under posttransplanting stress, the data on Chinese cabbage illustrate that favorable conditions after transplanting differentially favor the larger-sized transplanted seedlings. Restricted irrigation prevented medium and small-sized transplants from equaling yields of large transplants (fig. 8), but restricted fertilizer did not reduce yield potential of small more than that of large-sized seedlings.

Effect of Age and Chemical Composition of Legume Green Manure on Nitrogen Mineralization in Three Tropical Soils

Summary

Field trials were run during the hot humid summer and the cooler autumn to quantify biomass accommodation in four legume species planted at two densities and harvested on three occasions. Dried aboveground plant material from the summer harvests was incubated with soils from three sites (AVRDC soil and soil from two Philippine sites) to measure NH_4-N and NO_3-N release.

Highest biomass and N accumulation was achieved by soybean harvested 75 days after sowing (DAS) in both seasons, followed by Indigofera at 90 DAS. Mineralized N accumulated most rapidly as NO_3 without noticeable NH_4 on AVRDC soil, but pronounced peaks in early NH_4-N were evident on the other two soils. On all three soils, *Indigofera* from the 60 DAS harvest released the most N over a 10-week incubation period.

Introduction

Field experiments are being conducted at AVRDC and the Philippines from 1992 to 1995 to test the feasibility of partly substituting N fertilizer for the production of processing tomatoes in the tropics with legume green manuring. An optimal legume species for green manure use is a plant that accumulates high amounts of N and biomass in a short time, so that the farmer does not lose too much time and field space in producing this N source. Many reports mention the variability of legume growth depending strongly on the location and season of growth. Two field experiments monitoring the seasonality, the influence of the seeding density, and growth duration on the N and biomass accumulation of alfalfa (*Medicago sativa*), *Desmodium intortum*, *Indigofera tinctoria*, and soybean (*Glycine max*) were conducted at AVRDC in 1993.

Various incubation studies have reported the correlation of chemical properties of plant material with the inorganic N mineralization or immobilization in the soil. To understand the mineralization/immobilization patterns in relation to plant properties, two incubation studies were conducted to follow the release of N in different soils under controlled conditions. The major aim in the first incubation experiment was to determine the influence of age of plant material on the N release in the soil of four legume species. The variability of the N release of the same plant material was tested in three different tropical soils in a second incubation experiment.

Material and Methods

Field experiments

Growing legume green manure. Four legume species (*Medicago sativa*, *Desmodium purpureum*, *Indigofera tinctoria*, and *Glycine max*) were screened in a field experiment for biomass production and N accumulation at different growth durations (60, 75, and 90 DAS). The legumes were grown at two seeding rates (simple and double), which were 0.75 and 1.5 g/m2 for *Desmodium*, 0.5 and 1 g/m^2 for *Medicago*, 0.66 and 1.32 g/m2 for *Indigofera*, and 100 and 200 seeds/m^2 for soybean. The aim was to check whether by doubling the seeding density a high biomass production could be reached in a shorter time. The legume seed was inoculated with a Rhizobium strain mixture, specific for each legume species provided by the Soil Science Department of Chung Hsing University, Taichung. Fertilizer at 35 kg P/ha as superphosphate and 89 kg K/ha as KCl was applied to the soil before sowing.

The field experiment was performed twice, the first starting 19 March to 15 June 1993 (88 DAS), and the second starting 7 September to 9 December 1993 (90 DAS). The treatments (four legume species, two seeding densities, three growth durations) were arranged in a three-factor experiment in RCBD with four replications. The plot size was 6 m^2 and the field size 210 m^2. At each sampling date (60, 75, and 90 DAS), 1 m^2 of the plot was harvested to determine aboveground biomass production. Plant material was analyzed for total N and total carbon with a Carlo-Erba Gas analyzer, lignin content with the acid detergent fiber method, total phenolics with the Folin-Denis method, and condensed tannins with the vanillin assay method.

Statistical analyses were made using ANOVA with JMP (SAS for MacIntosh) program.

Incubation study

Soil and plant material. Soil was collected from the top 10 cm on the experimental farms of AVRDC, Taiwan, the Mariano Marcos State University (MMSU, Batac, Philippines), and Bukidnon Resources Corporation Inc. (BRCI, Manolo Fortich, Mindanao, Philippines). The AVRDC soil is of the Take series (loamy, mixed, hyperthermic, Fluvaquentic Eutrochrept, pH 8.2, total N 0.07%, total C 0.64%, NO_3-N ppm/g soil: 1.08, NH_4-N ppm/g soil: 1.33). The characteristics of the two other soils are presented for comparison in table 14. Soil was sieved (4 mm) and homogenized. Fresh legume shoot samples (stem with leaves) of 60- and 90-day-old plants of *Medicago sativa*, *Desmodium intortum*, *Indigofera tinctoria*, and *Glycine max* were collected from the field experiment on the AVRDC farm, the same site where the soil was taken. The plants were then oven-dried and ground (<1.5 mm).

Incubation procedure. For the first experiment 40 g air-dried AVRDC soil and 0.1 g dry and ground plant material were put into a 125-ml Erlenmeyer flask. This plant-to-soil ratio was to approximate a mulch or incorporation rate of 3.5 t plant dry matter/ha. Plant material was used from the double-density treatments of the field experiment in the hot and humid season (mentioned earlier).

Water in a quantity corresponding to 0.1 bar was added to the soil-plant mixture; the mixture was allowed to dry to 0.3 bar before being readjusted to 0.1 bar. Treatments are listed in table 14; a soil-only treatment was included as the control. The flasks were sealed with cotton balls and stored in the incubator at 25°C for 10 weeks. Three replicates of each treatment were randomly selected for extraction after 1, 2, 4, 6, 8, and 10 weeks of incubation. For extraction purposes, 60 ml of 1 N KCl solution was directly added into each sampling flask. The mixture was shaken for 1 h and the extract was filtered through Whatman 42. Then, NH_4-N and NO_3-N were determined with a gas-sensing ammonia electrode ORION 95-12.

Table 14. Comparative characteristics of the three soils used in incubation studies

		BRCI	MMSU	AVRDC
Clay (hydrometer)	%	59	50	16
Silt (")	%	25	33	52
Sand (")	%	16	17	32
pH H_2O (1:1)		6.1	8.0	7.6
CEC	meq/100 g	12.9	44.0	7.1
Organic C	%	1.95	0.5	0.7
Kjeldahl N	%	0.212	0.06	0.09

In the second experiment, air-dried soils from AVRDC, BRCI, and MMSU were weighed on an oven-dry basis at a rate of 39.3 g/flask, corresponding to the same amount of AVRDC soil as in the first experiment. A 0.1-g sample of dried and ground plant material of 60 and 90 DAS soybean and *Indigofera* from the same source was added to the soil into a 125-ml flask. The same incubation procedure was followed as for experiment 1. Treatments are listed in table 15.

Data analysis. The NO_3-N and NH_4-N contents per sampling date and treatment were analyzed using the JMP-SAS for Macintosh. By subtracting total extractable N in soil only from that in legume plus soil (apparent), net N mineralization and immobilization of inorganic N attributable to the N release of legumes was determined. Percent of original shoot N mineralized was calculated following the Constantinides and Fownes (1994)[3] method. Means and standard errors of percent of initial shoot N mineralized or immobilized were calculated for each sampling time. Multiple regressions between mineralized and immobilized inorganic N and initial shoot chemical indices were performed on untransformed data for each time period of the first incubation experiment (Statview program). Net N mineralization was considered the difference in exchangeable NH_4 and NO_3 between two samplings (Palm and Sanchez 1991)[4].

Results

Field experiments

Alfalfa, *Desmodium*, and soybean produced more dry matter and accumulated more nitrogen in a shorter time in the spring experiment compared to the autumn experiment (tables 15 and 16). The growth conditions in autumn were more favorable for *Indigofera* only which produced more biomass and accumulated more N than in spring.

The seasonal effect was especially strong for soybean which produced about 5 t dry matter/ha and 100 kg N/ha less in autumn than in spring.

The legume species, seeding density, as well as growth duration, had a significant effect on dry matter production and nitrogen accumulation in both experiments. Most biomass and N was produced by soybean followed by *Indigofera*, *Desmodium*, and alfalfa. In both seasons, legumes sown at double density had higher performances than those using simple seeding density. In the spring experiment most biomass and N was accumulated 75 DAS, whereas in autumn maximum yields were reached at 90 DAS. The interaction species x duration was significant in both seasons. This shows that for these four legume species a longer growth duration was not always correlated positively with higher biomass accumulation. Alfalfa and soybean grown in summer yielded the highest biomass within 75 days. After that, biomass decreased due to senescence for alfalfa, and to leaf dropping for soybean which entered the reproductive phase. In contrast, *Indigofera* and *Desmodium* produced the highest biomass at 90 DAS as their vegetative phase was longer than that of the two other legumes.

In spring, more dry matter and N was accumulated in double compared to single-density plantings with all four legumes (table 16). Doubling of the seeding density increased the biomass production of *Indigofera* strongly, whereas the other legumes were only slightly affected. N accumulation in soybean and *Indigofera* was raised by the double-density seeding; the same was true, for alfalfa and *Desmodium* in autumn.

[3] Constantinides, M. and Fownes, J.M. 1994. Nitrogen mineralization from leaves and litter of tropical plants: relationship to nitrogen, lignin and soluble polyphenol concentrations. Soil Biol. Biochem. 26(1): 49-55.

[4] Palm, C.A. and Sanchez, P.A. 1991. Nitrogen release from the leaves of some tropical legumes as affected by their lignin and polyphenolic contents. Soil Biol. Biochem. 20(1): 83088.

Table 15. Biomass accumulation (dry matter yields t/ha) of alfalfa, *Desmodium intortum*, *Indigofera tinctoria*, and soybean 60, 75, and 90 days after sowing grown in two seasons in Taiwan, 1993

Growth duration (DAS)	Seeding density	Hot and humid season (April–June 1993)				Cool and dry season (October–December 1993)			
		Alfalfa	*Desmodium*	*Indigofera*	Soybean	Alfalfa	*Desmodium*	*Indigofera*	Soybean
60	simple	1.4	1.2	1.1	5.3	0.6	0.7	1.3	2.8
	double	1.7	1.4	1.3	6.1	0.7	0.9	2.2	3.6
75	simple	2.0	2.3	2.4	8.2	1.0	1.5	2.8	3.3
	double	2.3	3.0	2.8	9.9	0.9	1.8	3.7	4.2
90	simple	1.5	2.3	3.0	7.7	1.3	2.4	4.0	3.0
	double	1.8	3.1	3.9	8.3	1.2	2.9	5.1	3.1
SED	species			SED	species				
	density				density				
	duration				duration				
	sp. x dur.				sp. x dur.				
					sp. x density				

Table 16. Nitrogen accumulation in biomass (kg/ha) with alfalfa, *Desmodium intortum*, *Indigofera tinctoria*, and soybean 60, 75, and 90 days after sowing grown in two seasons in Taiwan, 1993

Growth duration (DAS)	Seeding density	Hot and humid season (April–June 1993)				Cool and dry season (October–December 1993)			
		Alfalfa	*Desmodium*	*Indigofera*	Soybean	Alfalfa	*Desmodium*	*Indigofera*	Soybean
60	simple	50.0	39.5	50.8	187.7	16.4	18.7	58.2	97.4
	double	57.0	47.2	56.0	209.3	16.8	23.4	90.1	142.2
75	simple	61.6	55.1	93.0	276.4	29.1	40.9	105.6	121.7
	double	67.1	73.3	106.7	314.1	22.9	45.1	131.7	143.88
90	simple	33.7	44.5	83.2	256.2	42.3	69.8	124.0	155.6
	double	40.9	58.3	101.3	263.1	38.8	75.7	159.7	148.2
SED	species			SED	species				
	density				density				
	duration				duration				
	sp. x dur.				sp. x dur.				
					sp. x density				

Alfalfa, *Desmodium*, and soybean produced highest biomass yields 75 DAS and *Indigofera* 90 DAS in spring. N accumulation was highest 75 DAS for *Indigofera*, alfalfa, and soybean and 90 DAS for *Desmodium*. In autumn alfalfa, *Desmodium* and *Indigofera* registered the highest dry matter yields 90 DAS, whereas soybean reached its maximum 75 DAS. N accumulated throughout growth and reached its maximum 90 DAS.

These two experiments show that the optimal time to harvest highest biomass and N accumulation is 75 DAS for soybean and 90 DAS for *Indigofera* in both seasons.

Chemical plant properties from nonreplicated samples only are currently available. Percent lignin and C/N ratio mostly increased with the age of the plant, while N as well as polyphenol and tannin concentrations decreased.

First incubation experiment. In general, nitrate (plus nitrite) constituted over 95% of the extractable inorganic N. One week after incubation, NH_4-N values were as high as 1.7 µg/g soil in treatments with *Desmodium* 60 DAS (fig. 9). But generally, NH_4-N values dropped throughout incubation from 1.5 to 0.5 µg NH_4-N/g dry soil. The legume species and legume age strongly influenced the speed of net N mineralization in the soil. More nitrogen became available, and the release dynamics was faster when young plant material (60 DAS) was used. The addition of legumes lead to an initial NO_3-N immobilization in all treatments. The N immobilization lasted 1-2 weeks with 60-DAS-old plant material vs. 4-6 weeks for 90-DAS-old plant material. The greatest N release occurred with the addition of 60-DAS-old *Indigofera* and alfalfa, where up to 50% of the initial N in the plant material was mineralized after 10 weeks.

N released to the soil (% of the initial N added) with alfalfa, *Desmodium*, and *Indigofera* 10 weeks after incubation was higher in 60-DAS compared to 90-DAS-old plant material treatments by 37, 51, and 25%, respectively. In fact, neither *Desmodium* nor alfalfa at 90 DAS produced a net positive numeralization. The 90-DAS-old soybean material, however, released 6% more N than the 60-DAS-old soybean.

Results of the legume screening trial showed a reduction of soybean biomass production between 75 and 90 DAS, and for alfalfa in the hot humid season when % N also decreased, resulting in a strong increase in the C/N ratio. The first phase of decomposition might have taken place at this time. This phase is characterized by a retreat of important plant nutrients from the shoot to the roots, hydrolytic and oxidative splitting of polymeric substances, and an increasing microbial population on dying plant parts. These may partially explain the relatively quick N release to the soil of 90-DAS-old compared to 60-DAS-old soybean.

Second incubation experiment. Nitrate (+ nitrite) was the dominant form of extractable inorganic N in AVRDC soil. Initial values of 10 µg NH_4-N/g soil dropped to 0.5 µg NH_4-N/g dry soil within the first week of incubation. In BRCI and MMSU soils the addition of legume plant material generated a significant increase in NH_4-N levels as compared to the control reaching 20-40 µg NH_4-N/g soil 1 and 2 weeks after incubation (fig. 10-12). Treatments differed significantly in raising the NH_4-N content with the following ranking: 60 DAS *Indigofera* > 60 DAS soybean > 90 DAS soybean > 90 DAS *Indigofera*. During the first 2-4 weeks a strong immobilization of NO_3-N occurred in both soils, but especially striking in BRCI soil, where NO_3-N levels dropped from 30 µg/ g soil to 0 µg/ g soil within the first week. NO_3-N contents stayed below those of the control (immobilization) during almost 10 weeks in BRCI soil. In all three soils, younger plant material (60 DAS) released inorganic N attributable to the legume addition faster then 90 DAS. In MMSU soil, net NO_3-N mineralization took place after 2-4 weeks.

In all three soils most NO_3-N was released with 60-DAS-old *Indigofera*, followed by 60- and 90-DAS-old soybean in AVRDC soil, and 90-DAS*Indigofera* in BRCI and MMSU soils. The least was released with 60- and 90-DAS-old soybean in BRCI and MMSU soils, compared to 90-DAS-old

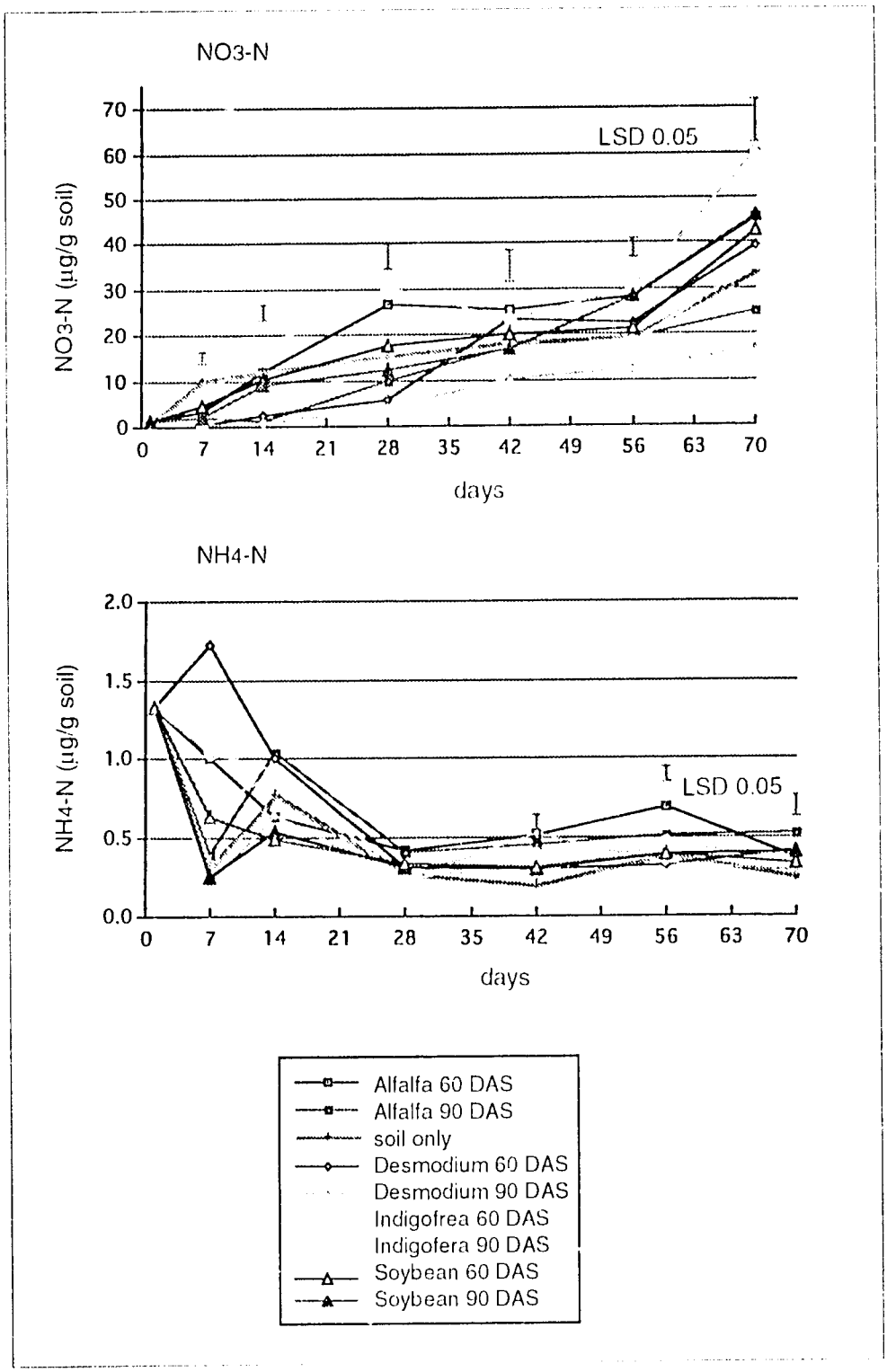


Fig. 9. Cumulative No_3-N and NH_4-N (µg/g soil) in AVRDC soil incubated with legume material of 60- and 90-day-old alfalfa, *Desmodium*, *Indigofera*, and soybean (Values shown are means of three replicates)

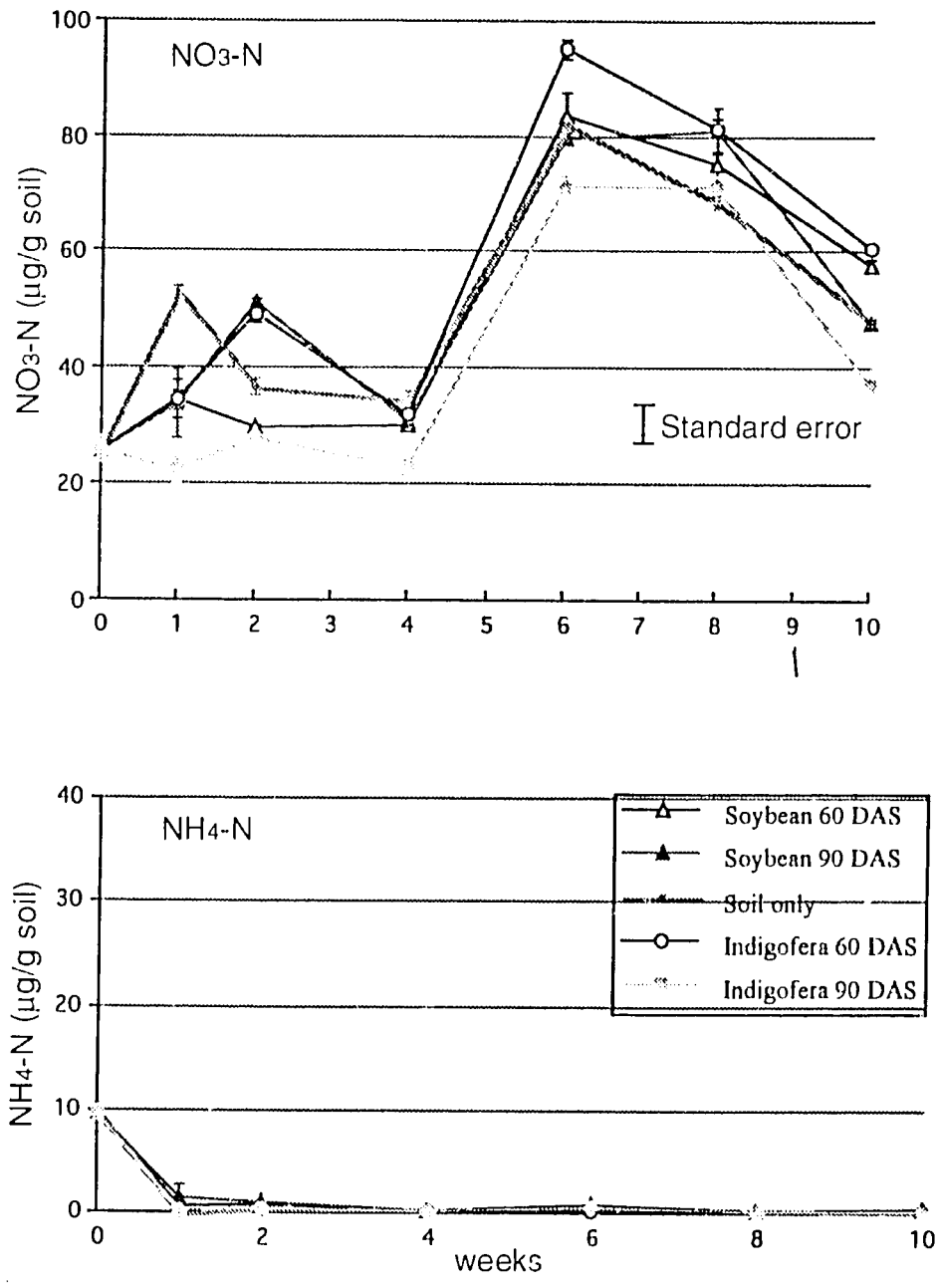


Fig. 10. Cumulative NO_3-N and NH_4-N (µg/g dry soil) in AVRDC soil incubated with 60- and 90-DAS soybean and *Indigofera*

Values shown are means of three replicates and standard errors

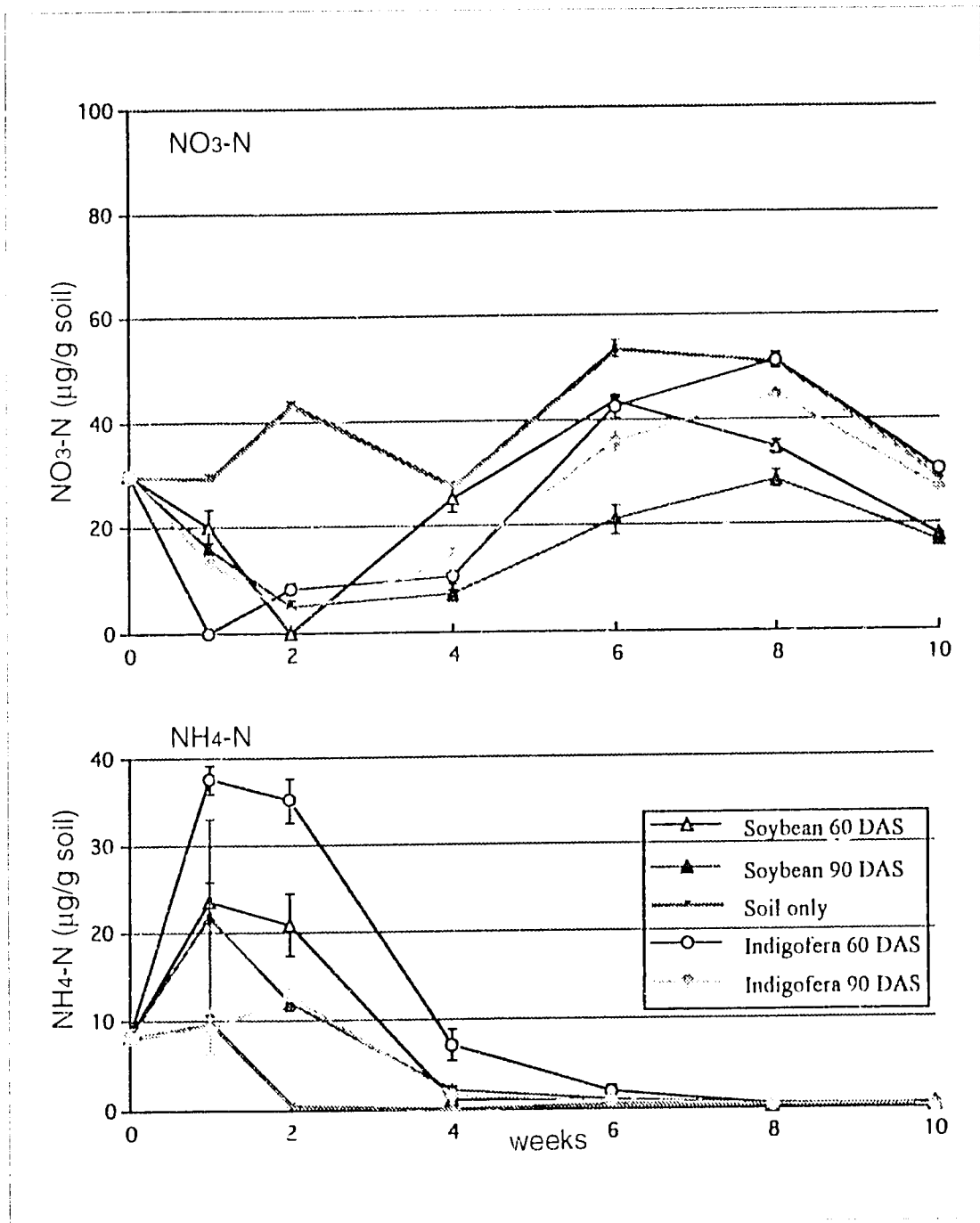


Fig. 11. Cumulative NO_3-N and NH_4-N (µg/g dry soil) in BRCI soil incubated with 60- and 90-DAS soybean and *Indigofera*

Values shown are means of three replicates and standard errors

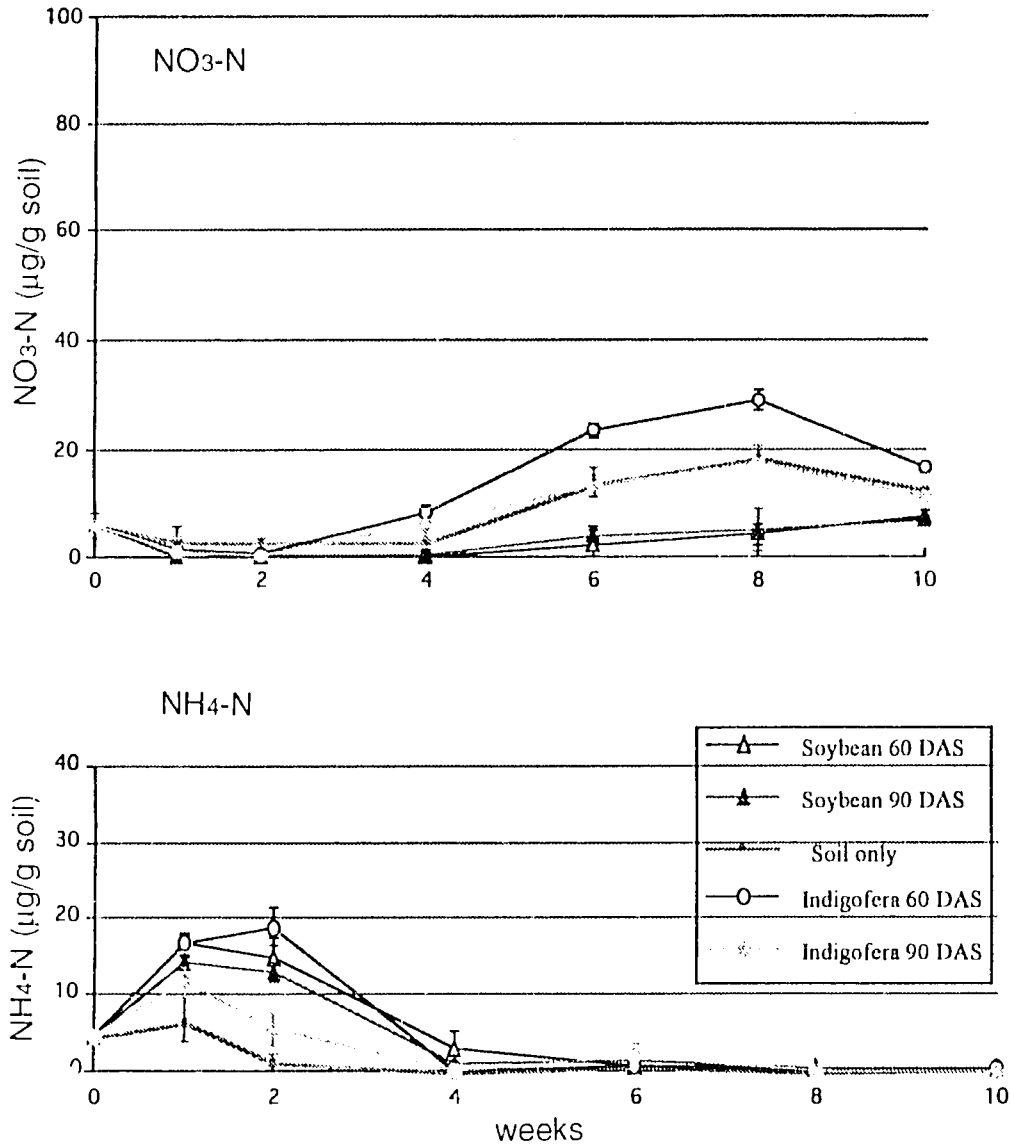


Fig. 12. Cumulative NO_3-N and NH_4-N (µg/g dry soil) in MMSU soil incubated with 60- and 90-DAS soybean and *Indigofera*

Values shown are means of three replicates and standard errors

Indigofera in AVRDC soil. The most NO_3-N released to the soil was 10% in AVRDC soil with 60-DAS-old *Indigofera* and soybean, about 5% in MMSU soil with 60-DAS *Indigofera*, and 0% in BRCI soil with 60-DAS *Indigofera*.

Conclusion

The legume species, seeding density as well as the growth duration (60, 75, and 90 DAS) affect biomass and N accumulation significantly. Highest biomass and N yields can be achieved for soybean at 75 DAS and *Indigofera* at 90 DAS in both hot-wet and cool-dry seasons.

N mineralized accumulated most rapidly without perceptible NH_4 + accumulations as NO_3-N in AVRDC soil, whereas in BRCI and MMSU soil an initial NH_4-N peak was followed by an NO_3 peak. Initial NO_3-N immobilization took place in all soils. Inorganic N was released faster with 60-DAS plant material compared to 90-DAS. In all three soils most inorganic N was released with 60-DAS-old *Indigofera*.

Environmental Physiology

Managing Nitrogen Fertilization to Maximize Resource-Efficient Productivity in a Continuous Year-Round Vegetable Cropping Sequence

Summary

Permanent high-bed cultivation systems are primarily used to overcome flood damage in vegetable crops cultivated in many lowland peri-urban production zones throughout Asia.

Overdoses of nitrogenous fertilizers in intensive tropical and subtropical vegetable production have created concern about their impacts on the environment and product quality, and have led to a demand for better N management practices. Within this context, we attempted to rationalize N fertilizer use.

The N_{min} method was tested in a 14-month continuous vegetable cropping sequence with five crops in two cultivation systems: flat planting beds and permanent high beds. Permanent high-bed cultivation and N_{min} method were compared with standard practices to test their potential for more resource-efficient productivity, particularly during the rainy summer off-season. Using plant petiole sap NO_3 testing, a promising new quick test procedure to detect N deficiencies, an integrated study of soil NO_3, plant sap NO_3, and crop yield was undertaken for a dry season crop of vegetable soybean and a rainy season crop of Chinese cabbage.

The influence of cultivation system (flat beds vs. high beds) on productivity was much more conspicuous than the effect of the fertilization method. Summer crops of Chinese cabbage and chili pepper in 1993 and Chinese cabbage in 1994 on high beds outyielded those on flat beds by 58, 240, and 161% for the standard N application rate. Winter crops of carrot and vegetable soybean were, however, slightly (8 and 14%) reduced.

Plant available N concentrations in soil (N_{min}) followed a seasonal pattern of fixation and/or immobilization of ammonium N fertilizer during the rainy summer, when water saturation inhibited nitrification most of the time. During winter, this bound nitrogen reappeared as soils dried out. This was particularly true for the more flood-prone flat beds.

The accelerated mineralization potential during the dry season led to N_{min} concentrations obviating the need for N fertilization for carrot and vegetable soybean crops in the N_{min} treatment. No yield reductions were observed compared to the standard N application rate. Although soil N supply was more limited during the rainy season, only yields of Chinese cabbage grown in summer 1994 on high beds were significantly reduced by the N_{min} method. This was attributed to heavy rain at head formation stage before the final harvest. A total of 360 kg N/ha (42%) was saved by the N_{min} method during 14 months for five vegetable crops.

The combination of hyperbolic relations of plant NO_3-N as a function of soil nitrate and yield as a function of plant NO_3 resulted in a good estimation of yield = f(soil NO_3) for vegetable soybean.

Small differences between measured sap NO_3-levels and calculated upper limits demonstrated that soil N was not a limiting growth factor for this dry season crop. Lower yields despite higher plant sap NO_3 contents on high beds were associated with excessive root growth which was favored by the physical soil environment on high beds.

A similar magnitude of soil nitrate absorption by summer Chinese cabbage, but limited efficiency of transformation into biomass in the former, resulted in significantly lower yields of the crop on flat planting beds. Large differences between measured sap NO_3 concentrations and regressed upper limits implied inadequate N supply from soil, for reasons stated earlier.

Permanent high-bed cultivation in combination with use of the N_{min} method was the most resource-efficient combination for out-of-season summer vegetable production. Plant sap NO_3 testing in combination with calculation of a mutual functional relationship --- soil NO_3 - plant NO_3 - crop yield based on the Michaelis-Menten model of saturation kinetics (hyperbolic approach) --- was a useful way to detect plant N deficiencies, and to explain crop performance in different cultural systems.

Introduction

Permanent high-bed cultivation is known to exist in many peri-urban lowland production zones in Asia ranging from China to India.

At present, heavy applications of N fertilizer are common practice in many of these and other intensive vegetable production areas in the tropics and subtropics. Increasing concern for the negative consequences of over-fertilization, particularly regarding nitrate contamination of groundwater and potentially harmful high nitrate contents in vegetables, has led to a demand for the development of innovative N management strategies to improve fertilizer use efficiency. One approach to improve N management is to fine-tune the amount of N fertilizer to better synchronize soil N availability with plant requirements.

Widely introduced in Central Europe is the N_{min} method that essentially depends on the measurement of soil N and takes into account crop needs only in so far that standardized critical values for plant available soil nitrogen are taken from extensive tables. Exclusively for basal N fertilizer application or more sophisticated for additional side-dressings, N fertilizer need for an individual dressing is calculated as follows:

N fertilizer need = crop demand for soil N (critical soil NO_3-N) - N_{min} (mineralized soil N; NO_3-N)

The limited use of N_{min} in commercial farms is attributed more to the immense requirement for time and labor to sample and analyze the soil than to the doubt that the soil analysis data would not reliably represent plant nutritional status. The first part of this study deals with the experience of the N_{min} method in a 14-month, five-crop continuous year-round vegetable cropping sequence in the subtropical environment of Southern Taiwan, where crops were either grown on traditional flat beds or on permanent high beds.

A suitable substitute to the slow and expensive N_{min} method is the sap nitrate test (SN-test) to improve N fertilizer management. This new and promising analytical procedure measures NO_3-N concentration in fresh petiole sap and has proved to be highly correlated with dry tissue NO_3-N concentrations in several vegetable species. Since nutrient concentrations decline most quickly in rapidly expanding new tissues, and the petiole acts as a storage and transport organ for nitrate-N, the petiole of recently matured leaves is a sensitive indicator of plant N status and N nutrition. The goal is to determine the minimum level of petiole sap NO_3-N associated with maximum yield, a "critical petiole NO_3-level" above which a crop would be adequately supplied with N and no additional N fertilizer would be needed, and below which the crop would be deficient in N nutrition and would require additional N fertilizer to ensure maximum yields.

At present, the calibration of the method poses the most significant hindrance to practical application since diagnostic standards for critical NO_3-N concentrations in plant sap are still lacking. Vegetable extension institutions in Europe and the U.S. are, however, beginning to use this technology and are providing the commercial grower with guidelines. New, portable nitrate-selective electrodes and quantitative reflectometric analysis procedures for test strips make it much easier to achieve reliable results.

The objective of the second part of this study is to describe a model that integrates (1) soil N status (soil NO_3-N), (2) crop N status (petiole sap NO_3-N), and (3) crop yield response to provide a theoretical background for further studies of its kind, and to apply this model to two field-grown vegetable crops, vegetable soybean and Chinese cabbage, cultivated either on flat beds or on permanent high beds.

Materials and Methods

N_{min} study

In spring 1993, an integrated permanent high-bed deep-furrow system was laid out and constructed in a randomized three-factor, split-split block design with four replications. Factors included: (1) high bed width, (2) with legume green manure living mulch or none, and (3) N fertilization method. The first two experimental factors will not be discussed here. The N fertilization method consisted of two levels, (1) traditional (standard) N fertilizer input, and (2) N_{min} method. The two levels of this factor were additionally randomized in four replications on 1.5-m-wide flat beds adjacent to the main experiment to represent a control to the permanent high beds.

In a 14-month continuous year-round vegetable cropping sequence, summer rainy season crops used were Chinese cabbage [*Brassica pekinensis* (Lour.) Rupr. variety ASVEG No. 1, AVRDC] and chili (*Capsicum annuum* L. variety Hot Beauty, Known You Seed Co.). Carrot [*Daucus carota* L. ssp. *sativus* (Hoffm.) Arcang. Red Judy, Known You Seed Co.] and vegetable soybean [*Glycine max* (L.) Merr. variety AGS 292, AVRDC] were grown during the dry winter. Aquatic crops cultivated in the continuously flooded furrows were taro [*Colocasia esculenta* (L.) Schott] and rice (*Oryza sativa* L.).

Carrot was sown in paired rows in place of single rows for direct sown vegetable soybean and transplanted Chinese cabbage and chili crops. Dimensions of cultivation systems and plant rectangularity are presented in fig. 1; other details are summarized in table 1.

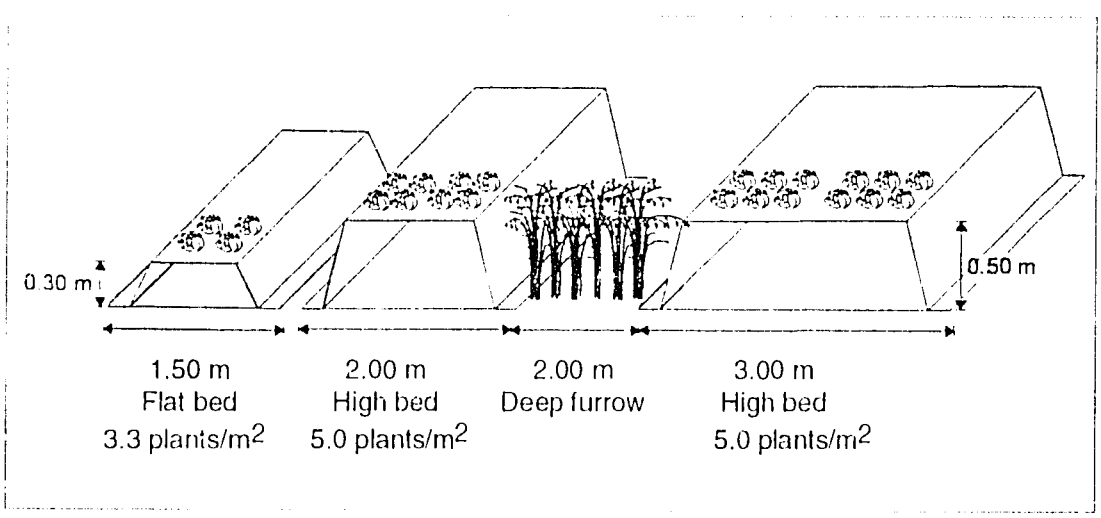


Fig. 1. Dimensions of cultivation systems and plant rectangularity

Table 1. Summary of cultural details

Crop	Chinese cabbage	Chili	Carrot	Vegetable soybean	Chinese cabbage
Cultivation period	May-Jun 93	Jun-Nov 93	Dec-Feb 93-94	Mar-May 94	Jun-Jul 94
Plant distance (cm x cm)	50 60	50 60	25 05	50 05	50 40
Plant density (plants/m^2)					
flat bed system	3.3	3.3	80.0	40.0	3.3
high bed system	3.3	3.3	80.0	40.0	5.0

[a]Nmin method only for basal fertilizer application

All nitrogen was applied as ammonium sulphate [$(NH_4)_2SO_4$], the less costly and most readily available N fertilizer source.

Soil N_{min} was measured before each N fertilizer application by sampling 30-cm-deep soil in every N_{min} treatment plot (no legume green manure living mulch). Extracted 1:2 by volume in 0.8 % KCl water solution, samples were analyzed for NO_3 using Merck's RQflex reflectometer and Reflectoquant nitrate analytical test strips. N application rate for the N_{min} treatment was calculated by subtracting the average N_{min} value from the traditional N application rate. All other cultural practices used were standard.

Statistical comparisons between main factors "N fertilization method" and "cultivation system" (means of two bed widths), and their interactions were performed using orthogonal contrasts on data from plots without green manure.

Integrated study of soil N, plant N, and crop yield

During the 1994 cropping of vegetable soybean and Chinese cabbage, soil NO_3 and plant nitrate data were also collected weekly. Soil was sampled from 0-30 cm and 30-60 cm layers in 4 flat-bed plots and 12 high-bed plots. Petioles were collected from about eight newly expanded leaves per plot for vegetable soybean, and five midribs of recently matured leaves per plot for Chinese cabbage. Petiole sap was expressed using a garlic press, diluted with deionized water to fit the range of the Reflectoquant test strips (5-225 ppm), and analyzed for NO_3-N with the RQflex.

To integrate soil N status, crop N status, and crop yield response, a functional relationship model was chosen based on theoretical and biological considerations and which was sufficiently well correlated with our data. The most widely used model in scientific publications is the polynomial approach ($V = a+b \times S+c \times S^2$ where V = crop yield, S = soil or plant N status). In contrast, the Michaelis-Menten model of saturation kinetics describes the velocity of an enzyme-mediated reaction as a function of substrate concentration. Analogous to the Michaelis-Menten model, the relationships (1) plant sap nitrate = f(soil nitrate), (2) crop yield = f(plant sap nitrate), and (3) crop yield = f(soil nitrate) were fitted to the hyperbolic model:

$$V = (V_{max} \times S) / (K_m + S)$$

where: V = plant sap nitrate concentration or crop yield, V_{max} = (constant) upper limit for V, S = soil nitrate concentration or plant sap nitrate concentration, and K_m = (Michaelis constant) affinity for S.

In an earlier study, the relationship plant sap nitrate = f(soil nitrate) has been well established for potato and peppermint. The same functional dependency may also be true for the two remaining models. For this analysis, however, since field experiments were not specially laid out for this purpose (N fertilizer rates were not systematically varied), it was expected that the regression equations would not provide very accurate predictions.

Yield as a function of soil N is usually poorly correlated. Substitution of (1) plant NO_3-N = [(a x soil NO_3-N)/(b + soil NO_3)] into (2) yield = [(A x plant NO_3-N)/(B + plant NO_3-N)] results in: yield = {[(Aa/B+a) x soil NO_3-N]/[(Bb/B+a) + soil NO_3-N]}. Comparison of this latter estimated function with the field-derived regression equation of yield = f(soil NO_3-N) can function as a control to govern whether the mutual relationship of hyperbole dependencies is sufficiently determined. Using the polynomial approach instead would result in a fourth-order polynomial function, implausible and unsuitable from a theoretical and practical standpoint.

Results and Discussion

N_{min} study

The cultivation system was much more influential on productivity than N fertilization regime throughout the 14-month cropping sequence (table 2). This tendency was at the same time much more pronounced for marketable yields than for total biomass production. Yields of the standard fertilization treatment on high beds surpassed those on flat beds by 58, 240, and 161% for the summer crops of Chinese cabbage and chili in 1993, and Chinese cabbage in 1994. Winter season crop yields of carrot and vegetable soybean were, however, slightly (8 and 14%) reduced compared to flat-bed cultivation.

Soil N_{min} contents were very similar in flat-bed and high-bed systems during the rainy season summer (table 2). In the dry season, however, high soil nitrification rates in both cultivation systems resulted in N_{min} concentrations that in the most part largely exceeded requirements of both crops of carrot and vegetable soybean. Very obvious is the accelerated mineralization potential on the flat beds during that season. Consequently, no additional N fertilizer was applied in the N_{min} treatment. No significant yield differences were finally recorded between N fertilization regimes for these cool season vegetables.

The tendency for reduced N fertilization to become a significant factor in crop production when yields reach higher levels can be summarized from summer crops of chili (1993) and Chinese cabbage (1994): with chili yields of around 0.2 kg/m^2 on flat beds the orthogonal comparison between fertilizer treatments is far from significant ($P = 0.88$). At higher yields of 0.7 kg/m^2 on high beds it comes close to significant ($P = 0.16$). For Chinese cabbage, low yields of 0.8 and 0.2 kg/m^2 did not differ on flat beds ($P = 0.10$), but it did on high beds (2.0 and 1.2 kg/m^2; $P < 0.01$). Higher N fertilizer application did not overcome the disadvantageous crop environment of the commonly used flat planting beds in summer vegetable production.

To summarize, N fertilization for individual crops was reduced by up to 100% and a total of 360 kg N (42% of the traditional rate) was saved in a 14-month continuous vegetable cropping sequence by use of the N_{min} method. Significant yield loss due to reduced N fertilization was only observed in the 1994 Chinese cabbage crop grown on high beds. The yield reduction occurred in the second harvest, after a first harvest that showed no yield differences between traditional N fertilization and N_{min} method ($P = 0.79$). Heavy typhoon rain between harvests may have denitrified or leached out plant available nitrogen essential for head formation. This thesis is supported by less pronounced differences in total biomass at the second harvest ($P = 0.08$).

Table 2. Influence of N_{min} fertilization method and cultivation system on vegetable yields

Cultivation period	Chinese cabbage May-June 1993			Chili June-November 1993				Carrot December-February 1993/94		Vegetable soybean March-May 1994			Chinese cabbage June-July 1994		
N_{min} content (kg N/ha)															
WAS/WAT	0	2	4	0	4	8	12	0	7	0	2	4	0	2	4
Flat bed system	43[a]	-	-	30[a]	-	-	-	132	213	43	120	51	22	32	21
High bed system	60[a]	-	-	34[a]	-	-	-	139	37	16	101	20	19	39	13
Fertilization (kg N/ha)															
WAS/WAT	0	2	4	0	4	8	12	0	7	0	2	4	0	2	4
Traditional fertilization	60	30	30	50	50	50	50	60	60	20	20	20	60	30	30
N_{min} method	0	30	30	20	50	50	50	0	0	0	0	0	20	0	0
Fresh biomass (kg/m²)	Flat bed	High bed	P level	Flat bed	High bed	P level	Flat bed	High bed	P level	Flat bed	High bed	P level	Flat bed	High bed	P level
Traditional fertilization	2.97	3.37	0.07	0.98	1.62	<0.01	2.41	2.31	0.83	2.16	1.80	0.01	2.76	4.46	<0.01
N_{min} method	3.06	3.42	0.10	0.80	1.46	<0.01	2.52	2.18	0.41	1.96	1.69	0.04	1.80	3.66	<0.01
P level	0.72	0.77		0.27	0.19		0.81	0.70		0.15	0.29		0.04	0.08	
P level fertilization	0.66			0.09			0.86			0.09			0.01		
P level cultivation	0.02			<0.01			0.46			<0.01			<0.01		
Marketable yield (kg/m²)	Flat bed	High bed	P level	Flat bed	High bed	P level	Flat bed	High bed	P level	Flat bed	High bed	P level	Flat bed	High bed	P level
Traditional fertilization	1.37	2.16	<0.01	0.22	0.75	<0.01	1.29	1.20	0.70	1.26	1.11	0.09	0.75	1.96	<0.01
N_{min} method	1.49	2.22	<0.01	0.20	0.63	<0.01	1.40	1.17	0.33	1.20	1.06	0.12	0.19	1.22	<0.01
P level	0.44	0.57		0.88	0.16		0.68	0.88		0.47	0.42		0.10	<0.01	
P level fertilization	0.36			0.22			0.91			0.23			<0.01		
P level cultivation	<0.01			<0.01			0.33			0.02			<0.01		

[a] N_{min} method only for basal fertilizer application

Level of soil N_{min} concentration depended largely on season. High nitrification potential was evident particularly for the flat-bed system during the dry winter season. Much less plant biomass was produced on flat beds compared to high beds during the preceding rainy season. Thus, since N fertilizer application rates were the same, much less N was removed from the soil system during that season when the soil was wet and partially anaerobic most of the time. Under anaerobic conditions, nitrification of ammonium fertilizer is largely inhibited and NH_4 is easily immobilized by soil microbes or fixed to clay minerals (Drury and Beauchamp 1991[1]). Following drying of the soil in winter and intensive aeration through management (e.g., bed construction), this immobilized or fixed N obviously reappeared in the oxidized N form, leading to immense NO_3 accumulation. We may infer from this preliminary evidence that excessive leaching of nitrates during the rainy season is not as important as assumed.

Since soil water content in the topsoil averaged less in the high than low beds, plant available N was produced more readily, and less nitrogen for current plant biomass accumulation was lost through immobilization and fixation. Permanent high beds in combination with use of the N_{min} method was the most appropriate management option for rainy season summer vegetable production in this experiment.

Integrated study of soil N, plant N, and crop yield

Vegetable soybean. The most satisfactory fit of yield and nitrate data was obtained for 4 WAS when plants set flowers and the first side-dressing was applied. While the fit was good for the regression plant sap NO_3 = f(soil NO_3), crop yield was only poorly related to soil nitrate content (0-60 cm depth; table 3). Scatter plots in fig. 2 illustrate significant differences between cultural systems in the ability to take up available soil nitrate and to reduce this absorbed NO_3 efficiently to amino acids. Although plant sap nitrate accumulation was significantly higher for vegetable soybean grown on high beds, these higher concentrations resulted in relatively low yields compared to the crop grown on flat beds.

Table 3. Regression equations of the hyperbolic form $V = (V_{max} \times S)/(K_m + S)$ for soil NO_3 (0-60 cm), plant sap NO_3, and yield of vegetable soybean and Chinese cabbage grown on flat beds and permanent high beds

Function type	Flat bed system Equation[a]	r^2	High bed system Equation[b]	r^2
Vegetable soybean				
plant NO_3 = f (soil NO_3)	$V = (2751^{*} \times S)/(115^{ns} + S)$	0.89^{ns}	$V = (4142^{**} \times S)/(62^{**} + S)$	0.53^{**}
yield = f (plant NO_3)	$V = (1.46^{*} \times S)/(299^{ns} + S)$	0.50^{ns}	$V = (2.12^{*} \times S)/(366^{**} + S)$	0.45^{*}
yield = f (soil NO_3)	$V = (1.30^{*} \times S)/(115^{ns} + S)$	0.33^{ns}	$V = (1.10^{**} \times S)/24^{ns} + S)$	0.17^{ns}
Estimated function				
yield = f (soil NO_3)	$V = (1.32 \times S)/(11 + S)$		$V = (1.12 \times S)/(29 + S)$	
Chinese cabbage				
plant NO_3 = f (soil NO_3)	$V = (7910^{ns} \times S)$	0.85^{ns}	$V = (4584^{ns} \times S)/(70^{**} + S)$	0.54^{**}
yield = f (plant NO_3)	$V = (7.65^{ns} \times S)/(5978^{ns} + S)$	0.82^{ns}	$V = (9.89^{ns} \times S)/(2721^{**} + S)$	0.51^{**}
yield = f (soil NO_3)	$V = (5.51^{**} \times S)/(112^{**} + S)$	0.99^{**}	$V = (4.63^{**} \times S)/(8^{ns} + S)$	0.04^{ns}
Estimated function				
yield = f (soil NO_3)	$V = (4.36 \times S)/(75 + S)$		$V = (6.21 \times S)/(26 + S)$	

[a]3 df, [b]11 df

**, significant at 1% level; *significant at 5% level; ns not significant

[1] Drury, C.F. and Beauchamp, E.G. 1991. Ammonium fixation, release, nitrification, and immobilization in high- and low-fixing soils. Soil Sci. Soc. Am. J. 55:125-129.

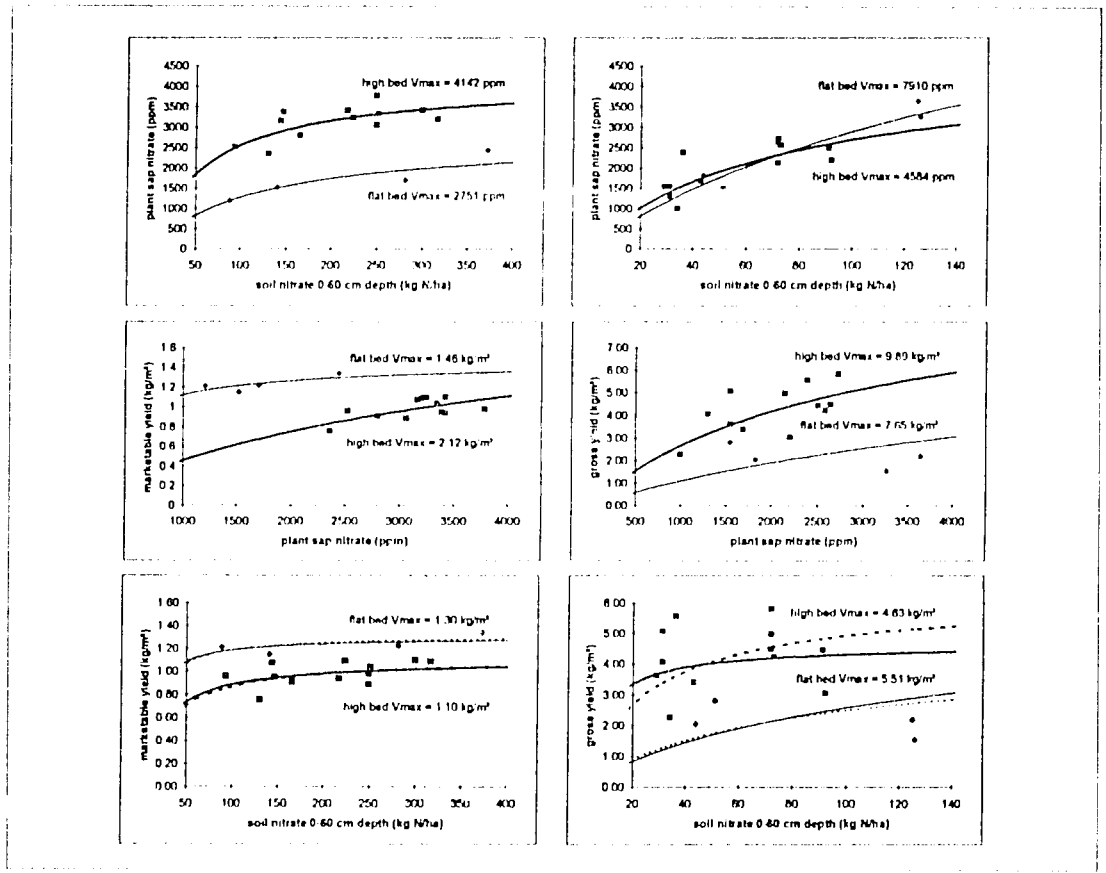


Fig. 2. Hyperbolic regression curves for functional relationships between soil NO_3, plant petiole sap NO_3, and crop yield

(left: vegetable soybean; right: Chinese cabbage; thick line: permanent high beds; thin line: flat beds; straight line: regressed function; dotted line: estimated function)

Regression equations for marketable yield as a function of plant NO_3-N overestimated yield potentials. Although low in r^2 and P, estimated functions of yield vs. soil NO_3 predicted upper limits of productivity that were almost precisely realized by the crop.

Small differences between yield and calculated yield potential suggest that soil N was not a factor limiting the growth of this crop, irrespective of cultivation system. This is confirmed by a lack of significant differences in yield between N_{min} and standard fertilization method (table 2). Excessive root growth particularly in the early growth stages at the expense of assimilatory leaf area (as indicated by crop cover and root distribution; data not shown), leading to a delay in crop maturity can explain these differences.

Chinese cabbage. Soil nitrate and plant sap nitrate fitted best to gross yield data when collected 4 WAT, before application of the second side-dressing. Marketable yields in Chinese cabbage during the rainy season are largely affected by soft rot (*Erwinia carotovora*) and poor head formation. Soil and plant analysis cannot account for these factors.

In contrast to the vegetable soybean crop, scatter plots of plant sap nitrate vs. soil nitrate (fig. 3) show that soil nitrate uptake of crops grown on either flat beds or high beds was not different, whereas

efficiency of transformation of absorbed NO_3 to biomass [yield = f(plant NO_3)] was. With similar NO_3 concentrations in plant sap, Chinese cabbage yielded much better on high beds than on flat beds. The regressions for plant sap nitrate = f(soil NO_3) produced upper limits for plant sap nitrate concentrations that were distinctly higher than those measured in the crop. The same is true for the relationship yield = f(plant NO_3). Regressions indicate yield potentials that could have been reached if plant sap nitrate concentrations were much higher. Soil nitrate content at the end of the cultivation period resulted in suboptimum plant sap nitrate levels that were possibly too little to support maximum yields. If the higher plant sap nitrate concentrations as recorded 2 WAT (4473 ppm in high beds, 5293 ppm in flat beds) were maintained, it may have been possible to achieve much better yields.

For the summer crop of Chinese cabbage, the limited NO_3 supply from the soil system most likely restricted yields. Since reduced N fertilization in the N_{min} treatment did not significantly reduce gross yields compared to the traditional N application rate, both nitrogen application rates were too low given the reduced nitrification potential (= low fertilizer efficiency) of the soils under rainy season conditions.

The differences between cultural systems for reducing this absorbed NO_3 efficiently to amino acids for accumulation of biomass and yield can possibly be attributed to soil-plant water relations. This indicates higher water stress (reduced water intake through anaerobic conditions in the root zone) of Chinese cabbage on flat beds and other physiological disorders.

To sum up, under our experimental conditions the integrated analysis of soil N, plant sap N, and crop yield using the hyperbolic approach of the Michaelis-Menten model of saturation kinetics resulted in a sufficiently well-determined, mutual functional relationship for vegetable soybean. This was expressed by the similarities of parameters of regressed and estimated functions for yield = f(soil NO_3-N). Besides highlighting deficiencies in plant nutrition, the significance of petiole sap NO_3 analysis lies also in using the dependency of plant NO_3 = f(soil NO_3), which is usually highly determined, to estimate the relationship yield = f(soil NO_3), which is commonly poorly correlated.

For vegetable soybean, the analysis was able to show that no limitations in plant nutrition occurred and that other factors contributed to the somewhat poorer performance of the crop on permanent high beds. For Chinese cabbage, however, large differences between calculated upper limits for plant NO_3 and measured concentrations revealed suboptimum nitrogen nutrition of this high N-demanding leafy vegetable during the rainy summer. High soil water contents inhibited nitrification of ammonium fertilizer, even when applied in large doses.

Nitrogen Management in Vegetable Cultivation and the Environment

Summary

The efficient use of nitrogen (N) by vegetable crops is the best way to reduce environmental problems due to N. Therefore, the behavior of three major mineral N components in vegetable cropping sequences was studied: (1) plant recovery, (2) leaching losses, and (3) residual amounts in soil at harvest. The following comprehensive experimental results were obtained.

Uptake and recovery of applied fertilizer N by Chinese cabbage were distinctly different in spring and autumn crops. Autumn cabbage utilized much more fertilizer N than that of spring, whereas the latter utilized much more indigenous soil N. Spring cabbage depended more on the soil N supply sources, suggesting that the mineralization process of soil N differed between two seasons.

Large nitrate leaching losses occurred in the onion-growing field, especially in plots with applied pig manure. As a result, the nitrate concentration in deeper soil layers increased over the limit established by the World Health Organization (WHO) for drinking water. During paddy rice growing, however, the enhanced level of nitrates in deeper soil layers decreased gradually due to the reduction of nitrates under anaerobic breakdown.

The residual levels of mineral N in soil were remarkably different between crops. Chinese cabbage growing resulted in huge residuals. A high level of residual N in soils is not only wasteful but also contributes to environmental pollution. Hence, measures to minimize it by better fertilizer management or recovery by succeeding croppings should be considered.

Introduction

Of the commonly used fertilizer elements, N is the most important one, being required by vegetable crops in a substantial quantity over and above the limited natural supply. It is directly responsible for plant growth and economic yields. Vegetable roots have a limited ability to absorb nutrients from the soil, yet a high nitrogen concentration in plant tissues is necessary to sustain their fresh look and softness. In vegetable cultivation, plenty of N is always applied, usually much more than is recovered. This means that only a part of the N is utilized by the crop, and that a large release of N occurs in the environment. This is not only wasteful but it also pollutes the environment.

Nitrogen is lost from vegetable fields by leaching of nitrates, by the denitrification to N_2O or N_2, by volatilization of ammonia, by runoff water of soluble N, and by erosion of soil. These losses create environmental hazards.

There have been many marked, detrimental environmental effects of N on a global scale. Today, many people in the world are very concerned about so-called "global change." Nitrogen dynamics is closely associated with this, and, hence, vegetable cultivation as well.

Most attention is on nitrates. NH_4-N applied in soil as fertilizer is transformed into nitrates by the action of nitrifying bacteria. The nitrates are leached to deeper soil layers and finally reach the groundwater used for human consumption. This contamination has led to increased interest to reduce nitrate leaching from agricultural lands. The leaching rate increases with the amount of N applied. Thus, intensive use of nitrogen fertilizer has contributed to nitrate accumulation in groundwater. The intake of nitrate can cause methemoglobinemia, which results in the inability of the blood to transport oxygen. This is of little significance to adults but can be fatal to infants. This problem is more serious in vegetable cropping, especially in the regions with high rainfall such as the tropics.

The addition of nitrate and ammonia to lakes and ponds can increase the growth of aquatic plants, giving rise to eutrophication. The enhanced growth of aquatic plants, including algae, can lead to the depletion of O_2 in the water so that fish and other animals cannot live.

Ozone is formed high in the atmosphere where it provides a protective shield against excessive ultraviolet radiation. The destruction of ozone is catalyzed by NO. A possible source of NO is N_2O, which is produced by denitrification of nitrogen in soil; it has a lifetime of about 200 years, can diffuse into the upper atmosphere, and not only contributes to the destruction of the ozone layer but also has long-term greenhouse effects producing global warming.

To reduce the risks to the environment, elucidation of the dynamic nature of mineral N behavior in the vegetable field is indispensable.

The behavior of a mineral N vegetable production system consists of the following three major components: plant recovery, leaching losses, and residual N in soil at harvest.

This study was conducted to demonstrate the behavior of mineral N in the vegetable field.

Materials and Methods

As shown in table 4, a field experiment composed of three cropping sequences, viz., (1) onion (autumn), vegetable soybean (spring), paddy rice (summer); (2). Processing tomato (autumn), Chinese cabbage (spring), paddy rice (summer); (3) Chinese cabbage (autumn), cherry tomato (spring), paddy rice (summer), was conducted at AVRDC. Processing tomato and Chinese cabbage (autumn) were transplanted on 6 October 1993. Chinese cabbage was harvested from 19 November to 1 December and processing tomato was harvested from 29 December 1993 to 2 February 1994. Onion was transplanted on 13 October 1993 and harvested on 4 March 1994. Vegetable soybean was sown on 7 March and harvested on 18 May. Rice was transplanted on 24 July and harvested on 5 October. Chinese cabbage (spring) was transplanted 2 March and harvested 18-19 April 1994. Cherry tomato was transplanted 18 February and harvested from 25 April to 31 May 1994. Plot area was 30 m^2 (5 x 6 m) and the number of replications was three.

The design of fertilizer treatments was as follows: (1) chemical fertilizer only, (2) chemical fertilizer plus pig manure, and (3) no fertilizer. According to conventional practice, 120 kg N, 65 kg P, and 100 kg K/ha of fertilizer were applied for Chinese cabbage (spring and autumn). In the plots with pig manure, 20 t/ha of manure was applied, while 169 kg N, 78.5 kg P, and 179.2 kg K/ha of fertilizer were applied for onion. In the plots with pig manure, 20 t/ha of manure was applied. For processing and cherry tomato, 147 kg N, 55 kg P, 150 kg K, and 10 kg borax/ha of fertilizer were applied. In the plots with pig manure, 20 t/ha of manure was applied. In vegetable soybean cultivation, 60 kg N, 26.2 kg P, and 66.4 kg K/ha were applied. Twenty tons per hectare of pig manure was applied in pig manure plots. For rice cultivation, 84 kg N, 27.5 kg P, and 99.6 kg K were applied per hectare. For rice, no pig manure was applied. All basal fertilizers were applied as bands prior to transplanting or sowing (soybean), and all top-dressings were applied as side-dressing. Detailed fertilizer treatments are shown in table 5.

The varieties of crops used were: onion: Granex 429; processing tomato: UC 204 and cherry tomato: Red Cloud; vegetable soybean: AGS 292, Chinese cabbage: ASVEG No. 1 and rice: Taitung No. 8. Spacing was 50 x 40 cm for Chinese cabbage, 15 x 15 cm for onion, 50 x 10 cm for vegetable soybean, 100 x 40 cm for tomato, and 25 x 20 cm for paddy rice. Total N was determined by the Kjeldahl distillation method and, using the condensed distillate, ^{15}N was analyzed by mass spectrometer. For the collection of leaching water, a porous cup was installed at definite depths of soil layers, viz., 10, 20, 30, 40, and 100 cm, respectively, in each treatment. Sample water collected in the cup was sucked into the sampling bottles by a vacuum pump as illustrated in fig. 3. NO_3-N was determined by ion meter (Horiba N-8M) immediately after sampling.

Table 4. Design of field experiment

Treatment			
		I. Cropping sequence	
No.	Autumn	Spring	Summer
1	Onion	Vegetable soybean	Rice
2	Processing tomato	Chinese cabbage	Rice
3	Chinese cabbage	Cherry tomato	Rice
		II. Fertilization	
1	Chemical fertilizer use only (inorganic)		
2	Chemical fertilizer + pig manure (organic)		
3	No fertilizer		

Table 5. Chemical fertilization treatments

Crops		N	P	K		Timing
Onion	Basal	84	47.1	99.6	band	at transplanting
	1st top	34	15.7	39.8	side	10 DAT
	2nd top	34	15.7	39.8	side	30 DAT
	3rd top	17	0	0	side	50 DAT
Soybean	Basal	20	26.2	66.4	band	at sowing
	1st top	20	0	0	side	at flowering
	2nd top	20	0	0	side	at seed development
Rice	Basal	28	27.5	49.8		at transplanting
	1st top	28	0	49.8		10 DAT
	2nd top	28	0	0		at panicle primordia initiation
Chinese cabbage	Basal	60	39	50	band	at transplanting
	1st top	30	13	25	side	10 DAT
	2nd top	30	13	25	side	20 DAT
Tomato	Basal	63	31.5	75	band	at transplanting
	1st top	21	0	25	side	15 DAT
	2nd top	63	23.5	50	side	30 DAT

Within the plus pig manure plots, 20 t/ha of the manure was applied prior to transplanting, except for paddy rice

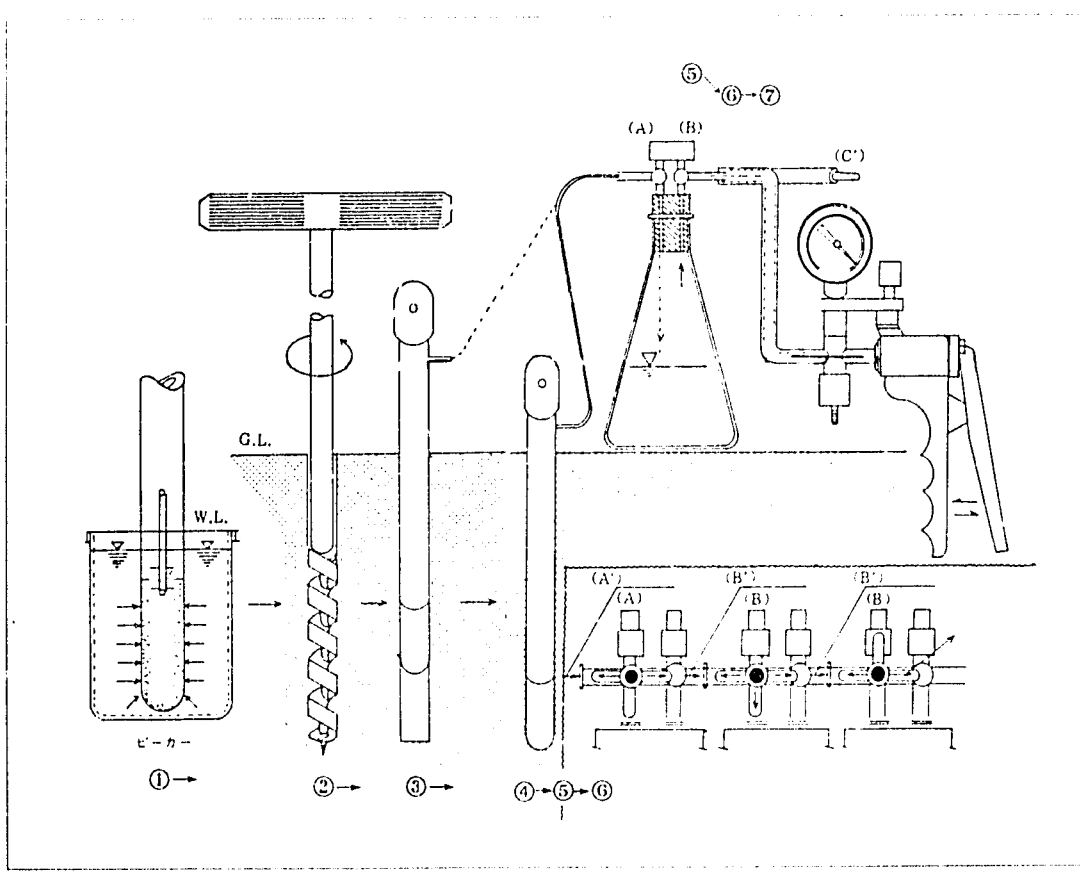


Fig. 3. Apparatus for collection of leaching water

Results and Discussion

Plant recovery

As illustrated in fig. 4 and 5, recoveries of N by Chinese cabbage from alternative sources were clarified by using the ^{15}N stable isotope tracer method. Data revealed that there were remarkable differences in fertilizer-N utilization by cabbage in the autumn and spring. Chinese cabbage grown in autumn utilized 68.3 kg N/ha from the fertilizer of which 28.7 kg was derived from basal application. Spring cabbage utilized only 33.3 kg of fertilizer N of which 15.9 kg was derived from basal application, and the rest from topdressing. Exactly the same amount of fertilizer N was applied in the same manner in both seasons. The difference was partly because there were large quantities of residual mineral N in the soil after tomato cropping (spring, Chinese cabbage), and mostly because yields were very different between seasons. In contrast, in chemical fertilizer plots spring cabbage utilized 51 kg N from the soil, whereas autumn cabbage utilized only 33.2 kg. The rates of soil-N mineralization differed between the two seasons.

Nitrate leaching losses

Nitrate leaching losses were studied throughout the onion-soybean-paddy rice cropping systems since poor fertilization management is responsible for nitrate groundwater pollution. Results obtained were as follows.

Nitrate leaching from onion field. As illustrated in fig. 6, remarkable NO_3 leaching losses occurred immediately after the beginning of the experiment in November from the N fertilizer applied in the interplots. ^{15}N enriched potassium nitrate was used as the N source. Nitrate leaching, however, declined rapidly thereafter with a gradual increase in NO_3 in deeper layers until January. This tendency was distinctly observed in pig manure plots; as a result, nitrate concentration in 100-cm-deep pig manure plots exceeded the WHO limit of 10 ppm for drinking water standards as shown in fig. 7. The reason for the acceleration of nitrate leaching with the addition of pig manure is still not clear, but Cl^- in the pig manure played a crucial role.

Nitrate leaching from soybean field. Overall nitrate leaching in the vegetable soybean field was sluggish (fig. 8) compared with that of onion because N input was low. In contrast to the onion cultivation, there was no remarkable difference in the nitrate concentration between upper and deeper soil layers because of the relatively higher levels of deeper ones. Data revealed that at the start of soybean growing, nitrate concentration at the 100-cm depth was as high as 60 ppm, suggesting that nitrate derived from the preceding onion cropping still remained in the deeper soil layers.

Nitrate leaching from paddy field. Fig. 8 shows that during the rice growing period from July to September, nitrate levels in all soil layers were extremely low compared with the preceding two croppings, except for the 100-cm depth of the early period. This is because nitrification does not proceed under anaerobic soil conditions, and the reduction of nitrates proceeds very fast, although dilution by irrigation water contributes as well.

It could be concluded that introduction of paddy rice into the cropping systems is very effective, not only in reducing the buildup of pest populations and pathogens, but also in preventing groundwater from being polluted with nitrate.

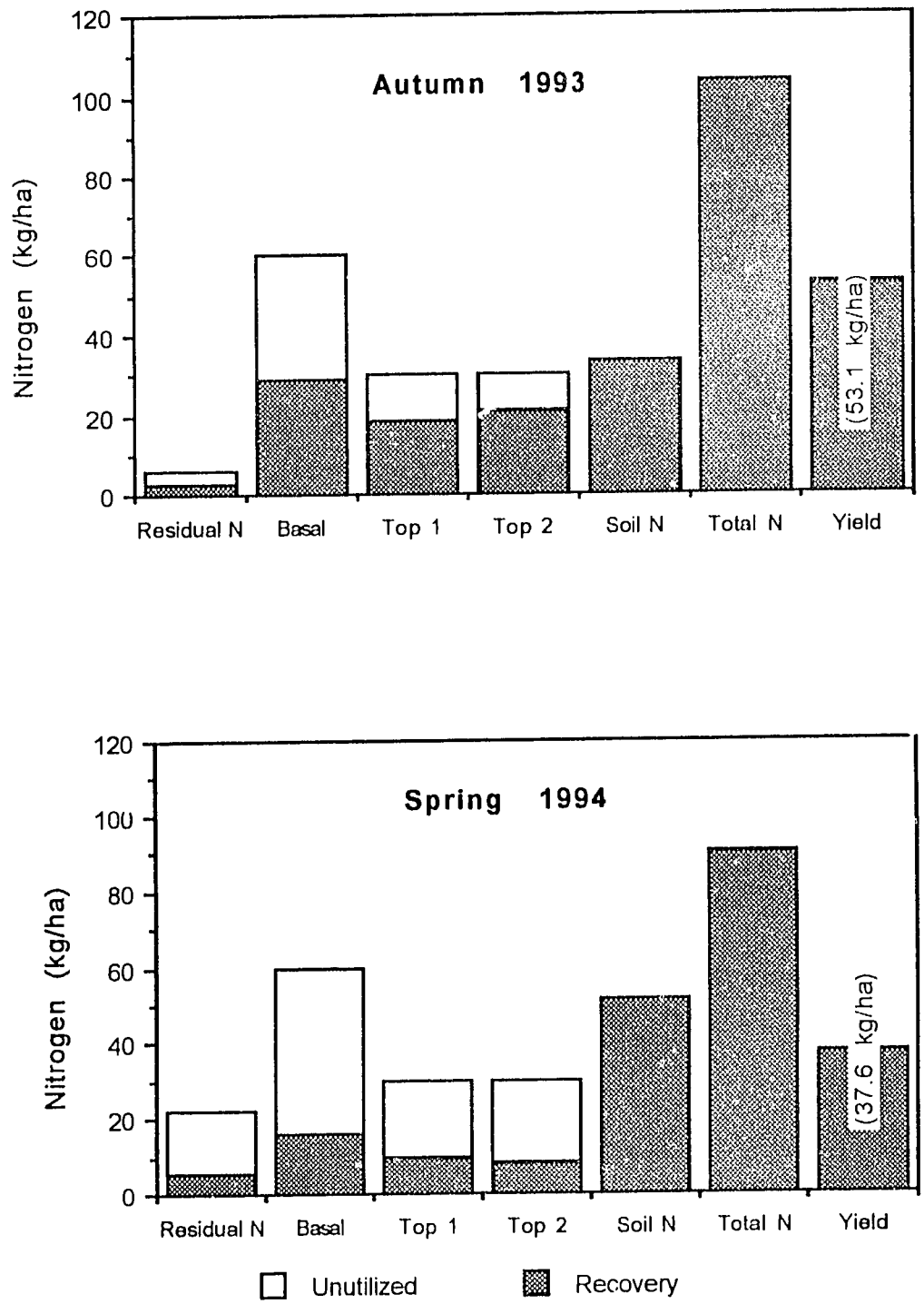


Fig. 4. Nitrogen recovery by Chinese cabbage (chemical fertilizer plot)

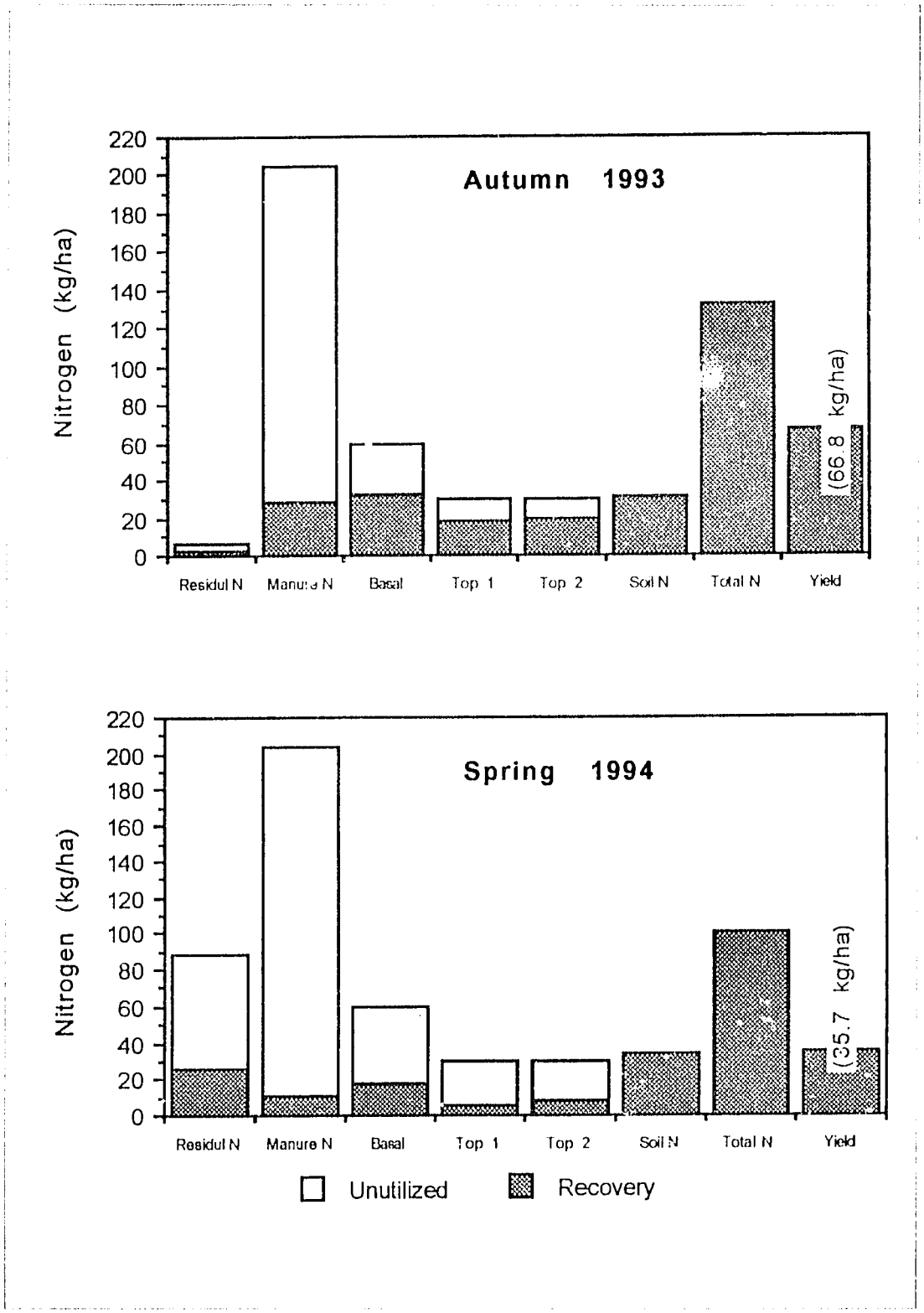


Fig. 5. Nitrogen recovery by Chinese cabbage (fertilizer plus pig manure plot)

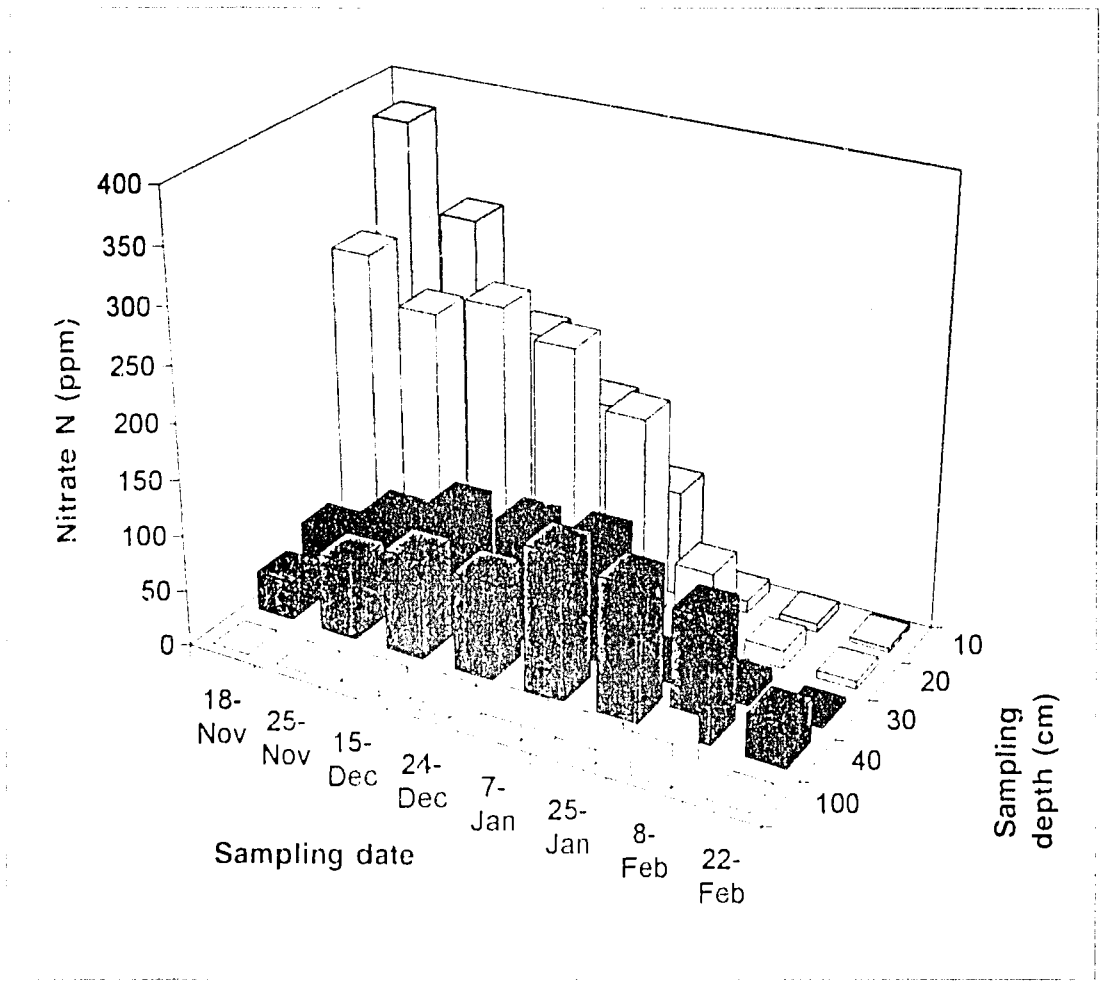


Fig. 6. Nitrate leaching from chemical fertilizer plots

Residual N levels in soil

High levels of N fertilizer are often needed by vegetable crops for maximum yields, but recoveries of this N are frequently small and considerable quantities of mineral N remain in the soils at harvest. This N is a potential environmental pollutant. Better N fertilizer management means minimizing the mineral N left in soil. Therefore, residual mineral N in soils at harvest was examined. It was found that large quantities of mineral N were left in the soil after Chinese cabbage growing; the amount was much less after onion cropping since much was leached out (fig. 6). Comparing among treatments, fertilizer plus pig manure exhibited an apparently higher remaining rate than chemical fertilizer, except for onion cultivation as shown in fig. 9. Tracer experiments clearly indicated that most fertilizer N was in the upper soil layer, and remaining rates were apparently higher in the case of basal application than those of topdressing (fig. 10). Since ^{15}N analysis is not yet complete, it is not known how much residual N was recovered by the succeeding crops. However, this study implied that an appreciable quantity of residual N could be recovered by succeeding crops, because spring cabbage after tomato revealed an unexpected higher utilization of untagged N composed of soil and residual N. The results suggest that in a vegetable production system it is necessary to take into account residual N at harvest prior to sowing additional crops.

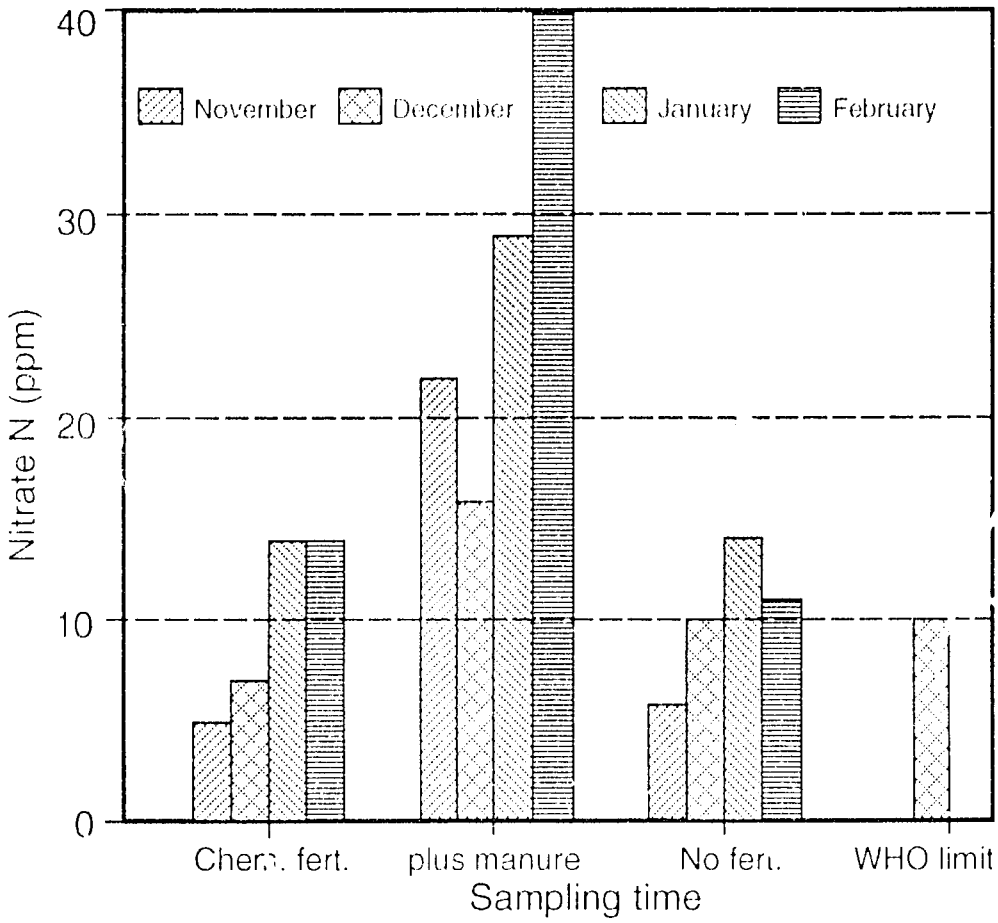


Fig. 7. Nitrate N concentration in 100-cm depth leachates

Souma[2] classified the growth characteristics of vegetables into three types: (1) only vegetative growth, (2) simultaneous vegetative and reproductive growth, and (3) initial vegetative growth, later to change to reproductive growth. Chinese cabbage belongs to type 1. Consumers use stems and leaves harvested at a relatively early growth stage. Since plant growth is still vigorous, yellowing occurs unless the N supply is sufficient at that time. Therefore, it is inevitable that a considerably large amount of mineral N remains in the soil after growing Chinese cabbage, but it must be minimized.

Tomato belongs to type 2, which produces fruit along with vegetative growth. Fruit production increases along with vegetative growth so that the total amount of N taken up by plants also concurrently increases until the final growth stages. Accordingly, a continuous N supply throughout the plant growth is indispensable. As a result, an appreciable amount of mineral N is always left in the soil at harvest.

[2] Souma, A. 1986. Present status of vegetable soils in Hokkaido and fertilizer management with response to growth characteristics of vegetable species. Tech. Bull. of Hokkaido Pref. Agri. Exp Stn. 56: 59-69.

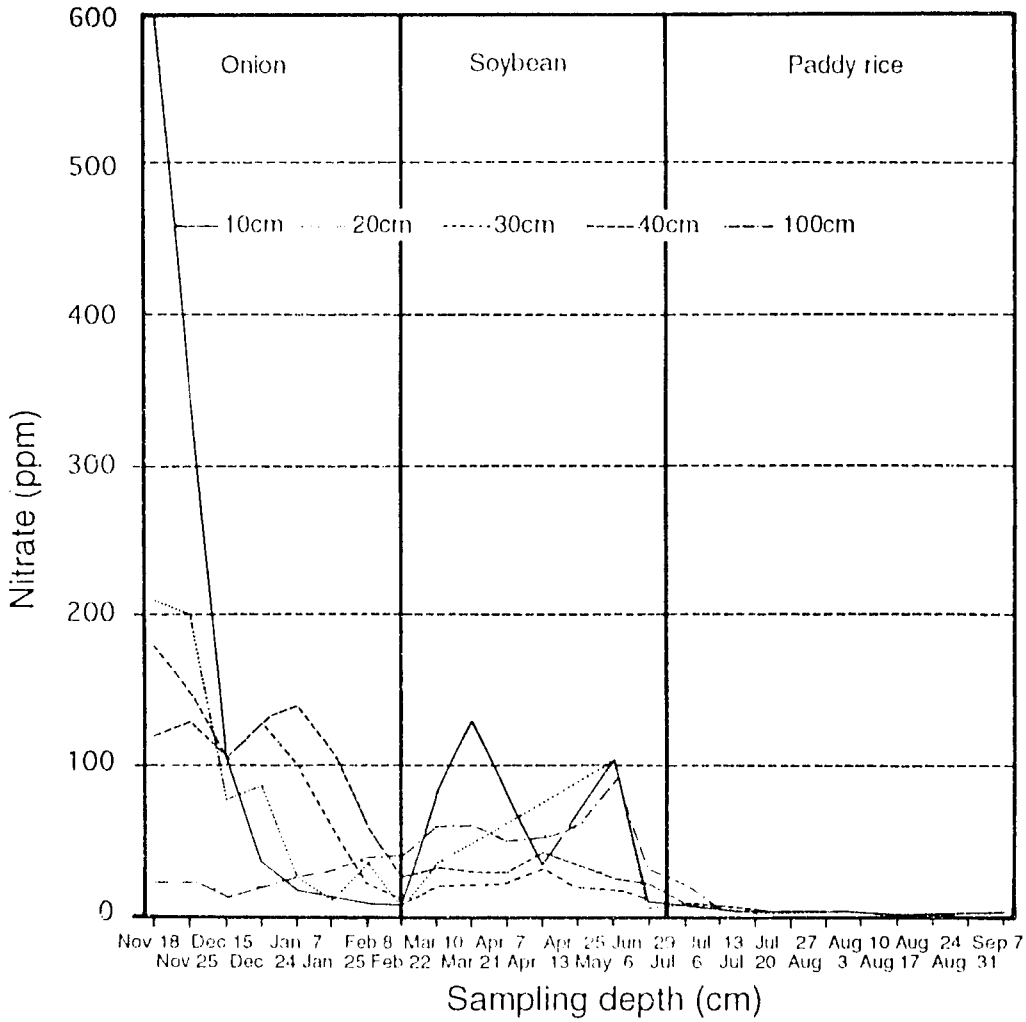


Fig. 8. Nitrate leaching from paddy field (plus manure)

Onion belongs to type 3. This type of vegetable accomplishes maximum N uptake during the transition period, which is from the vegetative growth to the reproductive growth stages.

Thus, sufficient N supply at the later growth stage is usually unnecessary. Therefore, the amount of mineral N in soil at harvest is usually very small.

Based on these findings, it is recommended that after Chinese cabbage growing, the succeeding crop be planted as soon as possible. Also reducing basal N or changing the cropping system, for example, relay-planting of Chinese cabbage and eggplant, is suggested.

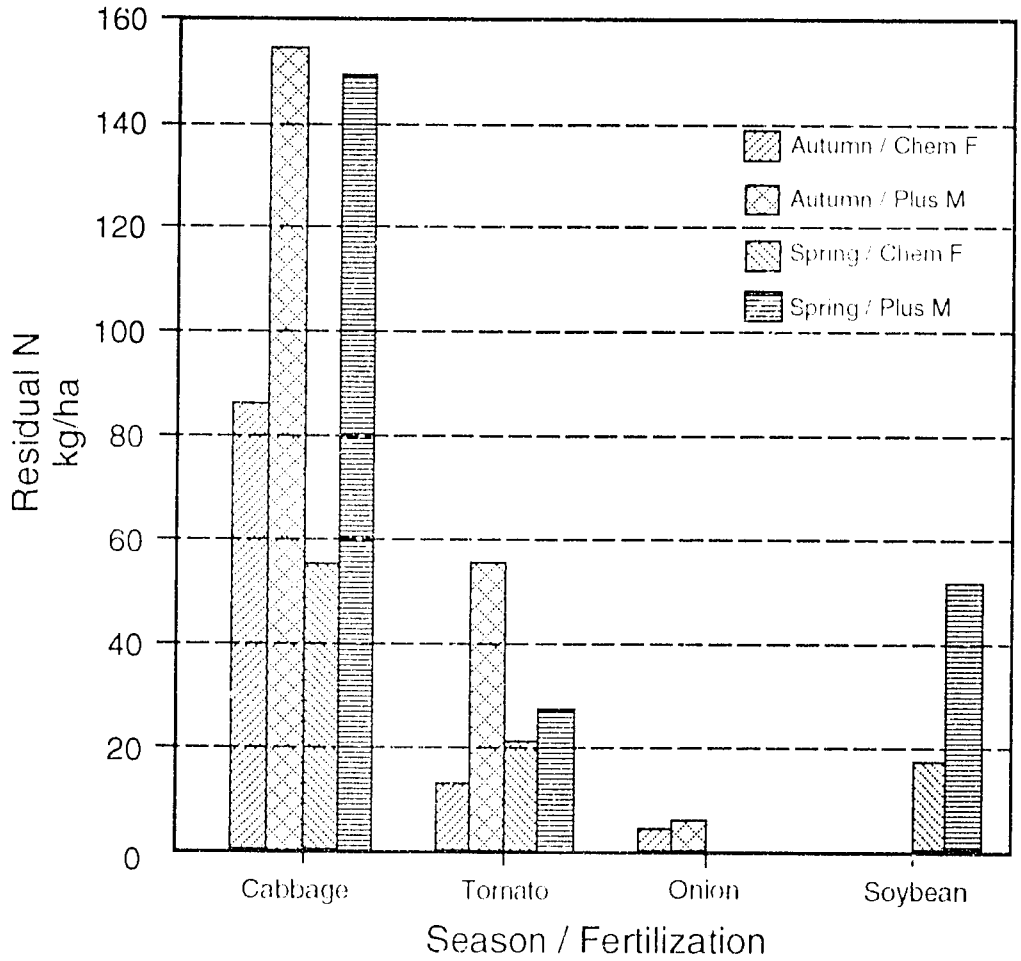


Fig. 9. Residual mineral N in soil at harvest

Relationship between yield and N recovery

Correlations were calculated between yield recoveries of N by Chinese cabbage from different N sources (table 6). Results clearly show that there was a high correlation between yield and recoveries of fertilizer N, especially basal applied N, while there was no significant relationship between yield and utilized soil N by plants.

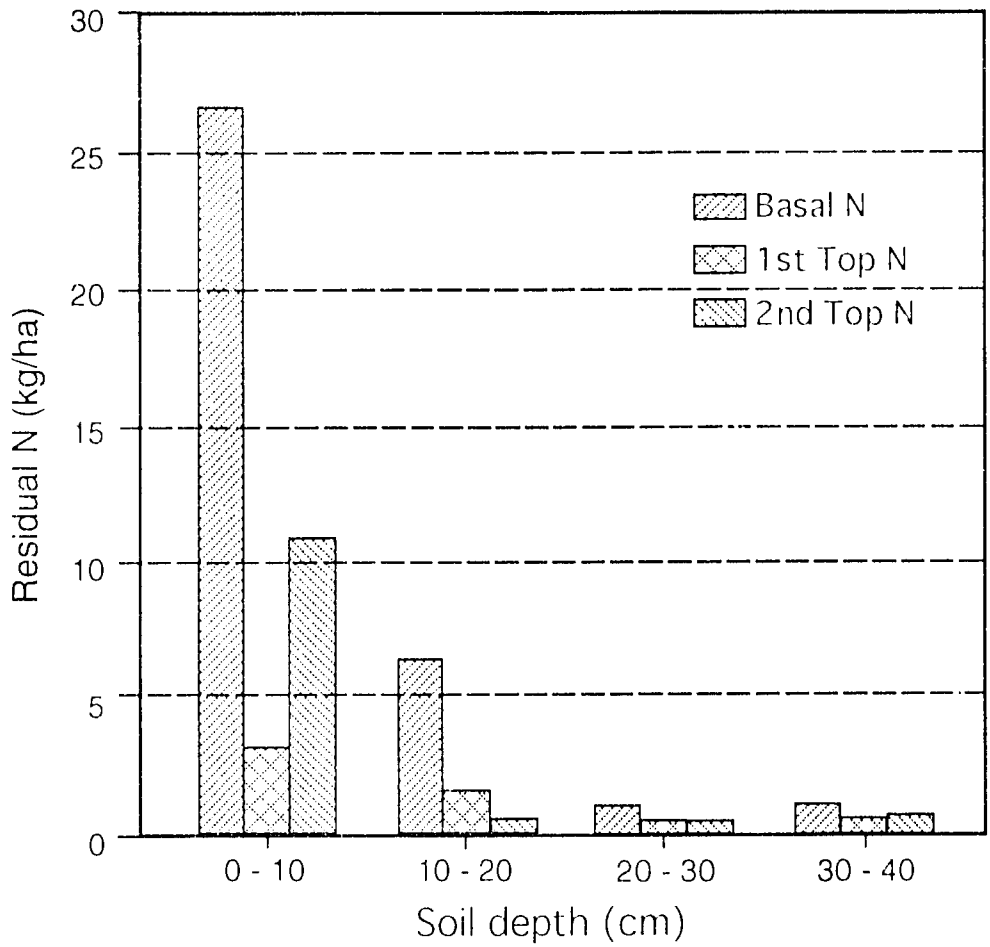


Fig. 10. Residual fertilizer N in soil after cabbage

Table 6. Relationship between different levels of nitrogen application and yield

Variables		A	B	Correlation
Yield x fert. N	12	-15.836	1.378	0.937***
Yield x soil N	12	55.38	-0.379	-0.582*
Yield x total N	12	53.23	1.107	0.906***
Yield x basal N	4	-1.892	0.526	0.964*
Yield x top 1 N	4	-6.200	0.398	0.902
Yield x top 2 N	4	-7.744	0.453	0.900

Linear regression equation: Y = a + bX, where Y = yield, X = nitrogen

* Significant at 5% level; *** significant at 0.1% level

Integrated Pest Management

Exploration for Entomopathogenic Fungi for IPM of Vegetable Pests

Summary

Integrated pest management entails the use of many different strategies for the management of vegetable pests. One integral component of this system is the use of biological controls. Insect-killing fungi are becoming increasingly important as viable alternatives to chemical insecticides. Explorations were done in vegetable fields in Taiwan to collect fungal isolates for future development. Over 400 isolates were recovered. Of these, 63 have been identified as true entomopathogenic fungi. An additional 110 isolates were classified as potential entomopathogens and warrant further testing to determine their utility.

Introduction

Most insect pests of vegetables have developed resistance to insecticides. Because of this and increasing demands by consumers for higher production and improved quality, alternative methods for pest management are being explored. Increased emphasis is being placed on biological control strategies to reduce reliance on agricultural chemicals and establish more sustainable systems.

The use of insect-killing fungi has been an underutilized resource. With recent improvements in mass-culturing techniques and new technologies for formulating isolates, their usage has become more feasible. We initiated exploration studies in Taiwan to develop a bank of entomopathogenic fungi as components for the integrated pest management of vegetables.

Materials and Methods

Exploration was confined primarily to agricultural lands being used for the production of eggplant, cucumber, onion, bottle gourd, and watermelon (fig. 1). These crops are commonly infested with *Thrips palmi* (Thysanoptera: Thripidae), a serious insect pest which has developed resistance to most insecticides. Three methods were used to locate entomopathogens. Leaves were sampled, dried at ambient temperatures in the laboratory for ~24 hours, and searched for thrips showing signs of fungal infection. Infected individuals appeared lethargic, moribund, off-color, have unusual dark spots on the epidermis (indicating points where fungi have entered the epidermis of the insect), or have obvious fungal growths arising from their bodies. Often sporulation was seen.

Second, soil samples were taken from a depth of 12 cm with a 7.5-cm-diam soil auger. The majority of *T. palmi* pupate at depths in the soil of 0-10 cm. These samples were then processed in the laboratory. Samples were extracted using a heptane flotation procedure. For this procedure, soil was washed with water through a series of three US standard sieves the top sieve (no. 18) with 1.00 mm mesh, the middle (no. 25) with 710 µm, mesh and the bottom (no. 170) with 90 µm mesh size. In

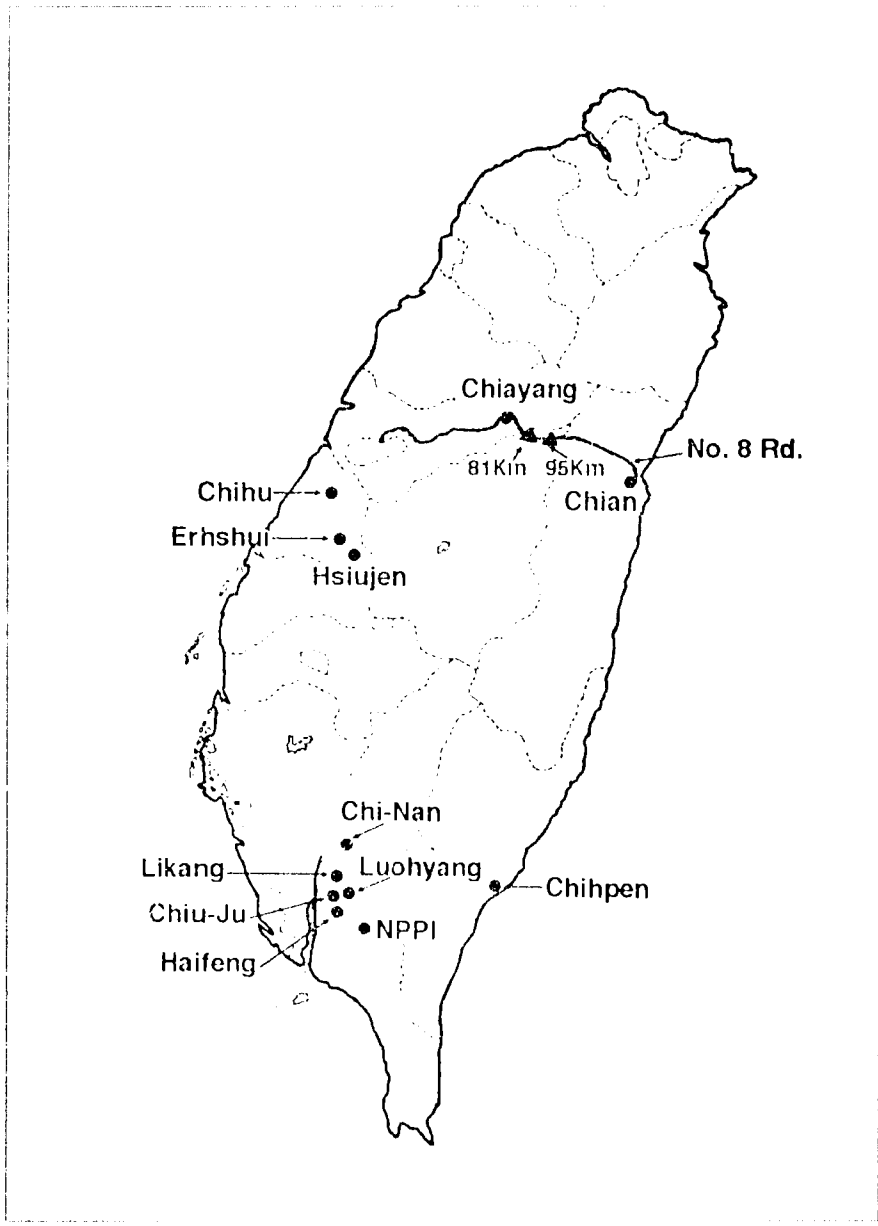


Fig. 1. Vegetable growing areas in Taiwan where entomopathogenic fungi were collected from *Thrips palmi* and soils

controlled experiments, 100% of the first instar *T. palmi* added to the bottom sieve and washed with water remained in the sieve. The residue in the bottom sieve was transferred to a 2-l separatory funnel. To the funnel we also added 1200 ml water and 50 ml heptane. The heptane formed a 3-mm layer over the water. The content was agitated for 10 sec, then after 1 min the lower water-organic matter layer was removed and discarded. Thrips remained in the heptane-water interface which was next drawn onto a filter over a Buchner funnel attached to a suction pump. Filters were examined under a binocular microscope and symtomatic thrips removed. These thrips were not surface-sterilized (as explained below) but immediately placed on selective media.

Last, additional soil samples were taken in an identical manner (as above), returned to the laboratory, and thoroughly mixed. From each sample three subsamples each weighing 20 g were placed in individual plastic cups. In each cup we placed three *Tenebrio molitor* larvae. These insects are extremely susceptible to fungal entomopathogens and very quickly show signs of infection. Because *T. molitor* normally remain at the soil surface, cups were inverted each day, causing the insects to crawl through the soil to the surface again. This behavior increased the chances of the insects coming into contact with any fungal spores in the soil. The insects in the cups were checked twice a week for 3 weeks and infected individuals removed.

Symptomatic *T. molitor* were then placed for 3-5 min in a 10% sodium hypochlorite solution for surface sterilization, rinsed in sterile distilled water, and placed on a selective medium. There are several selective media which help to eliminate nonentomopathogenic fungi. The ones used were:

I. Selective for *Metarhizium* sp. and other entomopathogens:

A/ Dodine stock solution:

Sylitt brand dodine fungicide	15.4 g
Absolute ethanol	100 ml

B/ Selective medium

Distilled H_2O	1 l
Agar	10 g
Oatmeal agar	36.25 g
Dodine stock	10 ml
Crystal violet	10 mg

Autoclave for 15 min, add 1 ml of antibiotic stock/liter of medium.

II. Selective for *Beauveria* sp.:

Mycological peptone (g/l)	10
Maltose	40
Yeast extract	10
Agar	15
Dodine	455 mg

The following antibiotics are filter sterilized and added after the above media have been autoclaved and cooled to 50°C:

Penicillin G	0.1
Streptomycin sulfate	0.5

Once growth on the selective medium was evident, standard pathological procedures were used in the laboratory for isolation, cleanup, and identification of isolates recovered. Fungal identifications were verified by Dr. Richard A. Humber, from USDA, ARS, Cornell University, Ithaca, New York, USA. Duplicates are in long-term storage in the USDA repository, Ithaca, NY, and AVRDC, Taiwan.

Results and Discussion

Four hundred-fifty isolates were collected. The vast majority were found using the baiting system with *Tenebrio molitor*. The major constraint during the fungal isolation procedure was contamination with bacteria which required the use of time-consuming cleanup techniques. The major species collected and where they were discovered are listed in table 1. Nearly all of the insect-killing fungi belong to Deuteromycetes.

Table 1. Summary of the fungal pathogens collected from agricultural fields in Taiwan

Fungal species	No. isolates	Location found
	True entomopathogenic fungi	
Metarhizium anisopliae	2	Hualien
var. *anisopliae*	1	Hsiujen
	1	Chiayang
	2	Hai Feng
	5	AVRDC
	2	Luohyang
	8	Pingtung
	13	Erhshui
Total	34	
Metarhizium anisopliae	6	AVRDC
(not identified to var.)	1	Chiu-Ju
	1	Hualien
	4	Luohyang
	4	Hai Feng
	10	Pingtung
Total	26	
Paecilomyces fumosoroseus	1	AVRDC
Paecilomyces farinosus	1	AVRDC
Paecilomyces sp.	1	AVRDC
Total	3	
	Possible entomopathogenic fungi	
Fusarium sp.	22	AVRDC
	1	Chiu-Ju
	8	Hai Feng
	13	Pingtung
	4	Chihpen
	7	Hsiujen
	5	Chihu
	35	Erhshui
	6	Chiayang
	9	No. 8 Rd.
Total	110	

Although not yet widely used, some entomopathogenic fungi are being mass-produced and sold commercially. *Verticillium lecanii* is sold for use in greenhouses in Europe. In parts of Asia, *Metarhizium anisopliae* is also commercially available. Isolates of *Beauveria bassiana* and *Paecilomyces* sp. will soon be on the market in the United States. The isolates collected in Taiwan may possess unique characteristics that make them suitable for use in tropical and subtropical areas for IPM of vegetables.

The next step in the development of fungi for IPM is the laboratory testing of each isolate for pathogenicity to major vegetable pests. Once pathogenicity levels have been established, each isolate will be characterized for responses to temperature and relative humidity. Select isolates will then be evaluated for growth and potential for mass production, then field-tested for pest management.

Development of a Procedure for Mass-producing Even-aged *Thrips palmi* Karney

Summary

Thrips palmi Karney is an important pest of vegetables in Taiwan and other countries in Southeast Asia. Its distribution is rapidly increasing. Within the last three years it has spread to North America and is considered a serious pest of vegetables in Florida. In the laboratory, trials were conducted to develop a mass-rearing technique for this insect on detached leaves of cowpea, mungbean, watermelon, and eggplant. Rearing on cowpea and eggplant resulted in higher production rates than on the other vegetables. At 25 °C, egg incubation, larval and pupal stadia took 4.5, 3.6 and 4.6 days, respectively. Approximately 40 even-aged larvae were produced from eggs laid by 20 adults on cowpea in acrylic rearing boxes.

Introduction

One of the major constraints to the production of vegetables is thrips. These tiny insects are difficult to control and are resistant to most insecticides. Alternative strategies are needed for implementing an integrated management system. Laboratory studies of biological mechanisms for management of thrips require large numbers of even-aged individuals. In addition, efficacy trials are more meaningful if the genetic stock of the test individuals is maintained and readily available for replication of investigations. No single method as published in the literature was adequate for mass rearing of *Thrips palmi* Karney. We studied a number of different techniques to develop a rapid, simple method for rearing this vegetable insect pest.

Materials and Methods

Developmental time

At the bottom of acrylic rearing boxes measuring 16.2 x 10.0 x 4.2 cm deep, we placed a layer of nonsurgical cotton. The cotton was saturated with water and eggplant leaves with short petioles were placed on top. The stems penetrated the cotton to facilitate rooting. To each of four boxes we introduced 20 adult *Thrips palmi* and allowed them to oviposit for 24 h. After adult removal, eggs were inspected regularly and the time of egg hatching recorded. Ten first instar larvae were then removed

and placed on fresh leaves in an identical box and their developmental time to second instar and pupation recorded. This study was replicated four times and done in an environmental chamber at 25°C and 12:12 LD.

Fecundity

In identical rearing boxes as above, but without the cotton layer, we placed an ELISA microplate template in the center and added water. To prevent thrips from drowning in the water, we placed a sheet of paper around the outer edges of the template. To each of four replicates we added either a single eggplant, cowpea, watermelon, or mungbean leaf. Next, we introduced 20 adults in each box and allowed them to oviposit for 48 h. Following egg hatch, immatures were counted. Rearing boxes were kept in an environmental chamber with the same parameters as the previous study.

Results and Discussion

Developmental time

Data on the duration of egg, larval, and pupal periods are summarized in table 2. The mean developmental periods for *T. palmi* on eggplant are 4.5 days for egg hatch, 3.6 days for larval period, and 4.6 days for pupation.

Table 2. Developmental periods for *Thrips palmi* reared on eggplant leaves in an environmental chamber at 25°C and 12:12 LD

Replicate	Developmental period (days)		
	Egg	Larvae	Pupae
I	4.5	3.2	4.7
II	4.5	3.6	4.6
III	4.5	4.0	4.2
IV	4.5	3.7	4.7
Mean ± SD	4.5 ± 0	3.6 ± 0.3	4.6 ±0.2

Fecundity

Even though the lids of the boxes were secured with rubber bands, many adults escaped. This influenced the number of eggs laid. Following 48 h of oviposition, only about 25% of the original adults released per box were recovered. With this recognized limitation, there were differences in the number of larvae found. The means were 39.7 larvae in boxes with cowpea leaves, 34.5 larvae in eggplant, 14.5 in mungbean, and 12.7 in watermelon (table 3).

Table 3. Reproduction of *Thrips palmi* on various host leaves held in an environmental chamber at 25° C and 12:12 LD

Host leaves	Replicate	No. of larvae
Eggplant	I	73
	II	22
	III	23
	IV	20
	Mean ± SD	34.5 ± 25.7
Cowpea	I	43
	II	49
	III	57
	IV	10
	Mean ± SD	39.7 ± 20.6
Watermelon	I	16
	II	16
	III	10
	IV	9
	Mean ± SD	12.7 ± 3.8
Mungbean	I	21
	II	13
	III	22
	IV	2
	Mean ± SD	14.5 ± 9.3

Effect of Application of a Soil Amendment on Bacterial Wilt Progress at Three Different Sites in Taiwan

Summary

Incorporating a soil amendment composed of urea (200 kg N/ha) and calcium oxide (5000 kg/ha) 2 to 3 weeks before transplanting tomato could reduce the disease progress of bacterial wilt at two sites in Taiwan. The disease rate (i.e., the slope of the logarithmically transformed disease progress against time) was reduced by half when the soil amendment was applied. This led to an increase in tomato yield of 150% compared to the nonamended treatment, but due to environmental interferences this difference was only significant at one of the two sites. At a third site, the disease progress ceased 2 weeks after transplanting, and by that time no effect of the soil amendment was evident.

Introduction

The use of a simple soil amendment composed of urea and calcium oxide is considered one of the key technologies besides crop rotation and the use of resistant tomato cultivars in the development of an integrated management of bacterial wilt. Bacterial wilt is a major soil-borne disease of solanaceous and other economically important crops in the tropics. Preliminary studies at AVRDC and CIP showed that this soil amendment can reduce the population of *Pseudomonas solanacearum*, which is

the causal agent of bacterial wilt in the soil. Field studies at AVRDC also showed reduction of wilt incidence in tomato 40 days after transplanting when the soil amendment was applied, but no information about the disease rate and the effect on yield is known yet. For this purpose, field experiments were conducted at three different sites in Taiwan during the summer.

Materials and Methods

A total of three field trials were conducted in summer 1993 at three different sites in Taiwan, i.e., AVRDC experimental farm (14 m above sea level); Puli Branch Station, Taichung DAIS (625 m asl); and Taiwan Seed Service (TSS, 470 m asl). Before starting the field experiment at AVRDC, the soil was artificially infested with *P. solanacearum*; the soils at Puli and TSS were naturally infested with *P. solanacearum*.

The soil amendment (SA) applied to reduce the population of *P. solanacearum* before transplanting tomato was a derivative of the SH-mixture developed in Taiwan. The two components of the SA were urea and calcium oxide (CaO). The rate used was 200 kg N/ha as urea (= 428 kg urea/ha) and 5000 kg/ha CaO.

The AVRDC field trial was designed as a split-plot, completely randomized experiment with three replicates; the two main treatments were infested and noninfested soil. Each main treatment was comprised of four subtreatments: SA, intercropping with cowpea, SA and intercropping with cowpea, and control. To infest the soil with *P. solanacearum* a bacterial wilt nursery was established in the plots of the main treatment "infested soil."

The field trials at Puli and TSS were conducted as a factorial experiment within a randomized complete block design. The two treatments were SA and control, and the number of replicates was five.

Two to three weeks before transplanting, the SA was broadcasted and immediately incorporated into the corresponding plots which had a bed width of 1.5 m including furrows. Two rows of cowpea were sown with a spacing of 0.6 m between rows and 0.1 m within the row. The transplanted tomato was an AVRDC line of determinate type (CL-5915-93-1-0-3). Four-week-old seedlings were transplanted in one row per bed with a spacing of 0.3 m between plants. At AVRDC, each subtreatment comprised of one plot of 1.5 x 6 m, and at Puli and TSS each treatment had three plots of 1.5 x 3 m (overall 4.5 x 3 m).

At AVRDC, the beds were covered with rice straw mulch after transplanting and furrow-irrigated. At Puli and TSS, the beds were covered with plastic mulch after incorporation of SA, and the irrigation effected by pipes under the mulch.

Disease ratings were made using the following scale (slightly modified after Winstead and Kelman 1953)[1]: 0 = no wilt symptoms; 1 = one leaf at least partially wilted; 2 = two to three leaves wilted; 3 = all but two to three leaves wilted; 4 = all leaves wilted; 5 = plant dead, i.e., at least half of the leaves completely dry. Disease index was calculated with the formula:

$$\frac{\text{sum of all reading}}{\text{number of all plant x 5}}$$

[1] Winstead, N.N. and Kelman, A. 1953. Inoculation techniques for evaluating resistance to *Pseudomonas solanacearum*. Phytopathology 42: 628-634.

Data were transformed with a "simple interest" transformation [ln 1/(1-x), where x = disease index] before calculating the disease rate for the SA and the control treatment. The slope of the linear regression of the transformed disease index plotted against time represents the disease rate. Only data after first visible symptoms of wilt were included for analysis. When two linear regressions at a location were significant, the two disease rates (i.e., their slopes) were compared with a t-test.

The yield data were transformed to relative yield basis with the control treatment mean = 100%. For the AVRDC trial, the control subtreatment mean of the infested soil was used as the basis for comparison. For disease ratings and for yield assessment, tomato plants at the end of each row were excluded.

Results

Disease progress

Intercropping did not affect the disease progress, neither with nor without the SA (fig. 2). The soil amendment slowed down the disease progress. However, the disease index of the treatments with SA was as high as 0.60 to 0.75 at 7 weeks after transplanting. Because of heavy infestation of black leaf mold, the tomato plants started to die weeks after transplanting, and rating of bacterial wilt was no longer possible.

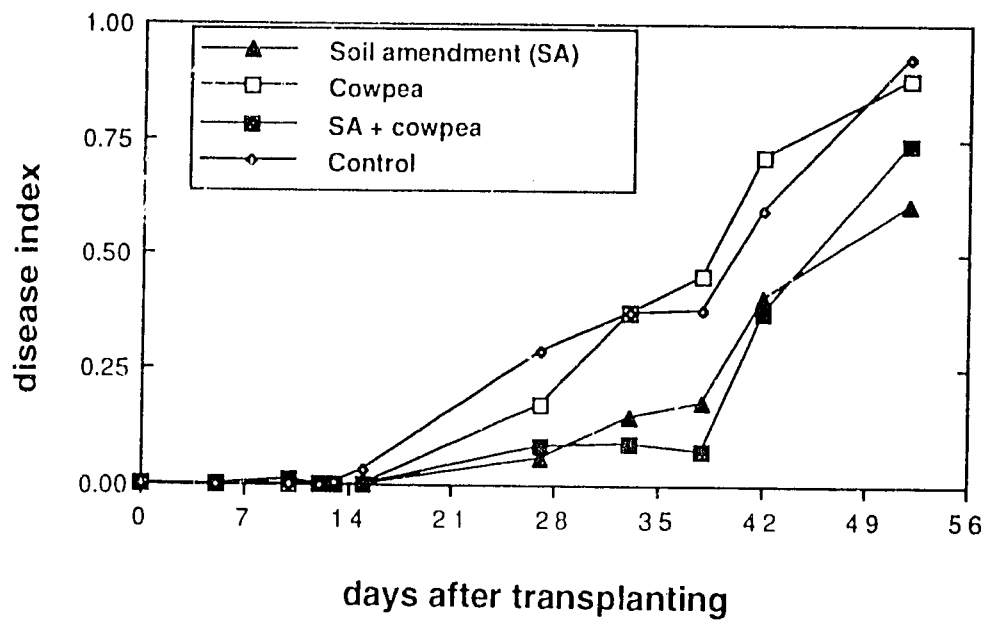


Fig. 2. Disease progress of bacterial wilt in the four subtreatments of the infested main treatment at AVRDC. Disease progress is expressed as disease index using the disease rating of Winstead and Kelman (1953)

A comparison of the SA treatment with the control showed differences among the three locations (fig. 3). At AVRDC and TSS, the SA reduced the disease progress, whereas at Puli no difference was evident between the two treatments. At Puli, wilting of tomato plants stopped after 2 weeks; initial wilting was more in the amended treatment. The reduction in disease progress by SA at TSS was clearly observable but not so marked as at AVRDC. Because of an error, the experiment could not be completed, and a final reading was not possible.

Disease rate

At AVRDC and TSS, the regressions for the determination of the disease rate were significant, but not at Puli (table 4). The transformation of the disease index fitted TSS data better with very high r^2 values than for AVRDC. The disease rate in the control in both locations was double that of SA, but the difference was significant only at TSS.

Relative yield

At AVRDC and TSS, the yield increase in the SA treatment compared to the control was about 150% (table 5), but the difference was significant only at TSS. No such difference occurred at Puli. At AVRDC, in infected soil the treatments with cowpea intercropping had higher yields than the control but lower yields than the SA alone. In the noninfested plots, the highest yield was measured in the cowpea intercropping treatment without SA where it was four times as high as in the infested control; this difference was significant at P = 5%.

Discussion

A reduction in the bacterial wilt progress in tomato with SA was demonstrated at two of the three locations in Taiwan. The yield was higher in two locations when SA was applied. The difference, however, was not significant at AVRDC, which is probably due to large variance. The reasons for this variance were the very low fruit-set and the incidence of black leaf mold which led to the premature end of the field experiment after 2 months.

At TSS, the second site with higher yield of the SA treatment, only a first harvest 8 weeks after transplanting was taken, yet the yield was significantly greater in the SA treatment. Analysis of the yield data at Puli showed that no significant difference occurred between the two treatments, neither for total yield nor for each sequential yield measured during the experiment (data not shown).

The absence of bacterial wilt at Puli 2 weeks after transplanting might be due to the moderate resistance to bacterial wilt of the tomato line used and the somewhat cooler conditions at Puli. The initial disease progress can be explained by injuries due to transplanting, which most probably increased the probability of infection of the tomato roots by *P. solanacearum*.

In the locations where the disease rates were determined, rates differed by a factor of 2 between the SA and control treatments. The higher number of replications and better environmental conditions at TSS might be responsible for the better fit of the data at TSS. For the same reason, probably the yield difference was only significant at TSS, even though the absolute difference between the two treatments was the same as at AVRDC.

In the subplots where intercropping of cowpea was combined with the SA, all cowpea seedlings died and had to be resown 1 week after adding the SA. This indicated the general toxic effect of the SA on living organisms. Further studies at AVRDC showed that the *P. solanacearum* population in the soil declined rapidly after mixing the amendment with soil. This presumably toxic effect might have also affected the tomato plants and was the reason for extending the time between adding the SA and transplanting tomato from 2 weeks at AVRDC to 3 weeks at Puli and TSS.

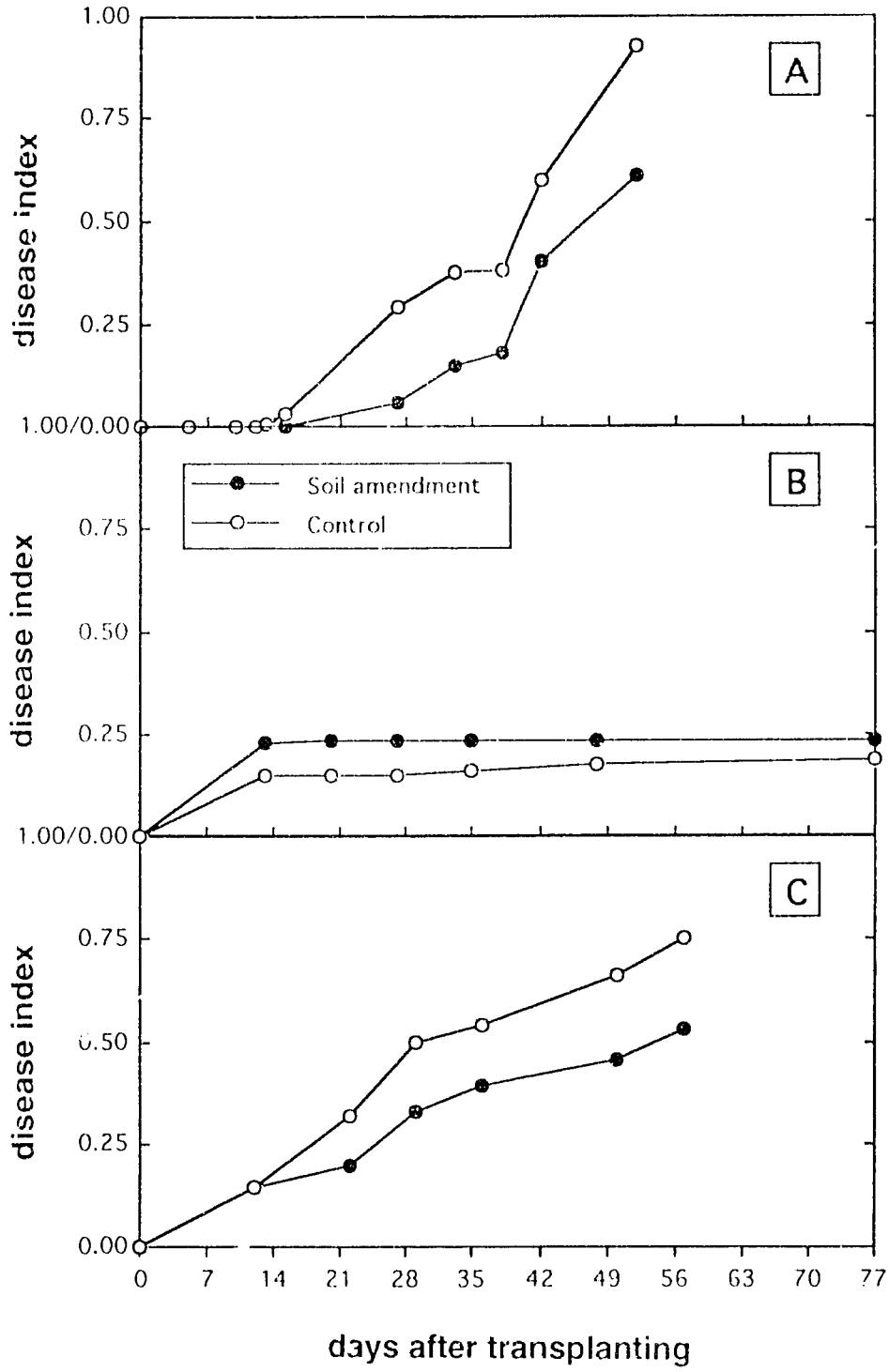


Fig. 3. Disease progress of bacterial wilt of the soil amendment and the control at three different sites in Taiwan: A = AVRDC; B = Puli; C = TSS. Disease progress is expressed as disease index using the modified disease rating of Winstead and Kelman (1953)

Table 4. Disease rate of soil amendment and control treatment after transformation by ln(1/(1-x)), where x is the disease index

Location	Treatment	Disease rate[b]	r^2 of regression[c]
AVRDC	Soil amendment	0.03 a	0.798 *
	Control	0.05 a	0.780 *
TSS	Soil amendment	0.01 a	0.977 ***
	Control	0.03 b	0.986 ***

[a] Regressions from Puli were not significant (P = 5%) and are therefore not included

[b] Values followed by the same letter are not significantly different at *P* = 5% when pairwise compared using t-test. Only treatments within the same location were compared

[c] * = P < 5%; *** = P < 1%

Table 5. Relative yield of tomato at three experimental sites[a]

Location	Treatment		Relative yield (%)
	Main	Subtreatment	
AVRDC	Infested	Soil amendment	250
		Cowpea	198
		SA + cowpea	143
		Control	100
	Noninfested	Soil amendment	356
		Cowpea	515
		SA + cowpea	121
		Control	358
LSD (5%) of subtreatment in same main treatment			240
LSD (5%) of subtreatment of different main treatments			578

	Treatment	
Puli	Soil amendment	103
	Control	100
		ns
TSS	Soil amendment	234
	Control	100
		**

[a] The control subtreatment of the infested main treatment and control treatment means are equal to 100%

[b] ns = P > 5%; ** = P < 1%

Conclusions

The use of a simple soil amendment incorporated 2 to 3 weeks before transplanting tomato reduced bacterial wilt progress and increased tomato yield. This effect might be due to one or several toxic substances generated by the SA once it is incorporated in the soil. Further studies to understand the action of the SA in the soil and to quantify its possible toxic derivatives have to be done. More field studies to quantify the reliability and site specifity of the amendment are needed, too, before it can be incorporated in an IPM program for bacterial wilt in tomato.

Socioeconomics

Peri-urban Vegetable Production Systems

Urban population growth rates are often double those of overall population growth in developing countries, hence, demand for vegetables is projected to increase rapidly within cities, creating great demand for adjacent production and supply systems with virtually assured markets. Increased peri-urban vegetable production by small-scale farmers close to major urban foci could significantly increase the availability of vegetables in urban markets, reduce malnutrition, reduce transportation costs, minimize produce spoilage while assuring farmers a steady income. Two years ago, AVRDC initiated a series of studies in close cooperation with national counterparts to characterize a number of contrasting peri-urban systems outside of Taiwan. These peri-urban systems are summarized in this report.

Three types of characterization of the peri-urban systems were undertaken: a) a structured questionnaire survey (pretested by AVRDC staff in situ) undertaken in and around Ho Chi Minh City (Vietnam), followed by a verification visit; b) a 1-week informal yet structured rapid rural appraisal (RRA) of the major vegetable production zones supplying Kathmandu (Nepal); and c) a 6-month structured questionnaire survey of the Bangkok peri-urban supply of vegetables. Each study used an interdisciplinary approach aimed at identifying limitations to the current supply and factors likely to limit sustainability of production in the future.

Ho Chi Minh City, Vietnam

The change from centrally planned and collective systems to a market economy has led to increases in vegetable production (sometimes a doubling of quantity), but lack of communal purchase of inputs and marketing limit the success of free-enterprise ventures.

Farms are small (~0.8 ha), two-thirds of which are de facto owned and approximately 0.42 ha dedicated to vegetables. On average, 60% of farmers also grow rice while 20% grow groundnut; cabbage is the vegetable crop most commonly grown by farmers (although cultivation of 26 different species was recorded in the survey). Vegetables provide about US$1,000 gross income, or US$650 net income (returns to family labor and land). Returns per labor day per hectare are double or more than those for rice and, additionally, provide employment for five or more times the number for rice. Major input costs were related to organic materials, pesticides, and fertilizers (although much variability existed between crops). Labor use varied from 2000 to 9000 man-hours/ha, with weeding and particularly irrigation accounting for this high labor use. A degree of specialization in production was evident, and the underlying reasons for this are currently subject to analysis.

Pests, and low and variable vegetable prices, were the most commonly cited constraints. The latter was subject to previously unknown market forces, while for the former some IPM is under way. There is a clear trough in vegetable supply during August to October following the hot-wet months, a clear average overuse of chemical fertilizers (8 to 10 times the N removed at harvest), and an apparently inefficient use of labor.

Kathmandu, Nepal

Vegetables in the three survey districts: Bhaktapur, Dhading, and Bara, are grown in many highly different cropping patterns. They vary from year-round vegetable cultivation in Bhaktapur to rice or maize-based systems in Dhading and rice-wheat systems in Bara. The use of inputs in vegetable cultivation is high and at times excessive, particularly for chemical fertilizers and insecticides. However, returns to vegetable growing exceed those for rice and wheat manifold, but risks and capital investments are also considerably higher.

Diseases and pests particularly seriously reduce yield, but most vegetable growers suffer from lack of information about pest and disease management with IPM practices practically unknown. Similarly, more and better information is needed regarding appropriate use of chemical fertilizers, and the performance of extension services could be improved. Both input delivery and output marketing systems are inefficient, resulting in scarcities or even lack of inputs at critical times and high costs for the farmer. In particular, seed quality and availability represent a major difficulty for many vegetable growers.

Stable-yielding varieties with durable pest resistance are given top priority, as are education programs aimed at promoting adoption of IPM and reducing pesticide usage. Economic yield response to fertilizer application, including enhanced input delivery services essential for optimum efficiency, and credible seed certification and marketing are called for. While some organized marketing efforts are under way, emphasis on lean periods of production may also help to stabilize supply and prices throughout the year.

Bangkok, Thailand

Cultivation of pesticide-free vegetables by farmers using the ditch and dike system was observed for 6 months. After pattern analyses, farmers were grouped according to farming styles which reflected the strategies and rationales for farm practices. Some examples of the farming styles are: the 'entrepreneur' who uses more hired labor than other styles and has high cash needs; the 'plantman' who focused on a range of crops with high inputs using only family labor; and the 'businessman' who is well integrated in the market and has a high degree of farm assets and cash costs.

An understanding of the basis for these styles ensures more appropriate technology development and extension. While the ditch and dike peri-urban system has existed for many years, the future is unsure due to high labor requirements (and competition with expanding industries) and limited possibilities for mechanization.

The three studies and data from Taiwan highlight the excessive use of chemical and even organic inputs for peri-urban vegetable production. Given the range of farming styles among peri-urban enterprises, to satisfy research and development demands, it will be necessary to: (a) develop decision-support programs and (b) increase linkage between producer and consumer, both for recycling of minerals and acceptance of pesticide-free but less perfect produce to that the consumer may be accustomed to.

Home Gardens

Studies outside of Taiwan aim to quantify benefits and constraints to homestead gardens, while those at AVRDC provide information on specific components (themes) of homestead garden, e.g., intercrop gardens, aquatic gardens, hydroponics.

Central America

With the Center for Research and Training on Tropical Agriculture (CATIE), Costa Rica, a research project to study traditional home gardens in Central America was initiated, with AVRDC monitoring economic and nutrition aspects. This involved much training of local staff. The study involves 60 household gardens in six communities in Costa Rica, Nicaragua, and Honduras. During the course of one year of field research, a wide range of data is being gathered: diversity of species, management practices, gender roles, contribution of garden production to household nutrition and income generation, intrahousehold consumption patterns, and principal constraints for improved garden development.

Preliminary findings show that vegetables play a minor role in traditional Central American home gardens, which are dominated by perennials, primarily tropical fruits, plantains, and coffee, as well as tubers, maize, and cucurbits. Furthermore, it appears that the role of the home garden in the household economy increases with deteriorating economic and family conditions. Thus, when fertilizer and seed are in short supply for planting grain, off-farm employment is unavailable, when members of the family are incapacitated because of health problems, and various other combinations of dire circumstances, the family relies more on home garden production for food and small amounts of cash. Nutrition and economic analyses are under way.

In another study in Central America, that of an NGO-promoted Home Garden Projects, a 10-page questionnaire is being applied to about 10 organizations each in Nicaragua, Honduras, and Guatemala, where vegetables are included as a garden component. Follow-up visits and interviews with project staff and beneficiaries provide access to further information on extension methodologies, numbers of gardens receiving assistance, and characteristics of the agroecosystems, which will all have a bearing on the relative success/failure of the Home Garden Projects.

Preliminary findings suggest that many projects are short-lived. Principal reasons for failure include: lack of year-round water supply, interference of chickens and other farm animals, poor quality and high cost of seed, pest and disease problems, and, in some cases, failure to convince families of the benefits of home gardening. It appears important to refrain, at least initially, from introducing 'exotic' vegetable crops unknown in the local cultural and agroecological environment (even at the cost of dumping donated exotic seed from foreign seed companies). Organizations that emphasize improvement of traditional home gardens through better management of existing species and gradual introduction of other vitamin-rich, locally adapted vegetables are more likely to achieve long-lasting impact. Furthermore, most NGOs involved in this activity believe strongly in the need for raising the level of awareness in nutrition, health, environment, and gender in rural communities before focusing on the technical aspects of home garden production. Thus, an integrated approach is essential for the success of any home garden project.

Asia

Analyses of studies in Asia are ongoing. Target families in Bangladesh, evaluated at the midterm of a 4-year Helen Keller International project, significantly increased their year-round production and consumption of vitamin-rich fruits and vegetables, compared with the control group (table 1). More

than 80% of vegetables consumed by the target households came from their home gardens at the time of the midterm survey, compared with 37% for the control group and 47% for the interaction group (i.e., the households adjacent to target households, but not exposed through the project to home gardening). Thus, although vegetable consumption declined somewhat for the interaction group over baseline levels, a higher percent of the vegetables consumed came from their home gardens, perhaps a demonstration effect from nearby target gardens. Furthermore, over half of the target families earned supplementary income from garden sales, which allowed them to buy basic staples and improve the overall food security of the household. A combined strategy of focusing on women as gardeners and group leaders, incorporating nutrition education at all levels of training, promoting low input, low-cost gardening techniques, and use of continuous interactive monitoring allowed the project to overcome difficult cultural and economic barriers.

Table 1. Household vegetable consumption[a]

	Target n = 980	Control n = 194	Interaction n = 95	T/C	T/I
Consumed vegetables last week (%)	100	100	100		
Mean quantity consumed (kg)					
baseline	5.8	5.1	5.9	1.1	1.0
midterm	7.5	5.4	5.0	1.4	1.5
Source of vegetables		%			
baseline	81	16	40		
market	16	67	48		
others[b]	3	17	12		
Mean daily per capita vegetable consumption (g)[c]	193	119	118	1.6	1.6

[a] During week previous to midterm evaluation in Panchagaor District, northwest Bangladesh

[b] Exchange, gift, roadside, others

[c] Calculated from quantity consumed in previous week. Estimated weight before cooking

Analysis in cooperation with a small consulting firm in Nepal (No-Frills Consultants) of home garden activities in three agroecological zones within the country led to the conclusion that home gardening enterprises are very diverse in their purpose and output. Male labor input was predominant in the lowlands, while in the hills it was equal to female input. Gardens varied in size from 100 m^2 to intercropped patches with vegetables up to 4000 m^2; the larger sizes are in the lowlands. Lack of irrigation and presence of pests and diseases were common constraints to garden vegetable production, as was seed and in adequate training in the more isolated hills. A full working paper on this study develops lines for future research.

A study on home gardens in Indonesia conducted with the Center for Agro-Socioeconomic Research is undergoing preliminary analyses.

AVRDC—UGA Collaborative Research Project on Commercial Vegetable Production in Tropical Highlands

Introduction

AVRDC in collaboration with the University of Georgia (UGA), USA, has started a vegetable research project under the Sustainable Agriculture and Natural Resource Education and Management – Collaborative Research Support Program (SANREM CRSP) in the Philippines. This project is funded by USAID. It aims to identify the key issues in vegetable production systems, quantifying the extent and magnitude of the problems, and finally designing alternative systems to stabilize commercial vegetable production in Lantapan, Bukidnon, Philippines. A preliminary study suggested that vegetable farmers encroach on virgin forest since soils lower down do not sustain production. Lantapan is a major highland vegetable growing area in Southern Philippines. A vegetable survey in collaboration with the Philippines Department of Agriculture was conducted from May to June. The survey covered 102 commercial vegetable growers and collected information on land tenure, vegetable crops, postharvest losses, IPM, input use, management practices, farmers' perceptions on water erosion, and marketing of fresh vegetables. Close interaction with the local community was ensured while designing the questionnaire and identifying survey sites. The major parcels of vegetable lands of the survey farmers were visited. Information on slope, altitude, and geographical coordinates of each of the parcels were obtained. Soil samples from each of these major vegetable parcels and some abandoned (or long fallow) lands were collected and were analyzed in the Bureau of Soils, Philippines. All the surveys are digitized and analyses are under way.

Vegetable Production in Lantapan

Vegetable production is practiced throughout the year in Lantapan. Major commercial vegetable crops cultivated are tomato, potato, cabbage, Chinese cabbage, sweet pepper, hot pepper, and cauliflower. Wet and dry seasons are the two major vegetable growing seasons. Almost all of the vegetable growers grow vegetables in the wet season, while only about half of them continue during the dry season (table 2). Except for sweet pepper, all the major commercial vegetable crops are grown during both the wet and dry seasons (table 3). Table 3 presents the average farm sizes for the rainy season first crops of tomato (0.9 ha), hot pepper (0.9 ha), and cauliflower (0.9 ha). These were somewhat higher than those for rainy season first crops of sweet pepper (0.4 ha), cabbage (0.7 ha), potato (0.4 ha), and Chinese cabbage (0.6 ha). Except for tomato and cabbage, there was a remarkable difference in yield between wet and dry season crops. Yields during the wet season are higher than in the dry season.

Table 2. Farmers growing vegetable crops in different seasons in Lantapan

Season	No. of farmers[a]
1. Rainy 1	43
2. Rainy 1 + Rainy 2	5
3. Rainy 1 + Rainy 2 + Dry 1	4
4. Dry 1	9
5. Dry 1 + Dry 2 + Rainy 1	4
6. Dry 1 + Rainy 1	35

[a] 102 farmers surveyed

Table 3. Average area, yield, and production of vegetable crops in Lantapan

Crops	Season	No. of farmers[a]	Area (ha)	Production (t)	Yield (t/ha)
1. Tomato	Rainy 1	18	0.88	10.4	13.7
	Rainy 2	1	0.15	1.5	9.6
	Dry 1	10	0.82	8.0	12.0
	Dry 2	1	0.36	3.8	10.5
2. Pepper	Rainy 1	10	0.44	4.1	10.7
(Susceptible)	Dry 1	4	0.78	9.4	9.3
3. Pepper	Rainy 1	4	0.90	4.7	5.1
(Resistant)	Dry 1	2	2.41	33.5	13.9
4. Cabbage	Rainy 1	28	0.69	7.1	13.4
	Rainy 2	2	0.59	2.5	4.2
	Dry 1	15	0.89	9.0	13.1
	Dry 2	2	1.05	6.3	6.9
5. Chinese	Rainy 1	36	0.58	5.7	9.8
cabbage	Rainy 2	4	1.41	17.9	12.5
	Dry 1	16	0.64	7.4	12.2
6. Potato	Rainy 1	38	0.40	4.7	13.5
	Rainy 2	2	0.51	3.5	6.7
	Dry 1	19	0.45	3.2	7.2
	Dry 2	1	0.50	2.5	5.0
7. Cauliflower	Rainy 1	8	0.88	5.2	6.6
	Dry 1	2	1.42	4.1	2.9

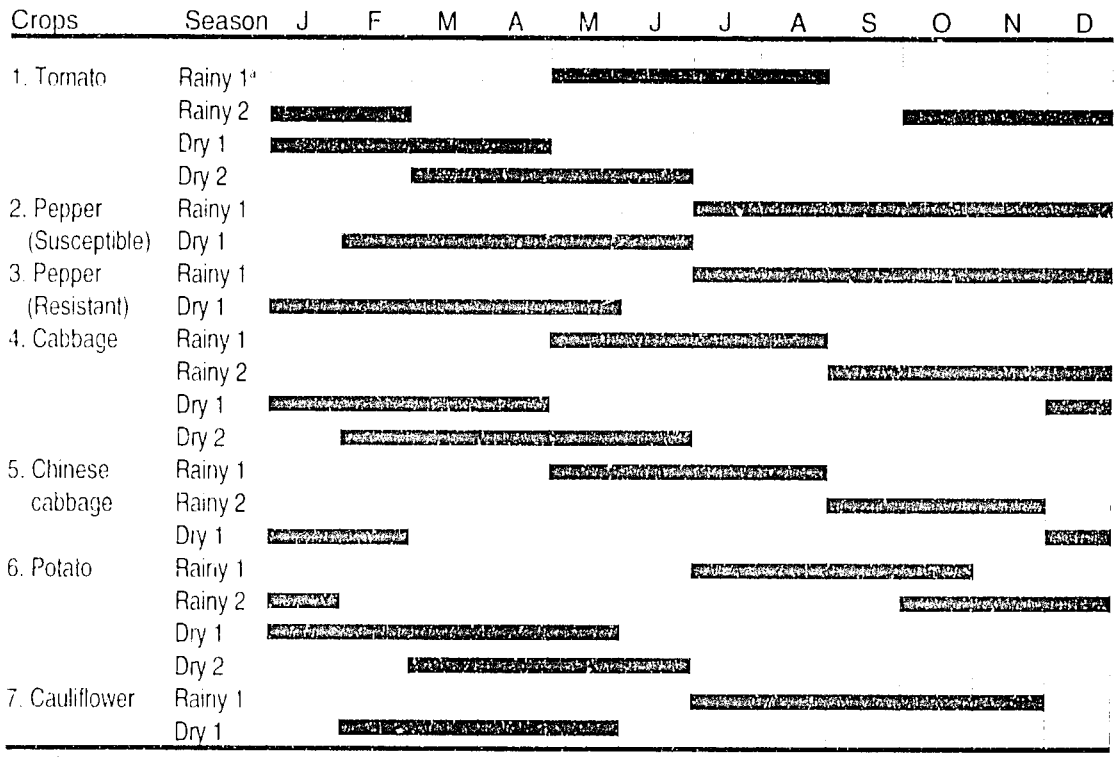
[a] 102 farmers surveyed

In general, the wet season planting starts in May and the dry season planting in January (fig. 1). However, vegetable crop plantings were found throughout the growing seasons. Vegetables are grown mainly on steeplands using an up-and-down cultivation method. Based on the survey, the average slope of vegetable fields was 18%, ranging from 2 to 54%. Given this slope steepness and up-and-down cultivation practices without conservation measures, we expect a lot of soil and nutrient losses due to water erosion from these vegetable fields. Evaluating the effects of different cropping systems and management practices under vegetable production systems is another research goal in Lantapan.

Land Abandonment

Abandonment (i.e., an indefinite fallow) of vegetable lands after a few years of cultivation is a common phenomenon in Lantapan. Based on survey results, about 22% of the total vegetable growers have abandoned land (table 4). The size of abandoned land ranged from 0.5 and 30 ha with a mean of 5.2 ha. On average, each land abandoner has two parcels of abandoned land. The majority of land abandoners belong to the large land holding group. Poor inherent productivity of the land (particularly after the organic matter has been removed from the soil) probably due to marginal labor and capital investment followed by unabated land degradation are the causes of land abandonment. Bringing these lands back into vegetable cultivation systems would potentially open up new employment opportunities, generate income, and uplift the standard of living of the population which in turn may enhance the sustainability of vegetable production in Lantapan. Setting up of rejuvenation plots to study the possibility of bringing back part of the degraded and abandoned land to vegetable cultivation in late 1995 is planned to reduce the need to open new lands for vegetable production.

Fig. 1. Vegetable crop calendar in Lantapan



[a] Rainy 1 and 2 and Dry 1 and 2 represent opportunities for two planting dates within a season for shorter cycle crops

Table 4. Distribution of vegetable growers who abandoned land, by barangay in Lantapan

Barangay	No. of vegetable growers[a]	No. of surveyed vegetable growers	No. of land abandoners	Farmers surveyed who abandoned land
Kapitan Juan	30	4	1	4.5
Kaato-an	80	13	5	22.8
Alanib	30	4	0	0
Sungko	105	21	7	31.8
Cawayan	120	11	1	4.5
Victory	90	8	1	4.5
Kibangay	150	25	3	13.7
Basac	70	16	4	18.2

[a] Number of vegetable growers (1993) reported by the Philippine Department of Agriculture, Lantapan, Bukidnon

Seasonality and Structural Changes in Food Consumption Patterns in Taiwan

Summary

The purpose of this study was to quantify the structural changes and seasonality in the prices and consumption of main food items which include cereal, livestock products, vegetables, and fruits in Taiwan. The study period was divided into two: 1974-84, when income was growing relatively slowly, and 1984-92, when income growth boomed; and three seasons: December-March (low vegetable prices but high fruit prices), April-July (vegetable prices start increasing and fruit prices start decreasing), and August-November (vegetable prices are high, and fruit prices start increasing). Inspite of the highly developed vegetable market of Taipei City, strong seasonality was observed in vegetable and fruit prices and consumption. Price variation for summer tomato in Taipei City has been reduced during the 1980s compared to the 1970s, mainly because of the increase in the availability of AVRDC summer tomatoes.

Introduction

Studying seasonality in food consumption is important to see how consistently bodily requirements are being met, as most of the food nutrients are dissolved everyday. Since basic food items such as cereals can be stored for a long time and transported long distances, the level of consumption rather than seasonality in consumption has been the main focus of both researchers and policymakers. The level of micronutrient consumption has a relatively low profile, due mostly to the seasonality in the availability of their main sources such as fruits and vegetables. Like any other crop, fruits and vegetables are seasonal, and are further constrained by their short shelf life. However, since many fruits and vegetables are available during different times of the year, it is not clear if the deficient supply in one fruit or vegetable is compensated by the abundant supply of other fruits or vegetables.

Like cereals which provide energy for the body everyday, and unlike livestock products which supply storable fats, fruits and vegetables need to be consumed daily to meet the micronutrient requirements of the body such as vitamins, calcium, and iron. However, because fruits and vegetables do not provide energy and satisfy hunger, the regular consumption of these commodities has been ignored. Seasonality of fruits and vegetables may cause an imbalance in the food consumption pattern as people try to substitute for deficiency in fruits and vegetables by some other food. The main purpose of this study was to quantify seasonality in different food items, and the effect of seasonality on consumption pattern. The consumption pattern in Taiwan, an advanced country, is analyzed, assuming that if seasonality exists here more will exist in less advanced markets.

One of the main goals of AVRDC is to reduce seasonality in the availability of vegetables. Thus, one of the concerns of this study is to see how far the center is successful in instigating such structural changes in the vegetable consumption pattern in Taiwan.

Material and Methods

Data on monthly household expenditure and prices collected by the Taipei Municipal Government for the period 1974-92, substantiated by the price data collected by the Provincial Government of Taiwan, was used in the study. All food consumption, excluding cola and alcoholic beverages, was grouped into four major items: cereal, livestock products including seafood, vegetables, and fruits which cover, respectively, 33, 83, 72, and 33 items in each category. Processed food items such as

juices and canned fruits and vegetables were converted into their respective raw form using the appropriate conversion factor. Average monthly per capita consumption and price indices of the major food items were developed separately for 1974-84 (when increase in per capita income was moderate) and 1985-92 (when per capita income increased).

Results and Discussion

The budget share allocated to different food items is shown in table 5. Livestock products had the biggest budget share of 54% in 1974, which continuously declined to 46% in 1992, but still maintaining the biggest share of the consumers' budget for food. Fruits had the lowest share of 8% in 1974, which continuously increased to 16% in 1992 and became almost equal to the cereal and vegetable shares. The latest figures show that about one-fifth each of the total food expenditure was devoted to cereals, fruits, and vegetables. The budget share of vegetables has slightly declined from 19% in 1974 to 17% in 1992, while the budget share of cereals first declined from 19% in 1974 to 16% in 1977, but again rose to 22% in 1992.

Table 5. Budget share allocated to different food items (%), Taipei City, 1974–92

Year	Cereal	Livestock	Vegetables	Fruits
1974	19.2	53.6	18.9	8.3
1975	17.8	55.7	18.4	8.1
1976	17.7	56.0	17.0	9.3
1977	15.6	55.9	19.2	9.3
1978	16.3	55.0	18.6	10.1
1979	16.4	53.6	17.7	12.3
1980	18.0	52.7	17.3	12.0
1981	16.7	53.5	18.3	11.6
1982	18.0	52.8	16.5	12.8
1983	16.7	53.1	17.3	12.9
1984	17.6	52.7	16.5	13.2
1985	17.7	51.5	16.9	14.0
1986	17.8	51.2	17.2	13.8
1987	18.3	49.4	17.6	14.8
1988	18.9	49.0	16.8	15.3
1989	18.9	49.7	16.3	15.2
1990	20.4	47.6	17.1	14.9
1991	19.6	47.3	17.3	15.8
1992	21.6	45.6	16.8	16.0

These adjustments in the budget share allocation are reflected in the changes in the per capita consumption pattern shown in fig. 2. The consumption of all food items, except vegetables increased during 1985-92 compared to 1974-84. For example, cereal, meat, and fruit consumption during 1974-84 increased from 7.4, 6.8, and 4.7 kg/capita per month to 8.1, 8.4, and 5.6 kg/capita per month in 1985-92, respectively, while the consumption of vegetables declined from 8.8 in 1974-84 to 6.9 kg/capita per month during 1984-92.

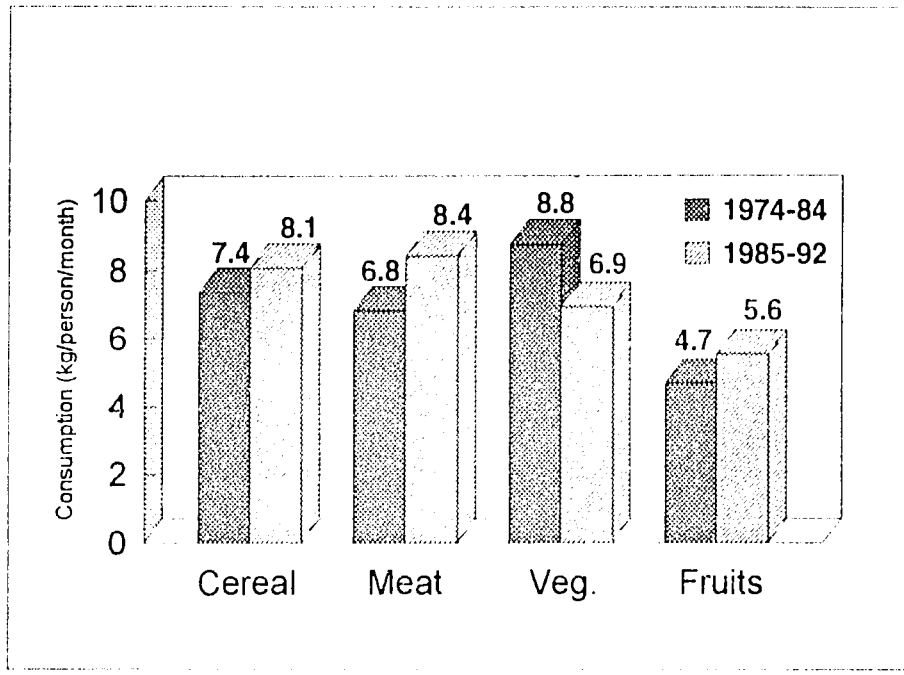


Fig. 2. Average monthly consumption per capita, Taipei City

It was observed that inspite of the highly developed vegetable market of Taipei City, the overall vegetable price index varied from 95 in March to the highest of 141 in September, while the overall fruit index varied from 73 in May to 103 in February (fig. 3). The vegetable prices remained high during August-October, while fruit prices were highest during September-March. The relatively high prices of fruits in November-March was relieved by the relatively low prices of vegetables in these months, but the high prices of fruits and vegetables coincided in September-October. As expected, there was no significant variation in the monthly average price indices of meat and cereal products.

Seasonality in consumption pattern was observed (table 6) to correspond to price seasonality. The consumption of vegetables was lowest in August-October when their prices were highest; it was highest during December-May when their prices were lowest. A similar seasonal relationship between price and consumption can be observed in fruits. The consumption of vegetable plus fruit was lowest (about 12 kg/capita per month) in October-November when the prices of both these items are simultaneously highest; consumption was highest in May-August (around or above 14 kg/capita per person) when the prices of fruits were lowest and the prices of vegetables were moderate. The consumption of cereals, meat, and vegetables are highest in January during Chinese New Year in Taiwan. The lowest consumption of fruits and vegetables during October-November may not have strong implications for micronutrient deficiency in Taiwan because of high per capita consumption of these items in the country. However, for those countries where per capita consumption is already low, similar seasonality in fruits and vegetable consumption may create a serious micronutrient deficiency during the months when fruits and vegetables are in short supply.

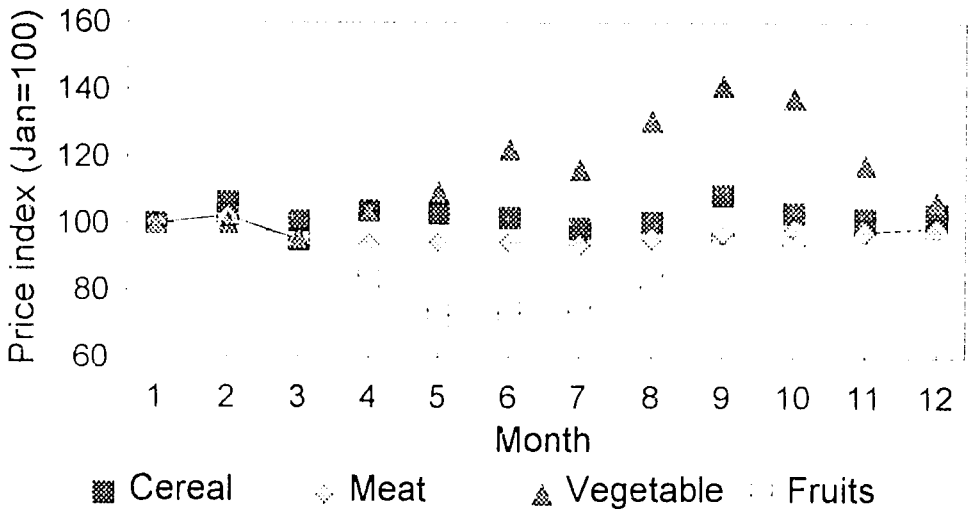


Fig. 3. Average monthly price index, Taipei City, 1974–92

Table 6. Average consumption (kg/month/person), Taipei City, 1974–92

Month	Cereal	Livestock	Vegetables	Fruits	Vegetables + Fruits
1	8.5	8.5	9.2	4.1	13.3
2	7.2	7.4	8.0	4.0	12.1
3	7.8	7.3	8.8	3.9	12.7
4	7.3	7.2	8.0	5.0	13.0
5	7.6	7.3	8.1	6.1	14.2
6	7.5	7.1	7.5	6.4	13.9
7	7.2	7.1	7.9	6.6	14.5
8	7.6	7.6	7.4	6.0	13.4
9	7.6	7.5	7.0	5.1	12.2
10	7.9	7.6	7.1	4.9	12.0
11	7.7	7.6	7.8	4.3	12.0
12	8.0	7.8	9.0	4.1	13.2

Price variation in Taipei City has been reduced during the 1980s compared to the 1970s, (fig. 4) mainly because of the increase in the availability of AVRDC summer tomato. The average monthly fluctuations in tomato prices has declined correspondingly (fig. 5).

The study is still looking at the substitution among food items and income, own- and cross-price elasticities over seasons and the two study periods.

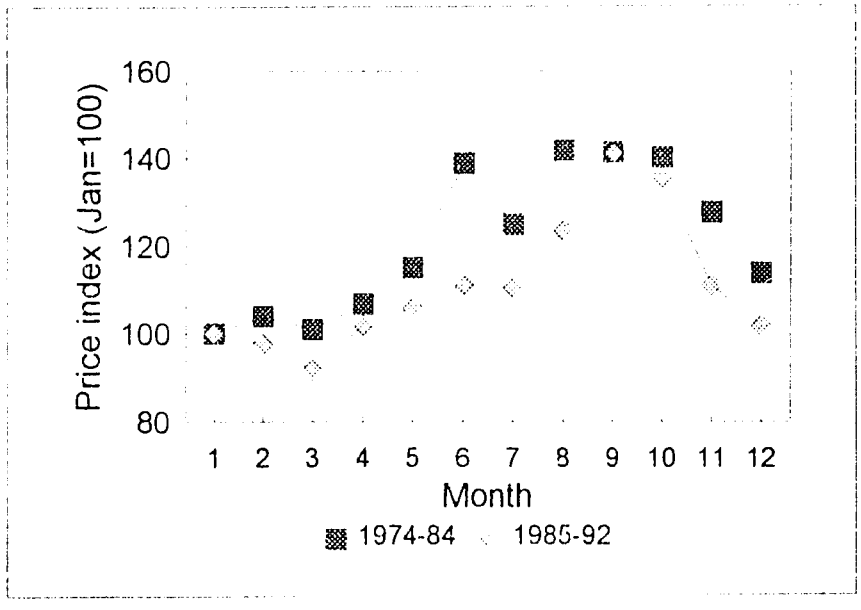


Fig. 4. Vegetable price indices, Taipei City

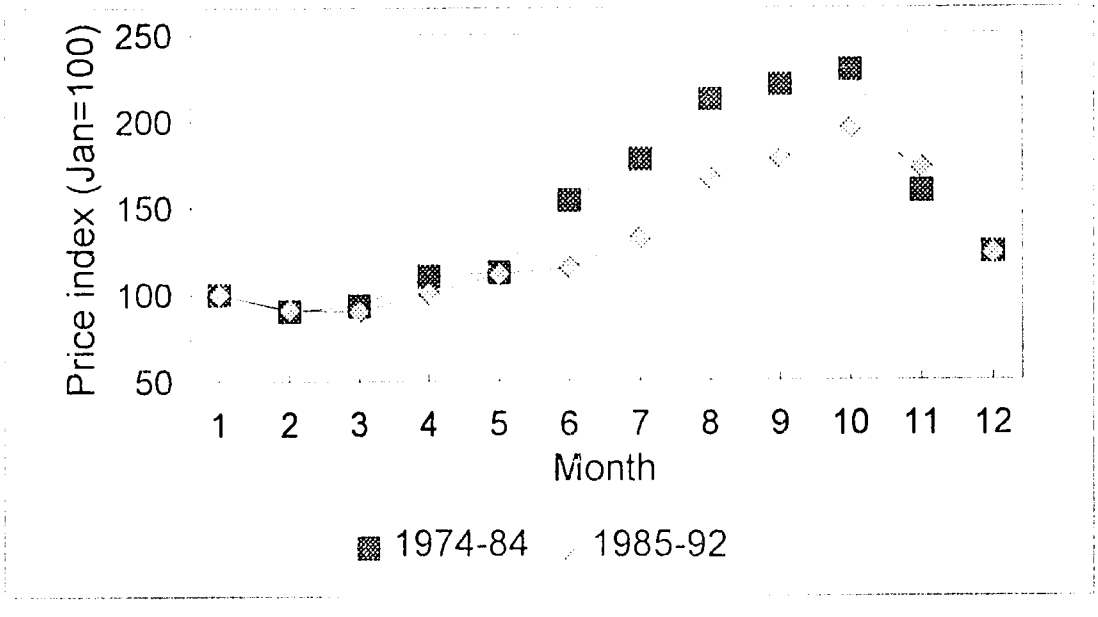


Fig. 5. Average monthly tomato price index, Taipei City

The Role of Vegetables in Combating Micronutrient Deficiency

Summary

The widespread prevalence of micronutrient deficiency is a major cause of poor health in many developing countries of Asia. Integrating micronutrient-rich foods such as vegetables, fruits, and livestock products into diets is the most practical and sustainable way to tackle micronutrient deficiency. Vegetables are efficient sources of many micronutrients both in unit cost of production and per unit of land area. However, in many developing countries numerous biotic, abiotic, management, and institutional constraints limit adequate vegetable supplies in the summer, resulting in high consumer prices and consumption significantly below the required levels. Policies to ameliorate micronutrient deficiency include decreases in the relative prices of vegetables, increases in income, enhancement of micronutrient contents in vegetables through breeding, and changes in consumer preferences through nutrition information campaigns. Each of these options requires a thorough understanding of site-specific vegetable production, marketing, and consumption constraints, as well as of socioeconomic factors that determine consumer preferences for major and micronutrients.

Introduction

The consumption of micronutrient-rich food such as vegetables is significantly below the required level of 200 g/capita per day in most developing countries of Asia, with corresponding serious levels of micronutrient deficiency. The deficiency can seriously hamper normal functioning of part or the whole body and can make the body vulnerable to diseases. Despite indications of widespread micronutrient deficiency and the clear clinical relationships between such deficiency and morbidity, mortality, and disease infections, research to enhance the supply of micronutrients has been largely neglected.

Possible solutions to the micronutrient deficiency problem include fortification of foods with micronutrients, micronutrient supplements, and integration of micronutrient-rich foods into diets. Success has been achieved in controlling goiter and cretinism by fortifying salt with iodine. However, only limited success has been reported in the adoption of fortified vitamin and iron foods or their supplements.

In view of the administrative and logistical constraints in supplementing and fortifying foods, most clinicians believe that improvements in the overall quality of diets by integrating micronutrient-rich foods such as fruits, vegetables, and livestock products into diets constitute a sustainable way of eliminating micronutrient deficiency.

The Potential of Vegetables to Overcome Micronutrient Deficiency

To show that vegetables are able to supply significantly more iron, calcium, and even protein per unit of land than cereals, standard micronutrients per 100 g of edible portion from different vegetables and cereals were multiplied with the respective per hectare yield of these crops grown in their optimum season in Taiwan (1990-91 data).

Potato, Chinese cabbage, sweet potato, onion, cabbage, tomato, potato, eggplant, and sweet pepper produced, respectively, 42, 31, 24, 20, 15, 4, 3, and 3 times more calcium than cereals per unit area. Similarly, these vegetables respectively produced 11, 5, 8, 2, 4, 4, 3, and 3 times more iron

compared to cereals from a given piece of land. In addition, some vegetables like potato and onion were more efficient compared to cereals in producing protein per unit of land, as reflected by vegetables:cereals nutrient-supply ratios significantly larger than one.

Calculations were done to compare vegetables, livestock products (milk and chicken), and cereals in terms of micronutrient supply per unit of production costs (all variable and fixed costs, including land rent). All vegetables were more cost-efficient compared to cereals in providing calcium and iron. Surprisingly, most vegetables are more cost-efficient compared to cereals in supplying protein as well. Milk was more cost-efficient compared to cereals in providing calcium, but less efficient than vegetables. Vegetables are more cost-efficient than chicken in providing all micronutrients.

The relative cost and land efficiencies of vegetables and cereals in providing different micronutrients will depend upon their relative yields which are determined by technology, policy environment, ecological zone, and season. Generally, efficiency ratios will be more favorable for vegetables than for cereals in temperate zones and during winter. Only an ecoregional analysis can decide which specific vegetable crops are most suitable to tackle a given micronutrient deficiency since a wide variation exists in the type of micronutrients deficient in a population and in the capacity of vegetables to supply different types of micronutrients.

Although vegetables are efficient sources of micronutrients, their regular supplies are not without problems. First, most vegetables are sensitive to high humidity, poor drainage, and high temperature, which translate into a risk production environment and high costs under tropical conditions. Second, most vegetables are more perishable than cereals. Vegetables are not available in equal amounts throughout the year because many cannot be stored. Third, transportation, packaging, and marketing are more expensive for vegetables than for cereals. Due to these problems, marketing margins (difference in retail and farm prices) for vegetables may be many times higher than for cereals.

Thus, even though the production of micronutrients through vegetables is more efficient than through cereals, the seasonal (year-round) availability of vegetables to consumers may be hampered by weather and postharvest problems, resulting in high prices. In addition, consumption of vegetables often shows considerable variation between the same seasons in different years (see Seasonality and Structural Changes in Consumption Patterns in Taiwan).

Integrating Vegetables into Diets

The consumer's decision to consume certain amounts of major and micronutrients depends on four factors.

Relative prices of vegetables and other food items

If prices of vegetables relative to other food items decrease, the consumption of vegetables will increase, assuming vegetables are not inferior goods. The relative price of vegetables compared to cereals depends on supply and demand with respect to these commodities. Both demand- and supply-side factors can act as constraints to the availability of vegetables to consumers and on the consumption of micronutrients (fig. 6). Demand-side constraints include low per capita income, high retail prices for vegetables, lack of information on the positive effects of micronutrients on health and on the potential of vegetables for alleviating micronutrient deficiencies, and social factors that translate into consumer taste and preference patterns.

Supply-side constraints can be divided into two groups: production and marketing constraints. Production constraints can be of biotic, abiotic, management, or institutional nature. High temperature and humidity during the summer are not conducive to vegetable cultivation and result in high losses

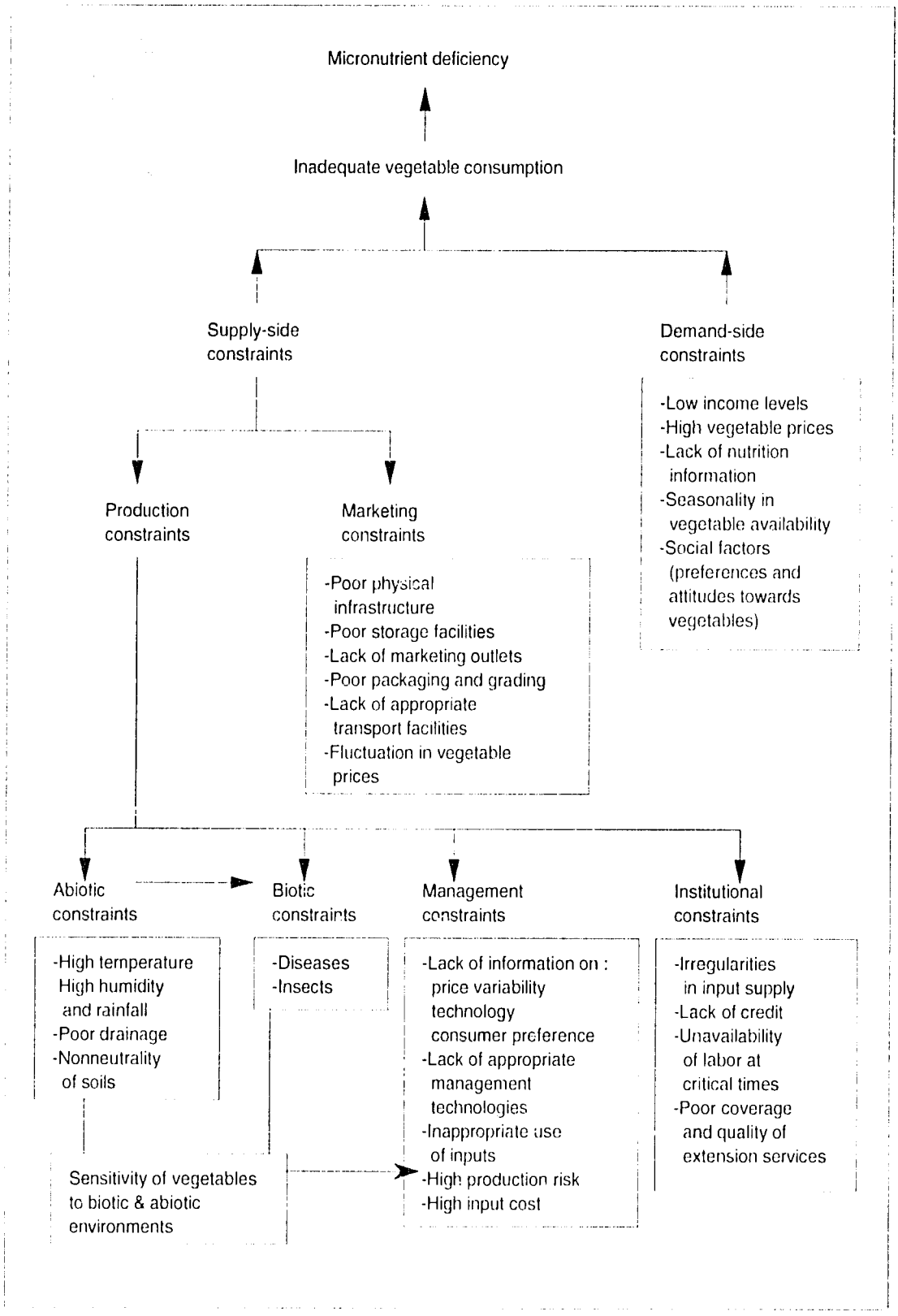


Fig. 6. Constraints to adequate intake of vegetables

due to abiotic stress which, in turn, often stimulate the occurrences of biotic stress, i.e., pest and disease infestation. The high sensibility of most vegetables to biotic and abiotic environments creates high production risks. Even during the cool winter when abiotic stresses are less constraining, insects and diseases are important yield reducers. This, combined with lack of information and technical skills among farmers, leads to excessive use of insecticides and fungicides, resulting in high financial cost to farmers and health costs to both farmers and consumers. Institutional constraints such as insufficient and untimely input supplies, poor coverage and quality of extension, and labor shortages during critical times are significant yield reducers.

Marketing constraints include roads, marketplaces, and storage facilities (all of which contribute to excessive postharvest losses and widespread differences between consumers' and producers' prices), poor grading, wide seasonal fluctuations and annual variations in vegetable prices, lack of information among producers on prices, and consumer preferences for different attributes of vegetables, poor packaging and grading, and lack of proper transportation facilities. To improve vegetable consumption, both supply and demand factors related to vegetable consumption need to be analyzed thoroughly, together with consumer decision-making processes responsible for allocating incomes to different foods.

AVRDC, besides developing improved vegetable varieties and production practices, is working to understand the constraints that limit year-round vegetable supplies. The main emphasis of AVRDC's research is on increasing vegetable production (particularly during the hot and humid summer) by overcoming biotic and abiotic stresses and to understand socioeconomic constraints in production, marketing (through improved varieties and production practices), distribution, and consumption. The ultimate goal is to improve year-round vegetable availability at the household level

Income levels

Micronutrient deficiency is usually considered indicative of a wider malnutrition problem and believed to be related to income levels. Higher income may not only result in increased consumption of vegetables because consumers can now can afford them, but also allows them to change preference patterns by substituting some of the major nutrient-rich foods with vegetables.

Income enhancements in several East Asian countries, including Taiwan, Japan, and South Korea, were associated first with an increase in consumption of both major and micronutrients, followed by a partial substitution of cereals with fruits and vegetables. Low consumption of food rich in micronutrients in Bangladesh is to a significant extent due to low per capita income. Recent microlevel studies in some developing countries, however, show that the demand elasticity of vegetables with respect to income is relatively small (0.2-0.6). Contrary to the expectation, therefore, increases in income may make relatively little difference in vegetable consumption.

To reconcile this contradictory evidence to the poverty-related malnutrition hypothesis, one has to consider the supply-side constraints of vegetable production which may limit vegetable consumption, despite substantial increases in income. This represents an area of research in which much empirical work remains to be done.

Another empirical question linked to the poverty-related micronutrient deficiency hypothesis concerns levels of vegetable consumption and income-related changes in the distribution of vegetable consumption across social classes and among deprived members of society. Evidence from a number of different household surveys indicates that the distribution of micronutrient consumption is biased against women and children. Increases in incomes, up to a certain level, may correct some of these biases. Again, these are empirical questions which need to be addressed by appropriate research.

Micronutrient content

An enhancement in micronutrient content without affecting the content of major nutrients may improve consumption levels of micronutrients without adversely affecting the consumption of major nutrients. Another possibility is enhancements in micronutrient contents of cereals at the cost of major nutrient availability. Theoretically, this will increase consumption of micronutrients and decrease that of major nutrients, although such technology would be hard to disseminate. Improvements in both micro and major nutrient contents through breeding has proven to be a notoriously difficult task.

Unfortunately, enhancement of the micronutrient content of existing foods by incorporating new genes or by screening for high micronutrient content usually involves certain trade-offs in taste, desirable color, or availability of major nutrients at higher costs. For example, increased vitamin A content in tomato through transfer of the beta gene can be achieved at the cost of a change in fruit color from red to yellow, which is not a desirable attribute from the consumer's point of view. In rice, high iron content changes its color to (again undesired) yellow, while increased vitamin A content means a reduction in calorie content. Therefore, even if breeders are successful in achieving a high micronutrient content, social scientists have to be involved in the research from the inception to assess the market implications of the trade-offs involved. Since micronutrient deficiency is a less visible form of malnutrition (and more difficult to diagnose as well) than calorie deficiency, consumers are usually disinclined to consume a commodity with a color or taste that they are not used to.

Consumer preference patterns

Consumer preferences are determined by the sociocultural environment and can be influenced through educational campaigns which provide information about the adverse effect of micronutrient deficiency on human health. Such increased awareness and preference changes are strongly associated with levels of economic development, perhaps because of the combined effects of increased information and ability to pay for micronutrient-rich foods. Occasionally, social, cultural, or religious factors may negatively influence consumption of micronutrients. Moreover, certain food habits may make the taste of certain vegetables unacceptable or reduce the bioavailability of micronutrients. In those instances, education about the serious negative health effects of micronutrient deficiency and its relation to particular food habits can help relax these constraints.

Information campaigns may induce consumers to increase consumption of specific vegetable types which are relatively rich in certain micronutrients. They may also induce people to change their food habits, thus improving the bioavailability of micronutrients. Alternatively, in societies where consumption of total nutrients is high enough efforts aimed at inducing consumers to reallocate a given budget away from foods rich in major nutrients towards foods rich in micronutrients may be considered. While both total expenditures on food and consumption levels of major nutrients may remain constant, nutritional status will increase.

Conclusions

Despite significant increases in the supply of major nutrients (particularly calories and protein) in Asia since the late 1960s, widespread micronutrient deficiency still prevails in many developing countries. The most practical way to tackle these deficiencies is to integrate micronutrient-rich foods in existing diets. Vegetables are efficient sources of micronutrients — both with respect to per unit cost of production and per unit of land area — if they are produced at appropriate times and locations. However, since regular supplies of vegetables are hampered by seasonal variation in weather and by numerous postharvest problems, vegetable consumption levels are significantly below the minimum requirement in many countries.

This study reviews a number of policy options to increase consumption of micronutrient-rich foods in general and of vegetables in particular. These include decreases in the relative prices of vegetables, improvements in income, enhancement of the micronutrient content in major food items through breeding, and changes in consumer preferences through educational campaigns. Each of these options involves a thorough understanding of vegetable production, marketing, and consumption constraints, as well as of socioeconomic factors that determine major and micronutrient preferences and food habits of consumers.

Wide variations in the types of micronutrient deficiencies may exist across regions. This, combined with differences in the cost efficiency of vegetables in providing various micronutrients, calls for an ecoregional approach in identifying appropriate vegetable species to combat such deficiencies. Because the relative importance of the various factors that limit vegetable availability are site-specific, a careful diagnosis, quantification, and prioritization of the constraints are required before designing any research agenda or policy alternatives aimed at ameliorating micronutrient deficiency.

Agricultural Economics Workshop

The Agricultural Economics Workshop on Vegetable Production and Consumption Patterns in Asia brought together prominent agricultural economists from 13 countries, representatives of regional organizations involved in vegetable research, and staff from AVRDC. Each country representative presented the vegetable production, marketing, distribution, and consumption trends in his respective country. This was followed by discussions on vegetable elasticities with respect to prices and income, production and marketing constraints (often detailed to the species level), issues related to the dynamics of trade in vegetables, comparative advantage of vegetable production across countries, and the role of peri-urban production. These were presented as a prelude to formulating a research, development, and policy agenda for vegetable production and consumption.

Concern for accuracy of some countries' production data spurred a request for standardization of field-level data collection procedures across countries and spot validation procedures with analytic rigor. More radical was the call to compare profitability of vegetable production in peri-urban vs. rural systems, in generalized vs. specialized vegetable production systems, and in production zones predefined through analyses of agroecology, market factors (distance, transport, demand), and the involvement of agroindustries. The call for cross-country studies on the comparative advantage of vegetable production and distribution was fueled by the prospects of future liberalization of vegetable markets in the more developed countries of Asia, and the new opportunities opened up due to market-oriented policies in countries previously with centrally planned economies.

Strong demand pressure on vegetable prices and stagnation in per capita vegetable consumption in most of the participant countries were observed. It was suggested that a comprehensive project be initiated on understanding the farm and household level constraints in vegetable production and consumption in developing countries. Such a project will help prioritize vegetable research at the national and international level. The group strongly felt a need to analyze the efficiency of alternative vegetable marketing systems to find ways to reduce marketing cost.

Participants also suggested technical and policy innovations to reduce seasonality in the availability of vegetables which is believed to seriously affect the status of micronutrient intake in most Asian countries. The consumer's tendency to demand for clean and pesticide-free produce stimulated discussion on the economic feasibility of protected production systems such as hydroponics and on the reduction of damage to the environment against the higher cost under these systems.

The full proceedings and recommendations of the workshop will be published by AVRDC in mid-1995.

Soil Science

Compost Effects on Yields and Nutrient Uptake of Continuously Cropped Soybean

Summary

Soybean fits well into the intensive crop sequences of tropical and subtropical Asia. The yield potentials of soybean cultivated continuously three times per year for numerous years have not been assessed nor have nutrient requirements of the crops, which mature earlier than those of soybeans grown at high latitudes, been quantified. After 10 years of continuous cropping (26 soybean crops harvested between autumn 1983 and spring 1993), an experiment with compost, cultivar and inorganic fertilizer treatments was terminated and yield and nutrient uptake data were analyzed and interpreted. Mean spring, summer, and autumn grain yields were 3.5, 3.3, and 2.7 t/ha. Gradual but significant grain yield declines with time were observed for plantings in the spring (0.2 t/ha per year) and autumn (0.1 t/ha per year). Cultivar effects were found within some planting dates but differences were not consistent over time. Compost increased grain yield by an average 0.2 t/ha per crop, but an increasing yield trend with time was not evident. In contrast, N, P, and K uptake from compost treatments increased with time. Evidence indicates, however, that nutrient supply from the compost contributed little, if at all to the 0.2 t/ha per crop grain yield response to compost. Nutrient applications from inorganic or organic sources could be adjusted downward as residual effects accumulated without diminishing grain yield. Soybean cultivars with potentials similar to those in this study, when grown in intensive systems with practices such as crop rotations, to reduce or prevent yield declines with time, should produce yields exceeding 3.5 t/ha in 3 1/2 months and exceeding 3 t/ha in 2 1/2 months in seasons with the most and least favorable photothermal conditions, respectively.

Introduction

Soybean is an Asian crop which can be cultivated in diverse cropping systems and all seasons at low latitudes. Within 23° N and S latitudes, many adapted cultivars are not only early maturing but of compact stature as well. AVRDC scientists have advocated plant densities of 35 to 40 plants/m^2 to compensate for small plant stature associated with rapid progression through growth stages. Nitrogen applications are required because, as the early maturing cultivars progress through early development rapidly, insufficient biologically fixed N is accumulated to satisfy maximum seed development before the crop self-destructs from N deficiency. Furthermore, most soils on which soybeans are cultivated at low latitudes have limited potential to supply N before nodule systems develop and again during grain filling when the developing seed becomes an overpowering sink for assimilate and N. A few scientists have advocated liberal organic and inorganic N applications to satisfy needs that cannot be met by biological fixation.

Scientists and farmers have had little experience with continuous low latitude soybean cultivation and documentation of the practice, to our knowledge, does not exist. Study objectives, therefore, were to determine the effects of continuously cropped locally adapted cultivars and of season within the continuous system on soybean grain and dry matter yields. Furthermore, N, P, and K uptakes were determined because the information is useful when deciding fertilizer management practices for intensive systems that are possible in low latitudes.

Materials and Methods

The study began in autumn 1983 and continued to summer 1993. Twenty-seven crops were planted and 26 were harvested during the study. Only spring and autumn crops were cultivated in 1984 and 1985. Crops were planted in subsequent summers except during 1988 when the field was purposely flooded for 2 months to reduce inoculum of charcoal rot (*Macrophomina phaseolina*), a disease that had infected the 1988 spring crop. In 1990, a typhoon destroyed the summer crop.

In autumn 1983 to autumn 1984 plantings, treatments were altered systematically to select practices that maximized yields. Treatments have been described in earlier AVRDC Progress Reports. They were arranged in two replicates of a split-split plot design. From spring 1985 to summer 1993, compost treatments (0 and 30 t/ha in 1985 and 0 and 50 t/ha thereafter) were in main plots and varieties were in subplots. Spent rice straw from mushroom cultivation was applied as compost until autumn 1985. Subsequently, sugarcane waste produced by a nearby mill was composted and applied. The waste was composed of filter slurry and finely chopped bagasse. The mean N, P, and K concentrations in the composts were 14.5, 5.4, and 8.4 g/kg (dry weight basis), respectively. AGS 129 (narrow leaflet, semideterminate) and Kaohsiung No. 8 (broad leaflet, determinate), were test cultivars in all crops except autumn 1983 and 1984, when only AGS 129 was planted, and in summer 1989 and 1991-93, when vegetable soybean types were cultivated to reduce exposure to typhoon damage as well as to increase time for field operations between crops.

The dry matter and shelling percentages of fresh vegetable soybean yields were used to estimate grain yield for the 1989 to 1993 summer crops. The estimation equation, GY = -0.568 + 0.371VSB (R^2 = 0.945) where GY was grain yield (t/ha) and VSB was vegetable soybean yield (t/ha), was derived from combined 1991 to 1993 data from all treatment combinations.

Sampled plants were separated into leaf, seed, pod, and stem components for dry matter and nutrient concentration determinations. Abscised leaves were collected, rinsed, and included with those remaining on the plant for the determinations. Nitrogen determinations were made separately on each component, starting with spring 1984. Phosphorus and potassium determinations were made on samples starting with autumn 1984.

Results and Discussion

Grain and dry matter yields

Median planting dates and contrasting photothermal and rainfall environments for each season are listed in table 1. In the autumn, photothermal conditions became progressively unfavorable as the crop progressed. Seasonal effects on grain yield were apparent although variability within each season was observed (fig. 1). The effects of season on grain yield and crop duration were consistent with prevailing photothermal environments. Except in the first planting (autumn 1983) when analysis of variance detected a small response to P and K (rates were confounded), no subsubplot factor had a significant effect on grain yield. Especially noteworthy were absences of responses to suspension of

fertilizer nutrients from spring 1990 onward and absences of interactions between subsubplot treatments and compost. The lack of response to N in treatments not receiving compost may be associated with accumulation of excess N in the humic fraction before spring 1990 followed by slow mineralization of the freshly humified N afterwards. The C:N ratios reported in the AVRDC 1993 Progress Report[1] showed that N in the soil organic fraction had increased even though organic C decreased in the treatment without compost.

Table 1. Median planting and harvest dates, photothermal characteristics at the beginning of the median seasons, mean rainfall, and mean grain and dry matter yields for each season

	Spring	Summer	Autumn
Median dates of			
Planting	7 Feb	11 Jun	26 Sep
Harvest	24 May	13 Sep	13 Dec
Growth duration, days	103	94	78
Daylength including civil twilight			
At planting hour[a]	12.4	14.3	12.5
At harvest hour	14.3	12.7	11.7
Mean daily growing season solar radiation, MJ/m^2	17.1	19.9	15.3
Air temperature (C) during planting week			
Maximum	22.5	31.5	30.6
Minimum	13.5	24.2	22.8
Harvest week			
Maximum	29.7	31.1	24.3
Minimum	22.6	24.3	13.6
Mean growing season rainfall, mm	260	919	35
Grain yield, t/ha	3.5	3.3	2.7
Grain yield/day, kg/ha per day	32.6	34.6	34.9
Dry matter yield, t/ha	7.6	10.3	5.9
Harvest index	0.38	0.20[b]	0.40

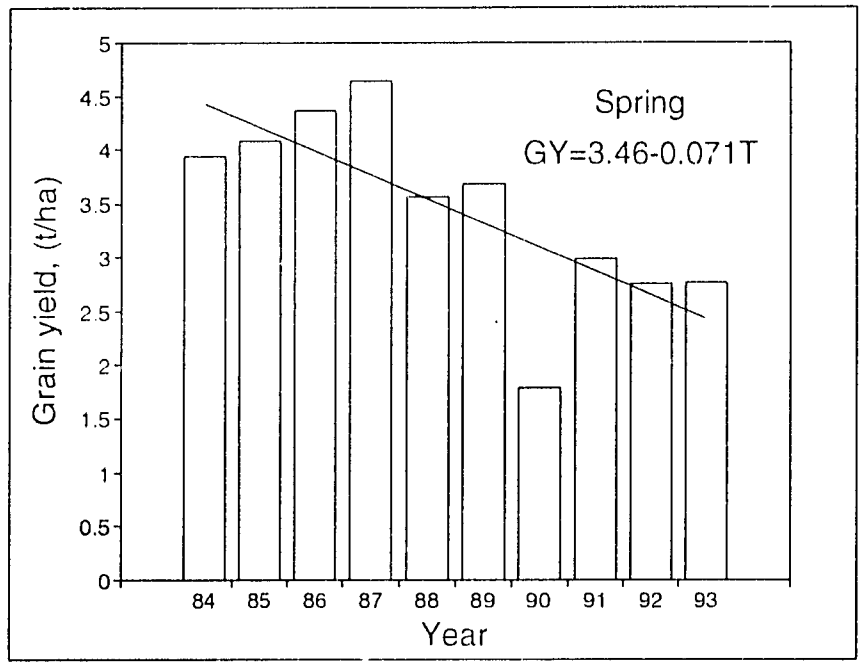
[a] Median dates

[b] Dry matter yield and harvest index means excluded vegetable soybean crops because these were harvested at R6

AGS 129 yielded significantly more than Kaohsiung No. 8 in 7 of the 11 harvests in which a cultivar effect was significant. A paired comparison of AGS 129 vs. Kaohsiung No. 8 across harvests was not significant, however, nor was there evidence that either cultivar was superior when grown under conditions in which yields were either worse or better than average.

Compost treatments were in main plots and therefore F-tests in analysis of variance of individual harvests were insensitive. The mean yield from compost treatments, however, was 0.22 t/ha greater than the mean of treatments without compost ($t = 2.90$, $P > 1\%$), but a significant linear effect of time was not detected suggesting that the compost effect was abrupt, not accumulative.

[1] AVRDC. 1994. AVRDC 1993 Progress Report. AVRDC, Shanhua, Tainan, Taiwan.



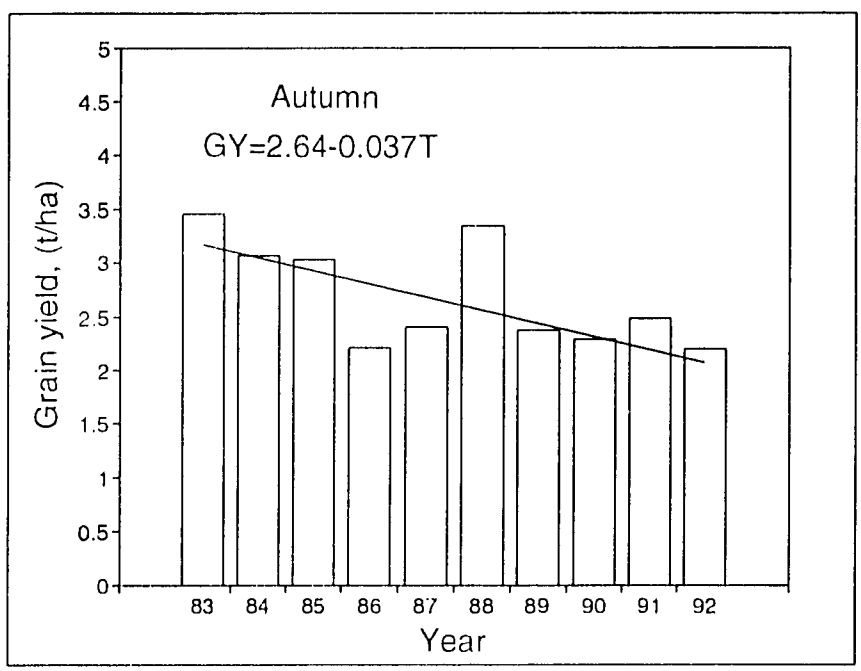


Fig. 1. Soybean grain yields in spring and autumn, time trends within season
The lines and equations were from the model described in the text, after adjusting intercepts and slopes for seasonal effects; GY = grain yield; T = year code: autumn 1983 = -14.5 and spring 1993 = 14.5

To examine grain yield trends within seasons, a regression model was fitted to the means from each harvest: $GY = a + b_iS + cT + d_iSxT$ where GY was grain yield (t/ha), T was a trend variable (autumn 1983 = -14, summer 1993 = 14) and S was a classification variable for spring, summer, and autumn. The effects of T and S and the SxT interaction were significant at $P < 1$, 2, and 0.2%, respectively, and $R^2 = 0.60$. Regression models with intercepts adjusted by b_i and trends adjusted by d_i are shown on fig. 1. Although *Macrophomina phaseolina* was observed frequently in the spring and may have contributed to the declining trend, the rate was not consistent with an abrupt increase in the disease inoculum level.

A significant positive trend for summer crops probably originated from superior adaptation of vegetable soybean cultivars grown during summers 1991-93 as replacements for Kaohsiung No. 8 and AGS 129 or from overestimates of grain yields from the fresh green pod yields of the vegetable soybeans. Therefore, the positive trend was regarded as aberrant.

The effect of season dominated dry matter yield even more than it did grain yield (table 1). Cultivar significantly affected dry matter yield only in summer and autumn 1986, and the spring crops 1988 and 1993. Neither time nor compost had significant effects on dry matter yield after removing the effects of season statistically. Dry matter yield means were less precise than the grain yield means, reducing the likelihood of detecting small differences. Coefficients of variation for subsubplots averaged 6.0% across plantings for grain yield and 8.0% for dry matter yields.

Autumn and spring harvest indexes (HI) were nearly equal but the mean summer HI was only 0.20. The high dry matter yield in the summer was attributed to growing conditions that promoted vegetative growth, but as noted, summer crops were vulnerable to typhoon damage as well.

Grain yields per day for each season were within a narrow range, averaging 34 kg/ha per day over all harvests. The mean grain yield/ha per day in this study required only 66 and 68 kg glucose-equivalent/ha per day of the new photosynthate for spring and autumn crops, respectively. When allowance was made for yield declines (i.e., when estimated spring and autumn yields in 1983 were taken as expected yields), the corresponding values were 85 and 96 kg glucose-equivalent/ha per day. These values are also lower than those reported from Northeast Asia. The autumn crop was more efficient even when mean daily solar radiation (table 1) was considered.

The study shows that yields exceeding 3.5 t/ha in spring plantings and 3 t/ha in autumn plantings are readily attainable at the latitude of this study. These yields would appear to be even more easily attained if soybean were grown in a sequence with other species. Typhoon damage, and probably excessive vegetative growth, contributed to low yields in some summer crops. Summer plantings should benefit from the better adapted vegetable-type cultivars now available.

Nutrient uptake

Uptakes of N, P, and K by spring and autumn crops were largely proportional to grain yield (fig. 2). Although mean uptakes for these two seasons differed, a common uptake-grain yield relationship was valid for both but not for the summer season. Therefore, only spring and autumn data were used to estimate coefficients for the regression models in fig. 2. Several factors explain the deviations of summer N uptake data from the relationships for the other seasons. Rain and wind damage from typhoons that occurred in 1986, 1987, and 1989 caused lodging and reduced grain yields below potential even though vegetative growth was abundant. For these harvests, N uptake by summer crops were above the trend for spring and autumn uptake. In summer 1987, dry matter yield was 14.7 t/ha but the HI was only 0.17. Furthermore, flowers were damaged by rainfall in most summers. Second, rainfall leaches N from leaves. The N uptake data for 1991-93 summers fell below the spring and autumn trend which was consistent with leaching losses caused by summer rainfall. Further discussion is restricted to the relationships between spring and autumn uptake and grain yield except where noted.

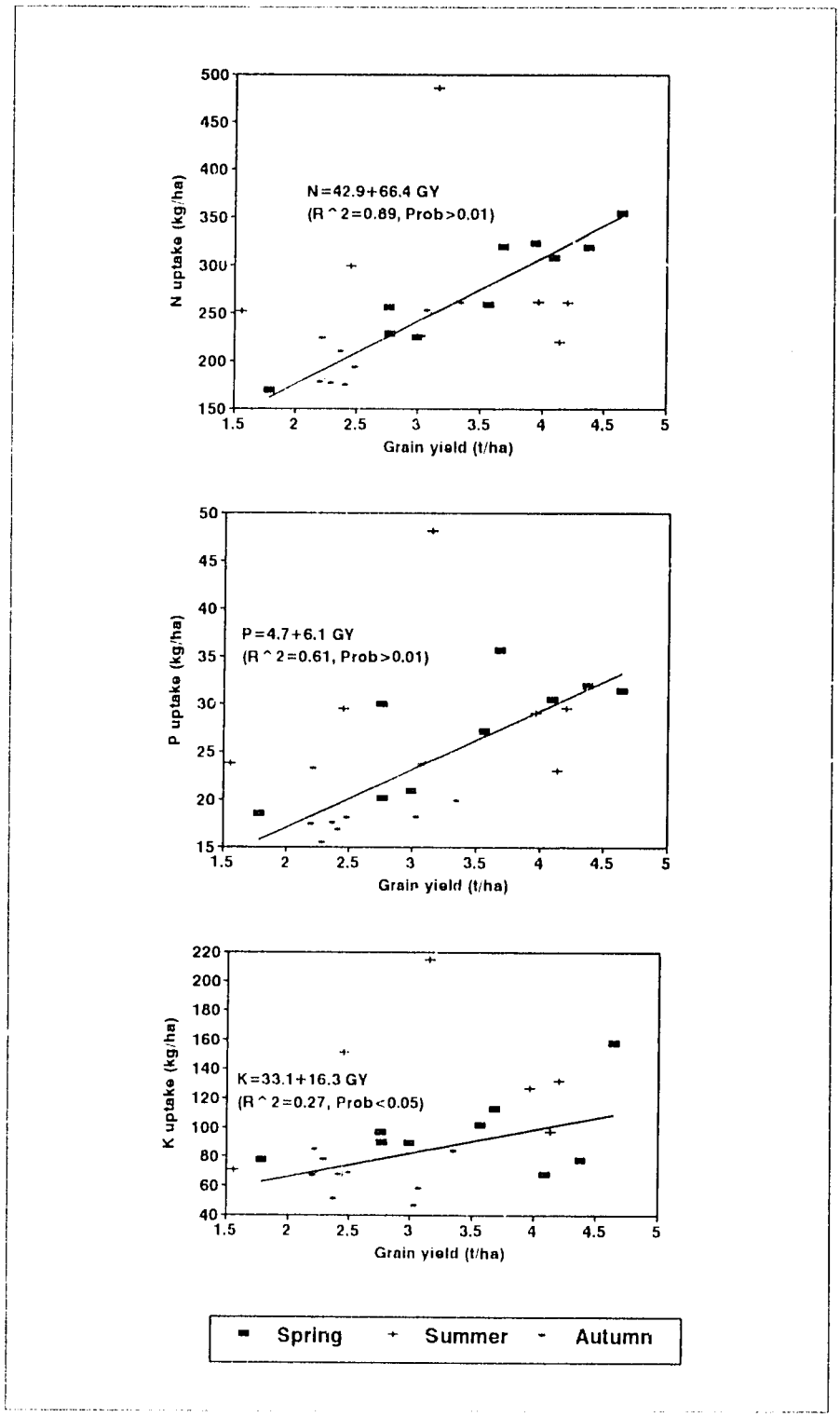


Fig. 2. N, P, and K uptake as functions of soybean grain yield (GY) Summer uptake and yield date were excluded for the regression equations

Nutrient uptake from compost applications increased with time when compared to uptake from treatments without compost, although considerable scatter was evident (fig. 3). The increases were undoubtedly due to accumulations of available nutrients from the large compost applications.

To estimate N uptake as a function of grain yield, compost application coefficients a_0, b_0, b_1, b_2, and b_3 for the following regression model were estimated: $N_{uptake} = a_0 + b_0(d_1) + b_1GY + b_2(d_1)(GY \times T) + b_3(d_2)GY \times T$ where GY was grain yield (t/ha), T was a scale for time (-1.3 = 1983 spring and +1.3 = 1983 spring), d_1 = 1 when compost was applied and 0 otherwise, and d_2 = 1 when compost was not applied and 0 otherwise. Corresponding models were used to estimate coefficients for P and K uptake. Estimated N and K uptake for grain yields from 1.8 to 4.7 t/ha at the beginning and the end of the study are shown in fig. 4. The N uptake model revealed that early in the study, an N contribution from compost was not evident and therefore the uptake-grain yield relation was similar for treatments with and without compost. By the end of the study, however, compost increased uptake and its effect increased as yield increased.

The model for K showed that uptake was not affected early in the study but increased with time regardless of grain yield. As for N, the compost effect was greatest at the high end of the yield range. Unlike N uptake, however, K uptake increased with time even in treatments to which compost was not applied. Applications of K fertilizer in excess of removals early in the study probably contributed to the increased uptake in these treatments as did small quantities of K in the irrigation water. Approximate quantities of K from irrigation, calculated from estimated rainfall deficits and concentrations of K in the irrigation water determined at irregular intervals (2.5 to 3.2 mg/l) were about 25 kg/ha per year.

Only b_1 was significant in the P uptake model and therefore differed little from the P regression on means in fig. 2. Although compost increased available P and its uptake P with time, the quantities apparently were neither sufficiently large nor consistent to be detected by the model.

From spring 1990 onward, K uptake decreased significantly from subsubplots on which K fertilizer applications had been suspended. However, grain yield did not decrease when inorganic K or other nutrient applications were suspended, evidence that K utilization efficiency was greater where K uptake declined. Where the inorganic N or P applications were withheld, significant effects on the uptake of these nutrients were not detected either in treatments with or without compost.

Compost contributions to nutrient uptake were probably not responsible for increased grain yields. As noted earlier, the mean grain yield response to compost was only 0.2 t/ha; there was no evidence that the response increased with time like nutrient uptake. Furthermore, grain yield did not respond to subsubplot treatments in which N, P, or K were suspended from spring 1990 onward. Therefore, the increased nutrient uptake probably contributed very little, if at all, to the small grain yield response from compost. The small but significant contribution to grain yield from compost may be due to non-nutrient effects on roots and shoots or to physical improvements of the soil. The absence of response to accumulated nutrient availabilities demonstrate a need for adjusting fertilizer rates in intensive cultivation systems for past applications and for the nutrient contributions from organic matter applications.

Conclusions

Three soybean crops per year can be cultivated continuously at low latitudes but yield declines, although gradual, should be expected. Soybean cultivars with characteristics similar to AGS 129 and Kaohsiung No. 8 perform well in spring and autumn. Yields should regularly exceed 3.5 t/ha in 3 1/2 months with spring and 3 t/ha in 2 1/2 months in the autumn if yield declines caused by continuous cultivation are avoided. In East Asia, summer cultivations are uncertain because of

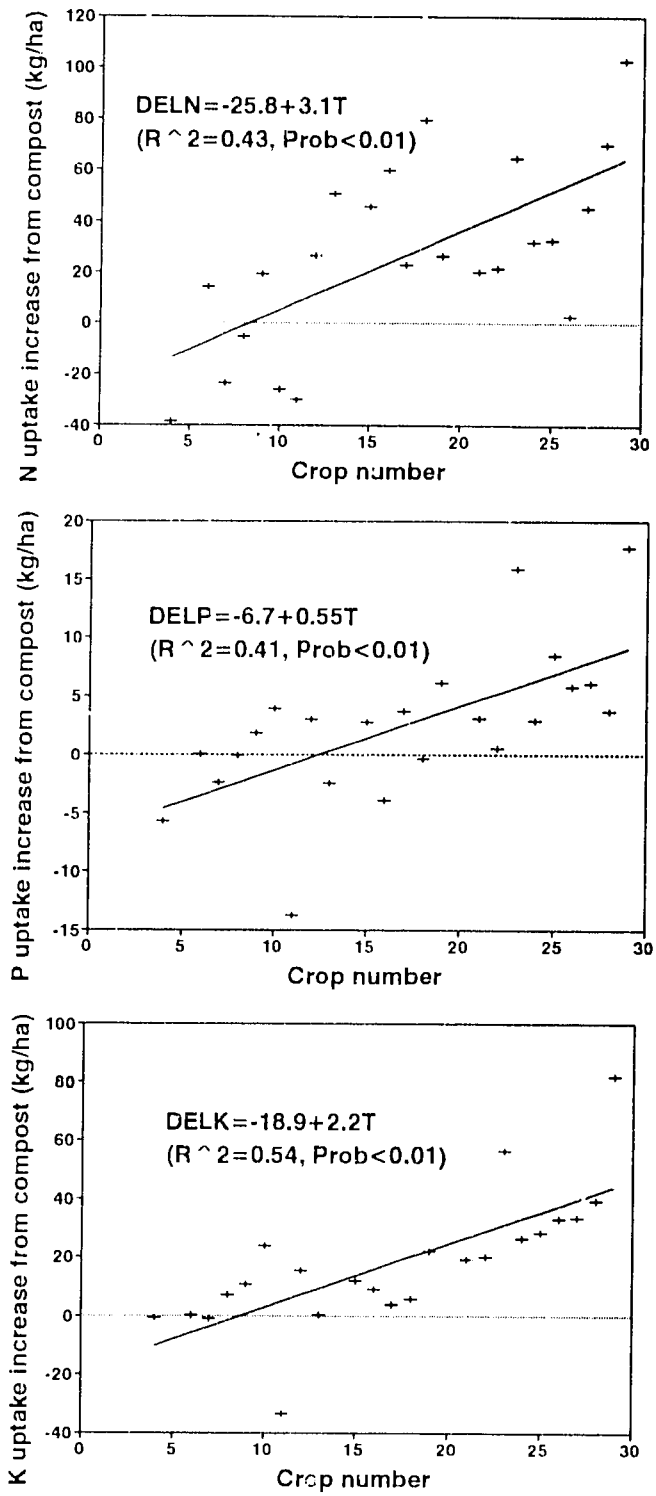


Fig. 3. N, P, and K uptake increases in response to compost applications
DELN = N uptake from compost treatments minus N uptake from compost treatments minus N uptake from treatments without compost; DELP and DELK were defined similarly; T = crop number (time)

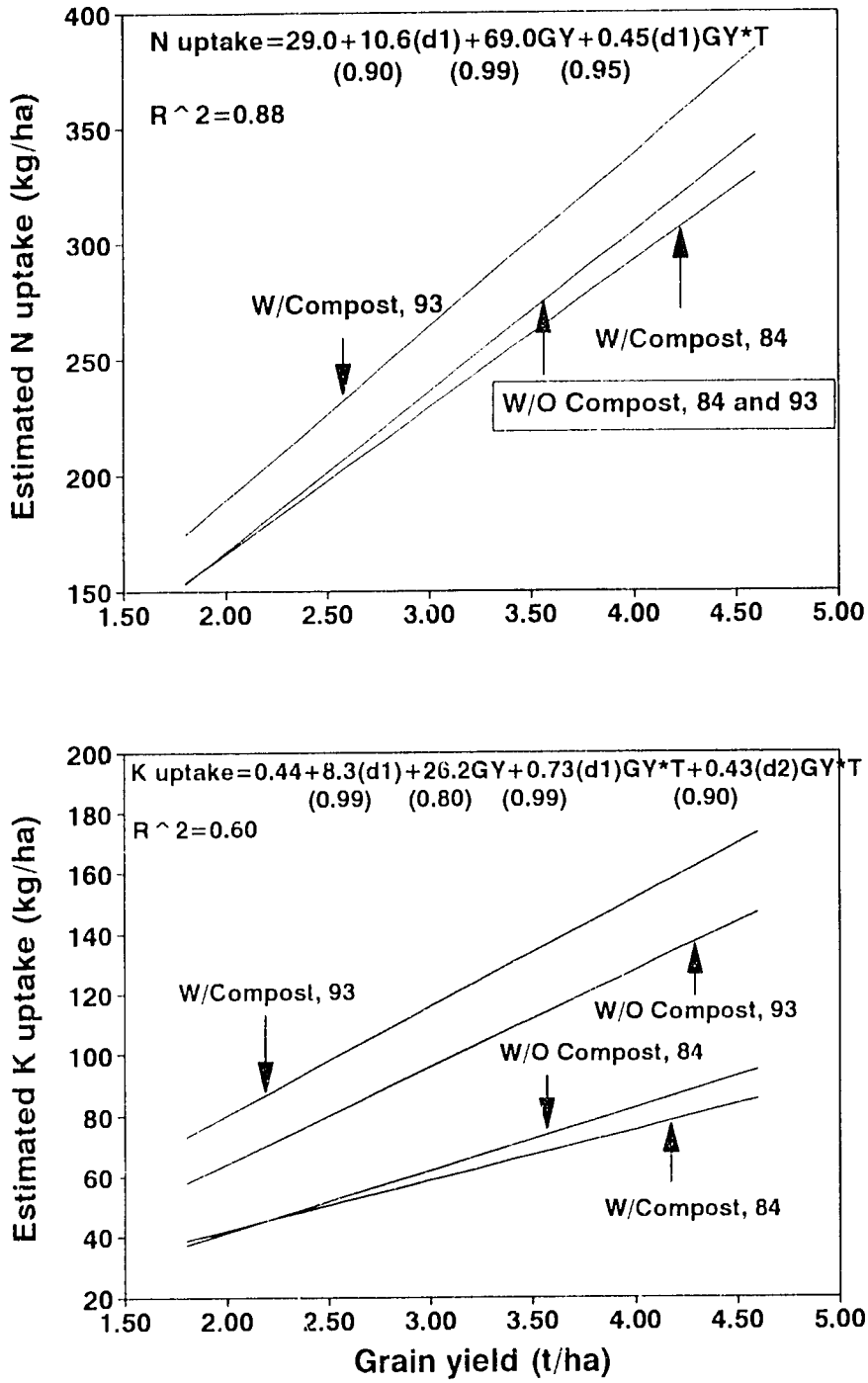


Fig. 4. Estimated N and K uptake from model described in text

GY = grain yield (t/ha); T = -13 for 1984 and +13 for 1993; d1 = 1 for treatments with compost,) otherwise; and d2 = 1 without compost, 0 otherwise. The probabilities for coefficient not equaling 0 are in parentheses

potential rain and wind damage and the summer photothermal environment probably requires cultivars different from those adapted to spring and autumn.

For a farmer, it would be economically unsound and probably agronomically unwise to permit soil fertility to increase to levels observed in this study. The initial and final nutrient availabilities, the absence of responses to suspension of N, P, or K applications to the final 10 crops of the study, and the increasing N, P, and K uptake with time from compost without corresponding grain yield increases demonstrate that nutrient applications were well in excess of crop requirements. Uptake requirements of the three nutrients, on a ton of grain per hectare basis, appear to be no greater and probably are slightly less than uptakes by soybeans cultivated at high latitudes. Soybean cultivars similarly adapted as those in this study, if included as components in intensive systems with other crops, with fertilizer applications for each species adjusted for residual effects of inorganic and organic sources, should be highly productive.

The Effects of Compost on Soil Chemical and Physical Properties After 10 Years of Continuous Cultivation

Summary

Soil data from the 10-year study of compost application to continuously cultivated soybean showed that much of the excess P was retained in the soil but the amount in available form was small. Evidence suggests that a large proportion of excess K leached. Excess N from compost accumulated in the soil organic fraction but the amount lost could not be satisfactorily quantified because the estimates of symbiotically fixed N from soybean could only be approximated. Compost had favorable effects on physical properties which promote root proliferation (bulk density, air permeability, water retention, and soil strength).

Introduction

The benefits of organic materials to cultivation are well recognized. Thus, organic materials are often advocated for intensive cropping systems in Asia. Moreover, if not recycled these materials are potential pollutants of the atmosphere and water resources. Research aimed at improving organic waste management is relevant to sustainable agriculture because of the nutrients these wastes contain and, furthermore, because C is intimately linked to N, P, and S cycling and cation retention through contributions to cation exchange capacity. The non-nutrient contributions to agricultural production systems from organic amendments are less evident.

Although organic amendments are often advocated in Asia, few quantitative analyses of their effects on soil properties have been reported from long-term experiments on intensively cultivated soils in warm climates. The objective of this study was to measure the effects on soil properties of compost applied two or three times per year in a 10-year study.

Materials and Methods

A study of soybean yields, in which the crop was planted two or three times per year on a fine sandy loam, was conducted from 1983 to 1993 to identify suitable management practices for AVRDC soybean lines adapted to the subtropics. Compost application was among the practices evaluated.

Treatments were described in the AVRDC 1993 Progress Report, and other information was presented in other sections of this progress report.

The total quantities of C, N, P, and K applied in composted wastes during the 10 years are listed in table 2. All treatments in the study, regardless of compost application, also received uniform applications of inorganic N, P, and K fertilizers. Furthermore, rice straw mulching was practiced until 1987. The quantities of N, P, and K contributed from the inorganic fertilizers and rice straw mulch are also listed in table 2.

Table 2. Quantities of C, N, P, and K applied, removed in crops, and remaining in soil to 60 cm, under treatments with (W) and without (W/O) compost, 1983—93

	C		N		P		K	
	W/O	W	W/O	W	W/O	W	W/O	W
Applied in compost	0	69600	0	9500	0	3600	0	6600
Total mulch applied	32400	32400	2700	2700	950	950	1900	1900
Removed in crops	0	0	5600[a]	6150[a]	550	650	1800	2400
Change in soil to 60 cm	-3600	+52400	-400	+7450	+50[b]	+30[b]	0[c]	+200[c]
Apparent loss	36000	49600	300	630	350	3600	100	5900

[a] Assumed N fixation was 50 and 67% of removed N for W/O and W treatments, respectively

[b] Change in the available P fraction (Olsen method)

[c] Change in exchange K

Surface soil P and K were determined after each crop on samples of six to eight composited cores taken from each 20-m^2 experimental unit. After the 24th soybean crop, determinations were made of C, N, P, and K in subsurface samples and of physical properties.

Available soil P and K were extracted with Na bicarbonate and Na acetate, respectively. Soil C and total N were determined by potassium dichromate combustion and sulfuric acid digestion, respectively.

An undisturbed core method was used to determine dry bulk density (BD) gravimetrically. Core volumes were 100 cm^3 and sampling depths were 5 cm. The same core sizes were used to determine air permeabilities by a permeameter (Daiki DIK-5001) after extracting water to 250 mm pressure. Moisture release curves were determined from 100 to 900 cm pressure on the Wide Range pF^2 Meter (DIK-3400). Infiltration rates were determined with 60 cm locally fabricated stainless steel infiltrometers fitted with mariotte syphons. Soil impedance was determined to 45 cm with a penetrometer (Penetrograph Stiboka).

Results and Discussion

Soil organic carbon and nitrogen accumulations from compost

The effects of compost on surface soil organic C and total N over time were described in the AVRDC 1993 Progress Report. Samples taken from 60-cm depth in early 1994 showed that after 10 years both elements accumulated below the depth of normal tillage (fig. 5). Leaching and root proliferation probably account for accumulations below 20 cm. The organic C and total N accumulations were not surprising in view of the large quantities of N applied (table 2).

High inorganic N concentrations, moreover, were found in plots to which compost was applied (fig. 5), but inorganic N accumulation below plots not receiving compost also was high, exceeding 250 kg/ha. The decomposing compost obviously contributed to the inorganic N pool as did the large applications of inorganic N and N fixation by soybean.

Because soybean fixes N, only approximations of contributions of compost N to the crop and soil were possible in this study. Fixation of more than 50% of the N accumulated by soybean was reported in the presence of 35 ppm inorganic N in solution from the AVRDC 1985 Progress Report. Assuming that one-third of the N removed in soybeans came from biological fixation when compost was applied in the long-term, a calculated 7450 and 4100 kg N/ha of the N from the compost and other sources accumulated in the soil and were removed by crops, respectively, leaving 630 kg N/ha unaccounted for. Assuming that one-half of the N taken up came from biological fixation when compost was not applied, the corresponding quantities were -400 and 2800 kg N/ha in the soil and crop, respectively, leaving 300 kg N/ha unaccounted for. The loss estimates differ only by 330 kg N/ha which is small considering that the compost and other N sources added 12,200 kg N/ha during the 10 years. Of this quantity almost 7500 kg N/ha accumulated in the 0 to 60 cm soil layer. Although the balances suggest that N losses were comparatively small, the bottom frame in fig. 5 shows that large quantities of inorganic N moved downward under the compost treatment. In view of the estimated small N loss and the large N accumulation below 20 cm even when compost was not applied, more N fixation probably occurred than was assumed in the balances. Furthermore, account was not taken of deposits of N from the atmosphere.

Available P and K accumulations from compost

The compost contained large quantities of P and K and, furthermore, large quantities were applied in inorganic form to all treatments. The contributions of compost to accumulations of available P and K are evident in fig. 6. Accumulations from inorganic P and K sources were small but fluctuated. Fig. 7 shows that compost increased the accumulations of both nutrients below the plow layer as well as in it. Compost applications, however, increased available P by a larger relative amount at all depths when compared to corresponding K increases with depth.

The K accumulation from crop residues and rice straw mulch during the first 10 crops rapidly declined once mulch incorporation ceased in 1987. After the 24th crop, the quantities of compost K plus K from inorganic fertilizers and rice mulch exceeded removal by more than 6000 kg/ha (table 2). The large contribution of K in the rice straw mulch is apparent in fig. 6. Calculations showed that in the plots receiving compost, crop removal of K and its retention in the soil in an available form accounted for only 31% (2600 kg/ha) of K from all sources. Most of the unrecovered K probably leached below 60 cm although fixation by clay minerals may have occurred. In the absence of compost, removal of K accounted for 95% of the K from inorganic fertilizer and rice straw mulch.

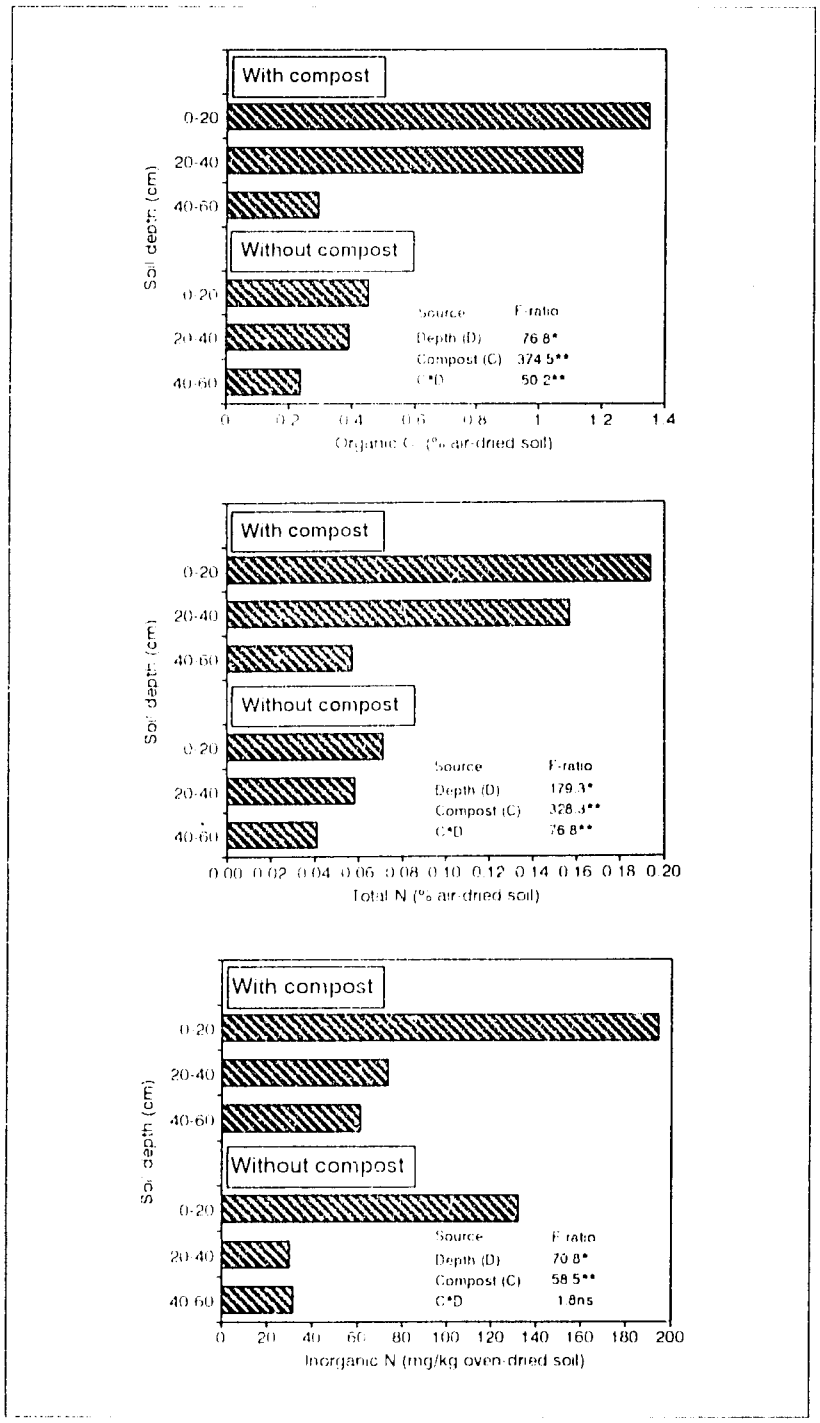


Fig. 5. Soil organic C, total N, and inorganic N in three soil layers after compost applications for 10 years

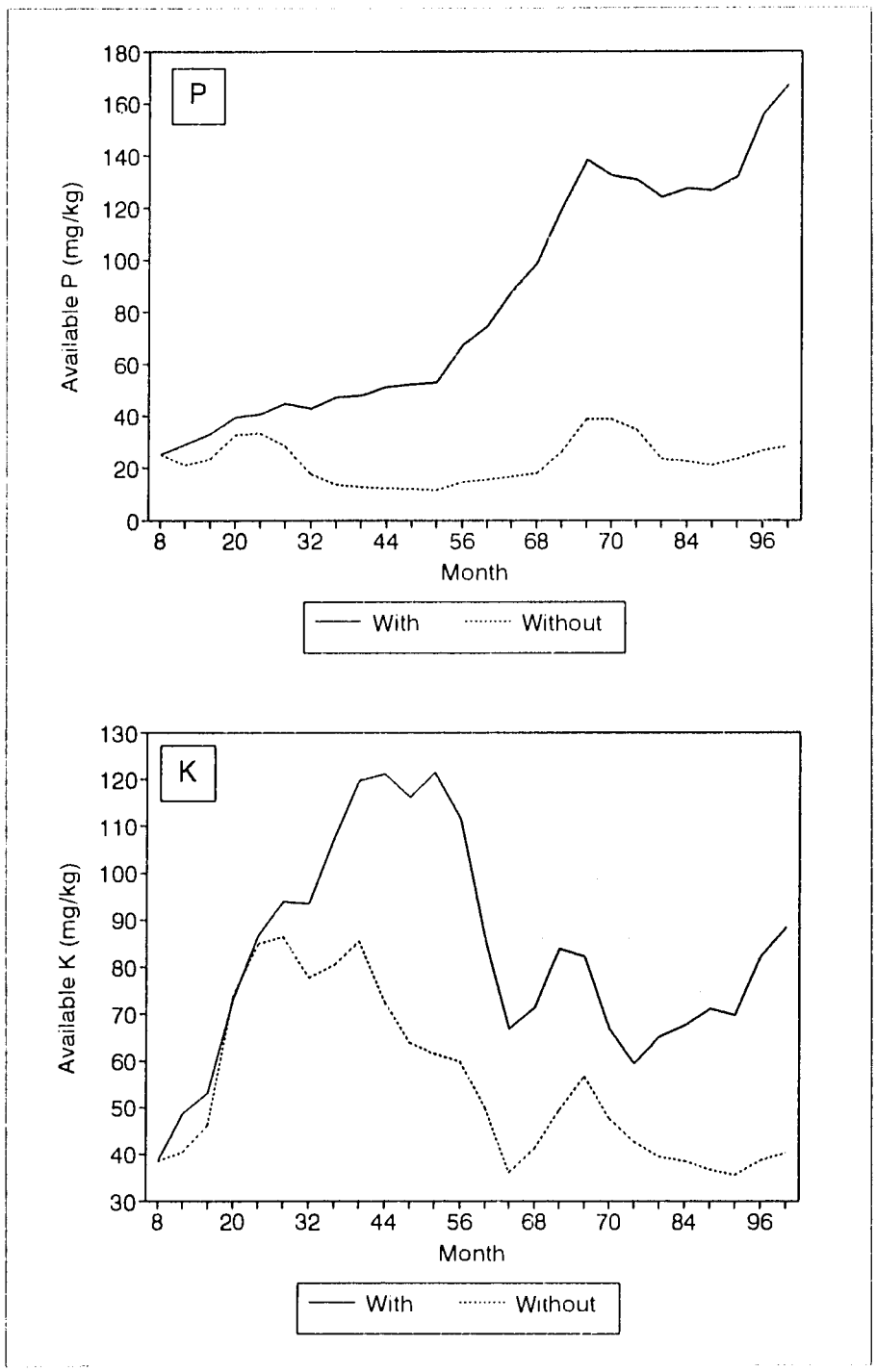


Fig. 6. Available soil P (top) and K (bottom) in surface soils with and without compost applications (lines connect moving averages over three seasons

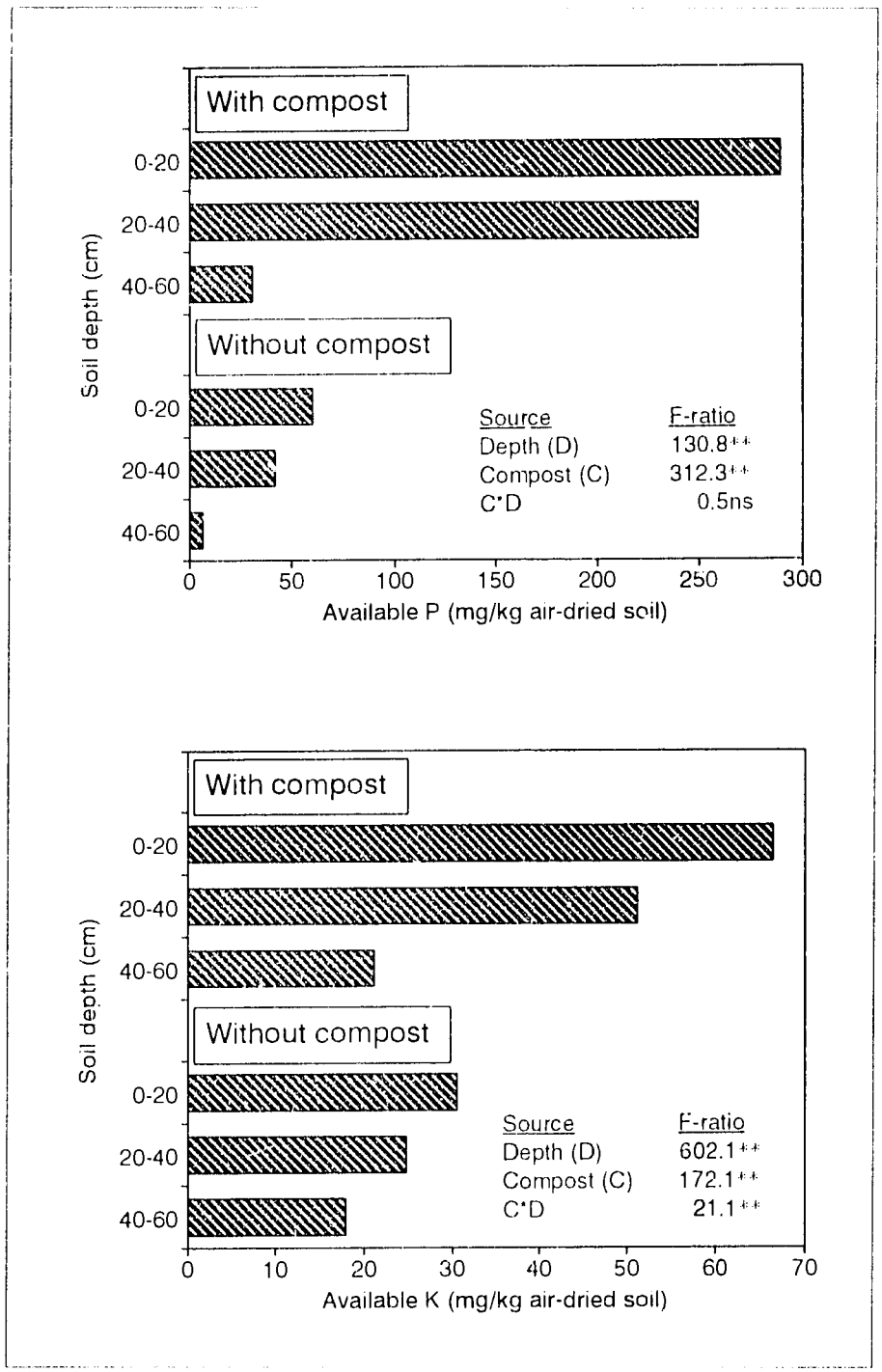


Fig. 7. Available P (top) and K (bottom) at three soil depths after 10 years

The quantities of P removed by the crop and accumulated in available form to 60 cm depth were 950 and 600 kg/ha for treatments with and without compost, respectively. Thus 79 and 37% of P applied in the two treatments were unaccounted for. Whereas much excess K was removed by crops, leached or fixed in clay minerals, P fractionation (AVRDC 1993 Progress Report) showed that inorganic P accumulated in the moderately labile and Ca-bound fractions. The difference between inorganic slowly available + unavailable P in soil receiving vs. soil not receiving compost was over 1000 kg/ha in the surface layer alone. Large amounts probably accumulated in the moderately available organic fraction of the surface layer and in both organic and inorganic fractions of the 20-40 cm layer.

Soil physical properties

The 10 years of compost application decreased bulk density by 12% (fig. 8). This physical response to organic matter accumulation, which is in effect an increase of soil porosity, was expected. Other physical properties affected by porosity, of course, were altered as well. Compost decreased the time required to pass one liter of air under slight pressure through a 100 cm^3 cylinder of soil by 49% (fig. 8). It increased the rate of water infiltration but only after the rapid initial absorption into dry soil was complete (fig. 9). Fig. 9 also shows that porosity determined by saturated water content (i.e., at 0 cm pressure) increased from 43 to 51% which is in general agreement with the difference determined from bulk densities.

Compost decreased mechanical impedance but the difference did not arise until the penetrometer passed through normal depth of tillage (fig. 10). Soil strength under both treatments, however, increased rapidly with depth to levels that make root penetration extremely difficult. Tillage by large tractors on soils that are in a moisture state suitable for the operation on the surface but which remain excessively wet below 20 to 25 cm probably contributed to compression pan formation. Impedance exceeded the penetrometer range at about 40 cm as the pan was encountered.

Conclusions

Applications of composted agricultural wastes created a soil containing large availabilities of N, P, and K, and exhibiting improved porosity and related soil physical attributes in the plow layer and reduced soil strength below it. These changes are favorable for crop growth. Moreover, such a soil should be less exacting in its management demands and require less energy to till. Reduced sensitivity to management is beneficial for intensive cultivation systems where competition for production resources and inclement weather occasionally obstruct the timeliness of operations. Furthermore, applications of inorganic N, P, and K can be reduced by the nutrient quantities in the compost. Complete elimination of inorganic sources can be obtained for some crops. Intensive agricultural systems offer opportunities to recycle agricultural wastes in an environmentally beneficial manner.

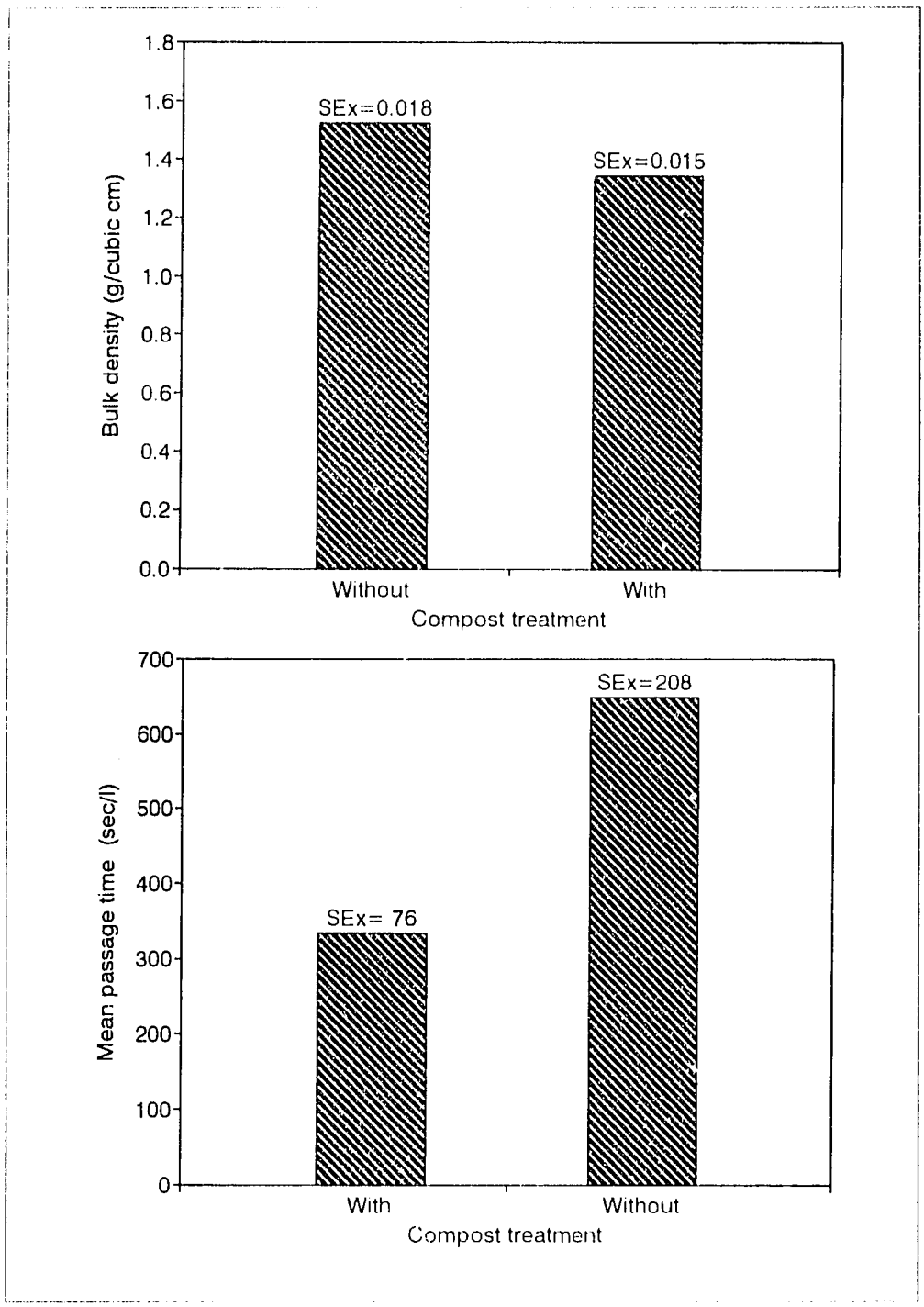


Fig. 8. Effects of compost on the bulk density (top) and air permeability (bottom) of surface soil

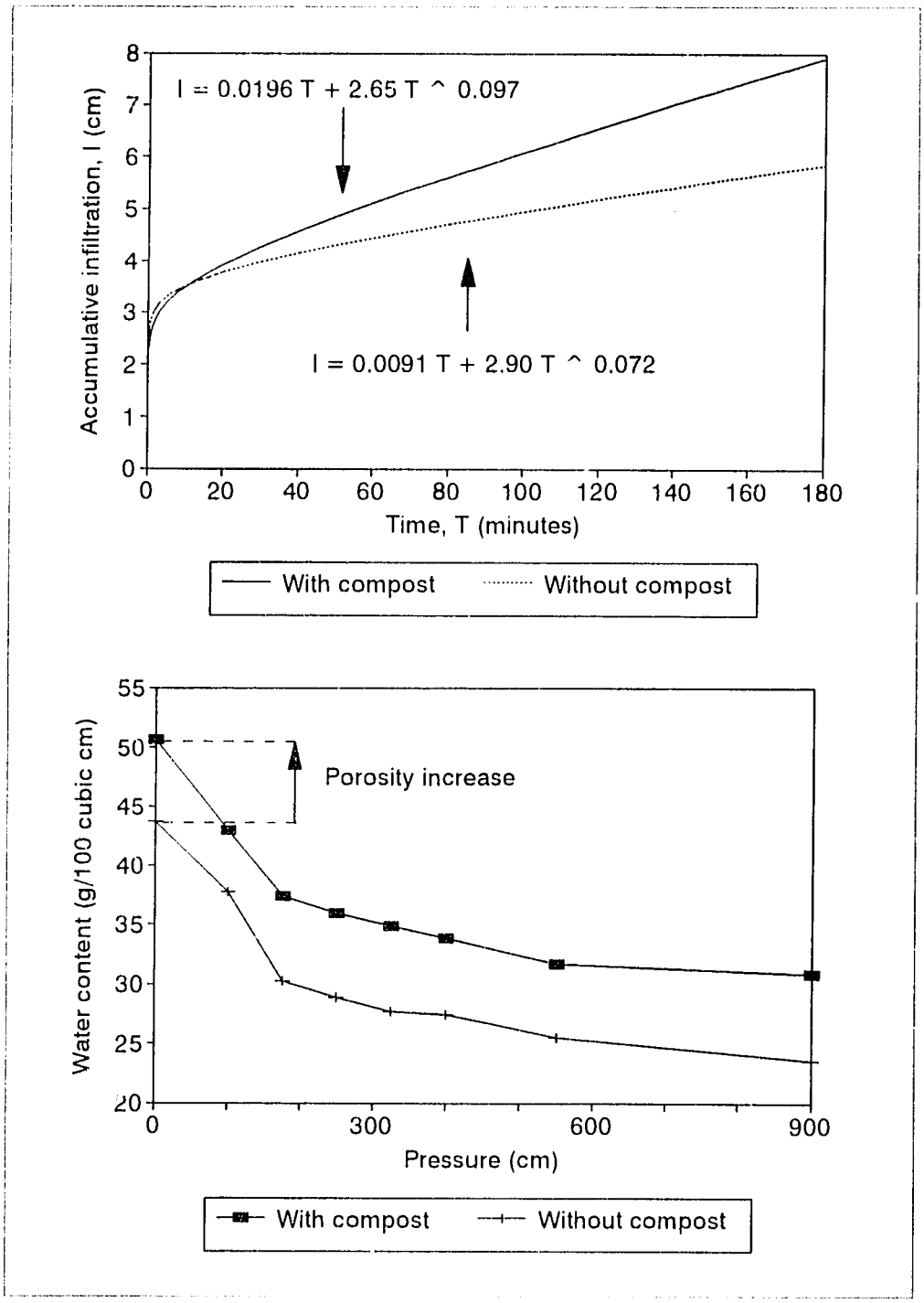


Fig. 9. The effect of compost on accumulative infiltration (top) and water retention under pressure (bottom)

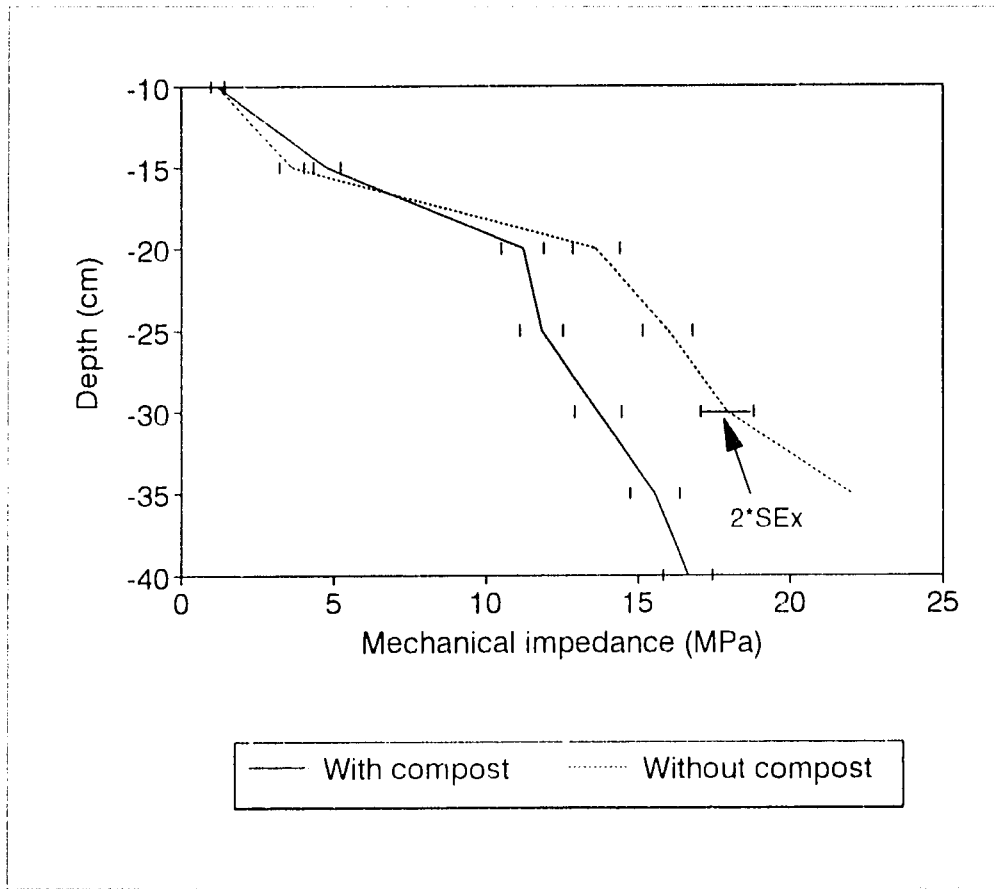


Fig. 10. The effect of compost on mechanical impedfance measured with a cone penetrometer

Organic Carbon in Crop Sequences

Summary

A descriptive model that predicts soil organic C accumulation from compost applications was modified by inserting into it a compartment for fresh organic decomposition. After model recalibration against data from the continuous cropping experiment, the model was used to estimate soil organic C observations from 11 crops cultivated sequentially in five rotations. Organic matter applications (crop residue, rice straw mulch, and compost) differed among the rotations. The model estimated organic C trends satisfactorily.

Introduction

The AVRDC 1993 Progress Report reported on a model that estimated soil organic C (SOC) accumulations for a study in which repeated compost applications had been made to soybeans cultivated continuously for 10 years. When we used the model this year to estimate SOC accumulations measured in rotation studies conducted from 1983 to 1987, however, agreement between observed and estimated SOC was poor. Loss predictions for the C in straw mulch and crop residues, when decomposed at the compost C rate, were too small. Therefore, a third C compartment was added to initial soil organic and compost C compartments, and the model was calibrated and tested to determine if it would satisfactorily estimate SOC accumulations from compost, crop residue, and rice straw mulch against a background of C loss from the initial SOC.

Materials and Methods

The three compartment model was recalibrated on data from the continuous soybean cultivation study used in the 1993 version. In that version, the effects of rice straw mulch and crop residues were ignored even though both were incorporated in the early years of the study. Rice straw mulch (10 t/ha) was applied to half the subsubplots in autumn 1983 and spring 1985 and to all plots from summer 1985 until autumn 1986 after which applications were discontinued. The mulch was incorporated during postharvest tillage in preparation for the succeeding crop. Soybean residues, averaging 3.9 t/ha, were incorporated after harvest of each of the first four crops. Residues, including most of the abscised leaves, were removed from the field as a disease control measure after subsequent harvests.

To estimate the C content of rice straw at the time of incorporation, the following were assumed: 10% moisture content and 40% C, of which one-third was lost during the season before incorporation. Soil organic C determinations were averaged across subsubplots within main plots. Therefore, before the summer 1985 only half the C in a 10 t/ha mulch was entered in the accumulation model. The full quantity was entered when mulch was applied to all plots. A 40% C concentration was assumed for soybean residue.

The three independent C compartments were designated ISOC (initial SOC), COC (compost C), and ROC (crop residue and straw mulch C). As the model progressed through time, the C in each compartment decreased according to sets of coefficients. Each time step was a 4-month interval corresponding to one season (including fallow time between crops). To establish the coefficients for COC decomposition, SOC measurements on the -compost series to which a single 20 t/ha mushroom compost residue was applied in autumn 1984 were fitted to the first order function $SOC = \underline{a} \times e^{-k \times (t+b)}$. Parameters $\underline{a}$, $\underline{k}$, and $\underline{b}$ were estimated by a nonlinear regression routine. To facilitate model calculations and presentation of output, SOC and the quantities of C in the soil and organic amendments were expressed in percent with 2250 t/ha of dry soil as the base. The time variable t was in months. For these estimates, mean ISOC concentration of the -compost series was 0.58%.

Because C from the straw mulch and soybean residues contributed to the SOC measurements during the early intervals, SOC values from autumn 1984 to summer 1987 were adjusted to account for the quantities applied. The amounts subtracted from the measured quantities were computed by using an equation that estimated the C residues remaining under tropical conditions as a function of time. Starting with the quantity applied in each season, the ratios of amounts remaining were 20, 86, 81, and 89%/%. The measured and adjusted SOC are shown in fig. 11. The same ratios were used in the spreadsheet to calculate ROC decomposition for estimating SOC accumulation as a function of time.

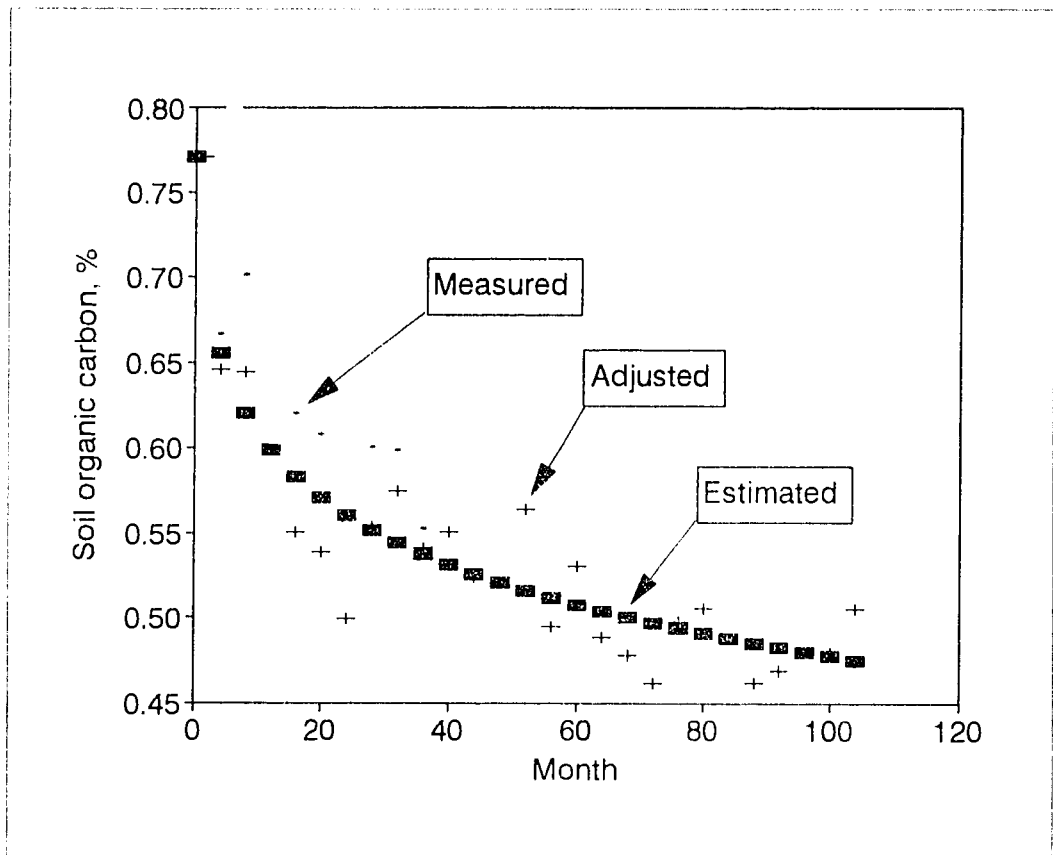


Fig. 11. Measured soil organic C (SOC) before and after adjustments for rice straw mulch and soybean residue incorporation SOC estimates from fitting Equation 2 to adjusted observations

Results and Discussion

Model calibration

The estimate coefficients a = 0.7718 (0.7715, 0.7720), b = 0.282 (0.244, 0.319), and k = -0.133 (-0.145, 0.120) where upper and lower asymptotic confidence levels (95%) are in parentheses. The estimated SOC means from the single 20 t/ha application are shown against the measured and adjusted means in fig. 11.

The parameter a, 0.7718%, is the estimated SOC concentration when t = 0 months. In this study, the SOC at t = 0 was 0.77% (0.58% in the soil plus a combined 0.19% from compost, straw mulch, and soybean residue). From t = 4 to 40 months, SOC was measured but adjusted for residue additions as described previously. The parameter k was the estimated loss rate on base e, and the parameter b decreased the loss rate as t increased.

To predict C accumulation from compost, the "1-decomposition rate, COC" coefficients (row 11 of table 3) were calculated from the SOC estimates from the first order function. The coefficients were obtained by substituting the parameters into the equation, subtracting 0.43% from the estimate at each

Table 3. Decomposition rates and calculations for C accumulation from compost and residue applications for 48 months

Row		A	Month after application											
		0	4	8	12	16	20	24	28	32	36	40	44	48
	ISOC compartment													
1	1 - Decomposition rate, ISOC		0.975	0.976	0.977	0.978	0.979	0.980	0.981	0.982	0.983	0.984	0.985	0.986
2	Remaining ISOC	0.560	0.546	0.534	0.524	0.513	0.504	0.495	0.486	0.479	0.471	0.464	0.458	0.452
	ROC compartment													
3	1 - Decomposition rate, ROC		0.200	0.760	0.810	0.820								
4	ROC incorporated, Autumn, 84	0.125	0.025	0.019	0.015	0.013								
5	Spring, 85	0.103		0.021	0.016	0.013	0.010							
6	Summer, 85	0.208			0.041	0.031	0.025	0.021						
7	Autumn, 85	0.000				0.000	0.000	0.000	0.000					
8	Spring, 86	0.167					0.033	0.025	0.021	0.017				
9	Summer, 86	0.107						0.021	0.016	0.013	0.011			
10	Autumn, 86	0.107							0.021	0.016	0.013	0.011		
	COC compartment													
11	1 - Decomposition rate, COC		0.598	0.872	0.903	0.918	0.927	0.933	0.938	0.941	0.943	0.945	0.946	0.948
12	COC incorporated, Autumn, 84	0.066	0.040	0.035	0.031	0.029	0.039	0.037	0.034	0.032	0.030	0.029	0.027	0.026
13	Spring, 85	0.099		0.059	0.052	0.047	0.043	0.050	0.047	0.044	0.041	0.039	0.037	0.035
14	Summer, 85	0.000			0.000	0.000	0.000	0.000	0.021	0.019	0.018	0.017	0.016	0.015
15	Autumn, 85	0.099				0.059	0.052	0.047	0.043	0.040	0.037	0.035	0.033	0.031
17	Spring, 86	0.133					0.080	0.069	0.063	0.058	0.070	0.066	0.061	0.058
18	Summer, 86	0.111						0.066	0.058	0.052	0.048	0.055	0.052	0.048
19	Autumn, 86	0.127							0.076	0.066	0.060	0.055	0.062	0.057
20	Spring, 87	0.163								0.097	0.085	0.077	0.070	0.065
21	Summer, 87	0.114									0.068	0.059	0.054	0.049
22	Autumn, 87	0.114										0.068	0.059	0.054
23	Spring, 87	0.097											0.058	0.050
24	Summer, 87	0.117												0.070
25	Estimated accumulated C	0.560	0.611	0.668	0.679	0.704	0.786	0.831	0.886	0.933	0.953	0.974	0.987	1.011

t, and calculating ratios between successive intervals. These ratios were the coefficients, expressed as C remaining, in row 11 (table 3). The difference between the SOC estimate and 0.43% was an approximation of young SOC that decomposed at rates established by the first order parameters. Thus, the resultant ratios were approximations of successive relative quantities of young SOC remaining as decomposition progressed. The significance of young organic matter component in decomposition rate studies is well established.

The estimated COC loss, calculated from decomposition coefficients in table 3, was 53% during the first year (t = 4, 8, and 12 months). Allowing for a 50% loss of initial C in the sugar mill waste during open composting, a total C loss of about 75% would have occurred during composting followed by 1 year of incorporation. A loss of about this percentage agrees with expected decomposition rates of materials with similar composition.

The loss rate coefficients for C in mulch and crop residues (ROC), as given earlier, were used to estimate the fraction of ROC remaining (row 3). The decomposition rates reduced the incorporated ROC quantities by 90% in 16 months. After the 16th month, the remainder was added to the COC compartment and decomposed at the COC rate.

The ISOC was composed of unknown proportions of young and old SOC. The loss rates, expressed as "1-decomposition rate, Initial SOC", are listed in row 1 of table 3. In an earlier model, SOC decomposition loss rates were estimated as ratios of regression model SOC estimates at t = 240 to 350 months. These were found to underestimate losses at early intervals probably because young SOC in the ISOC was not taken into account. Therefore, larger loss rates (i.e., smaller C remaining rates) were tested at t = 4 (row 1). The tested ISOC remaining rates decreased linearly from a low (at t = 4) to a high remaining rate, 0.998 (at t = 104). An ISOC remaining rate of 0.975 at t = 4 most closely modeled SOC for the -compost series. Estimated annualized net SOC losses from this rate were 7.0% at the beginning and 0.6% at the end of the series.

The three compartments of the spreadsheet model are shown in table 3. The organic C inputs, decomposition coefficients, and relevant calculations are given only for the first 48 months. The remainder of the model is a simple extension of the spreadsheet rows and columns. In the spreadsheet, ISOC, ROC and COC were entered in the shaded cells of column A. For the -compost simulation, all COC entries in column A were 0 except that for autumn 1984.

The SOC estimate for each interval is the sum of ISOC (row 2), ROC (rows 4 to 10), and COC (rows 12 to 24) in the ith column. The sums for each t are placed in row 25.

The resulting good agreement between unadjusted SOC observations and model estimates is evident in the bottom half of fig. 12 (- series). This agreement was expected because the observations were used to estimate the model coefficients. Estimated accumulations were sensitive to the quantity of "old SOC" assumed and, therefore, the quantity was systematically altered until the SOC estimates shown in the upper half of fig. 12 were obtained. These estimates exhibit the gradually increasing accumulations of the observations.

Sensitivity analysis

The sensitivity of estimated SOC accumulations to model coefficients was examined by increasing and decreasing each by 2%. The analysis showed that SOC accumulations were very sensitive to an increase or decrease in the ISOC decomposition rate. Multiplying 0.975 by 1.02 at t = 4 decreased annualized decomposition during the first year from 7.0 to 1.6%. Multiplying it by 0.98 increased it to 12.3%. The effects were largest during early intervals but cumulative (fig. 13). Furthermore, because ISOC was large compared to COC and ROC, small changes in the coefficient effected relatively large changes in SOC estimates at t = 104.

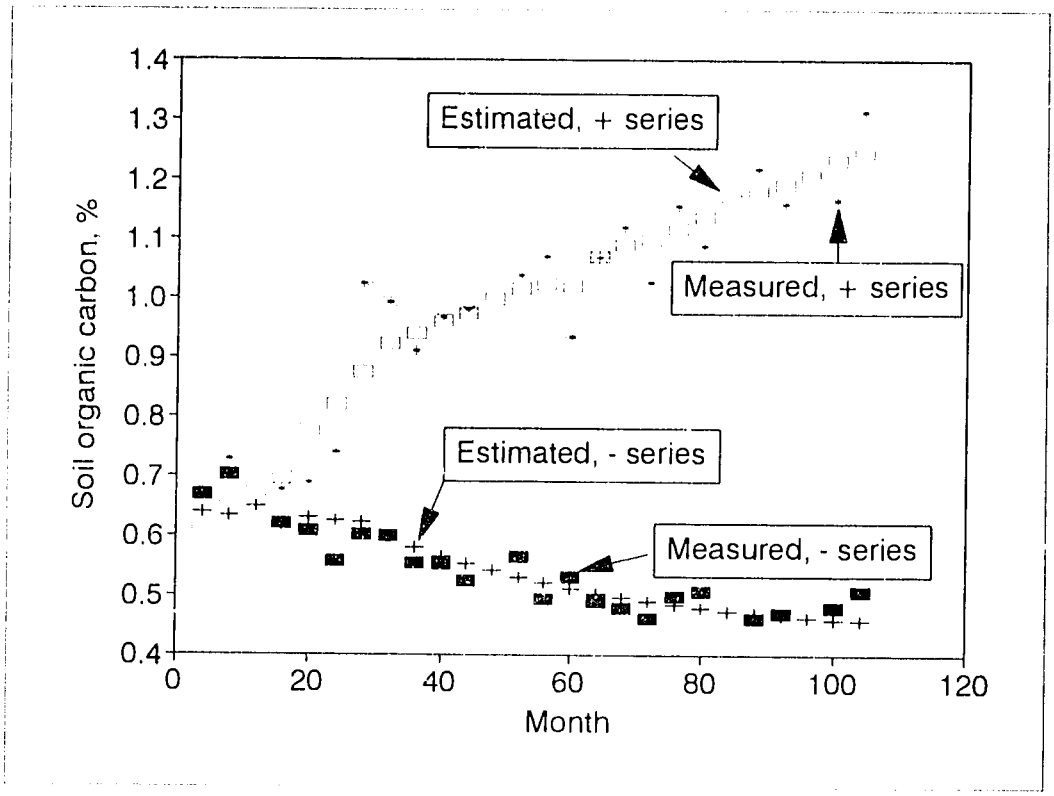


Fig. 12. Measured soil organic C (SOC) from - and + compost series vs. SOC estimates from the spreadsheet model

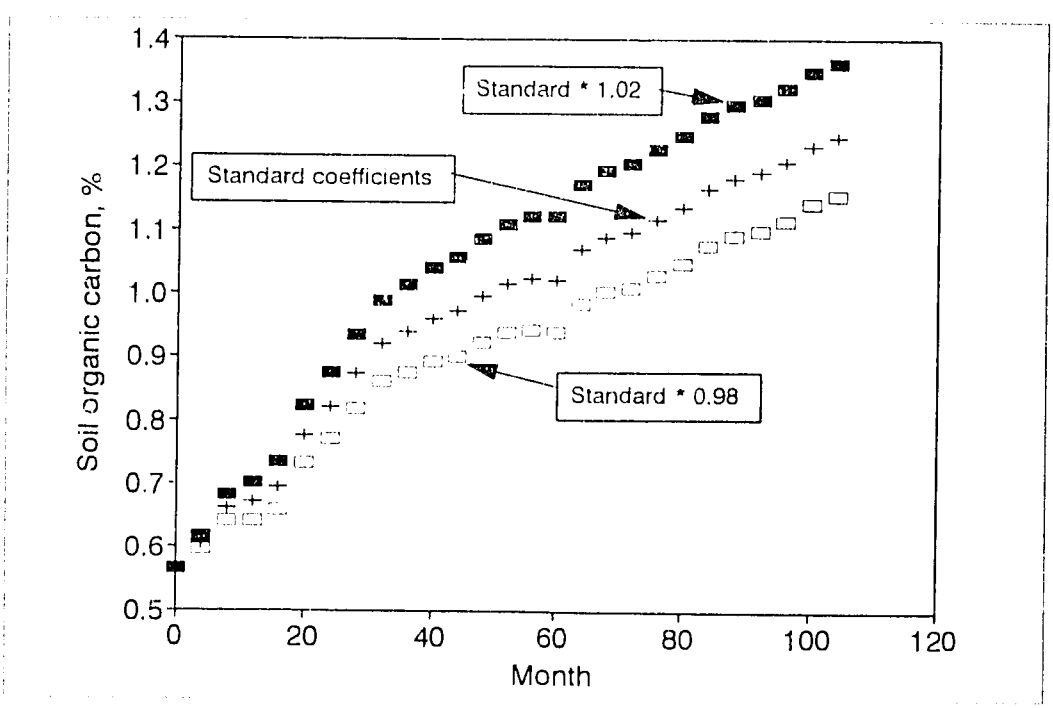


Fig. 13. SOC accumulations from the model with standard ISOC coefficients and coefficients increased and decreased by 2%

To test sensitivity to the portion of old SOC assumed in the calculation of the COC coefficients in row 11, ratios of successive estimates from the first order function were recalculated after increasing the quantity subtracted, 0.43% for the original ratios, by 2%. Accumulation estimates were moderately sensitive to the proportion of old SOC assumed.

To test sensitivity to k and b, the coefficients in row 11 were recalculated after 2% increases and decreases. The effects on both b or k was moderate, but negative when the parameters were increased. Decreasing the parameters had effects on estimates that were approximately equal to but opposite to those from increases. Effects of changes in k or b were cumulative with time but were less sensitive than changes in the proportion of old SOC assumed. When judged in conjunction with rather narrow confidence intervals of the two parameters, the moderate sensitivities of SOC estimates to changes in b and k add confidence to the use of the coefficients in row 11.

The sensitivity of SOC estimates to ROC decomposition rates was examined by multiplying each of the ROC coefficients in row 4 of table s by 0.98 and by 1.02. The changes had nil effect on SOC accumulation at t = 104. Moreover, effects on estimates for t = 4 to 32 never exceeded 1%. Reasons for the low sensitivities are, first, that residue and mulch incorporations were not sustained and, second, the material decomposed rapidly.

Model Validation

The SOC model in table 3 was used to estimate SOC changes for two three-crop/year sequences [A: Chinese cabbage (spring) — tomato — soybean and B: rice (spring) — tomato — soybean] tested in a 4-year study from 1983 to 1987. The crops cultivated in each sequence and the residue, straw mulch, and compost management of each rotation are shown in fig. 14. All rotations were components of a larger study which was reported in the AVRDC 1983 to 1987 Progress Reports. The ISOC of sequence A was 0.74% and that of sequence B was 0.76%. Apart from higher initial C concentrations, the soils on which these sequences were grown were similar to that of the 10-year soybean experiment.

A reduction in the ISOC "1-decomposition rate" from 0.975 to 0.945% at t = 4 produced the estimates for the three rotations in sequence A in the upper frame of fig. 14. The annualized loss in the first year from the adjusted ISOC coefficients was 15%. The ISOC of sequence A apparently contained a large young C content which accounted for the rapid initial loss. The ROC and COC decomposition rates for the residue, straw mulch, and compost were unchanged from those in table 3. Estimated and measured SOC from the rotations of sequence A are shown in the upper frame of fig. 14.

The SOC of the two sequence B rotations are shown in the lower frame of fig. 14. The 0.76% ISOC of sequence B rotations required that the "1-decomposition rate" coefficient be decreased to 0.92 which corresponded to an annualized loss rate of 21%. This suggests that a somewhat larger component of young SOC occurred in this sequence than in sequence A.

Deviations between observed and measured SOC were larger than deviations in the continuous soybean cultivation study probably because each point was a mean of soil samples from only two replicates of 50-m^2 plots. In the soybean study, means were from 16 20-m^2 plots. Although the deviations were large, fig. 15 shows no evidence that the discrepancy increased or decreased as SOC increased, adding confidence to the model. Thus, the SOC estimates demonstrated more forcefully than the highly variable SOC measurements that when crop residues alone were incorporated, SOC declined. The downward trend in rotation 6 of sequence A showed that the decrease was not trivial even when residues from two of the three crops were returned.

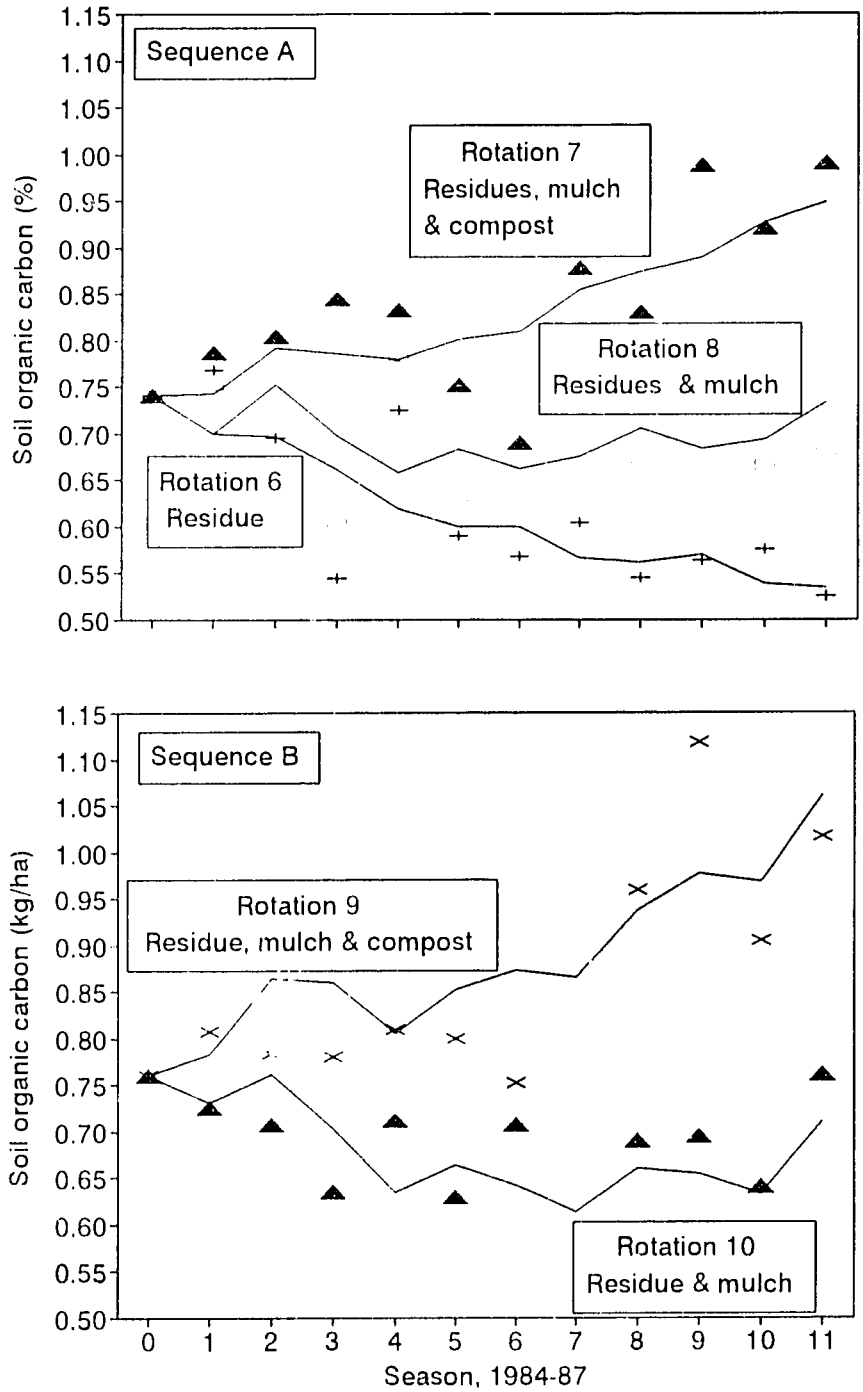


Fig. 14. Measured (symbols) and estimated (lines) soil organic C from three rotations in sequence A (Chinese cabbage—tomato—soybean) and two rotations in sequence B (rice—tomato—soybean)

Organic amendment applications differed

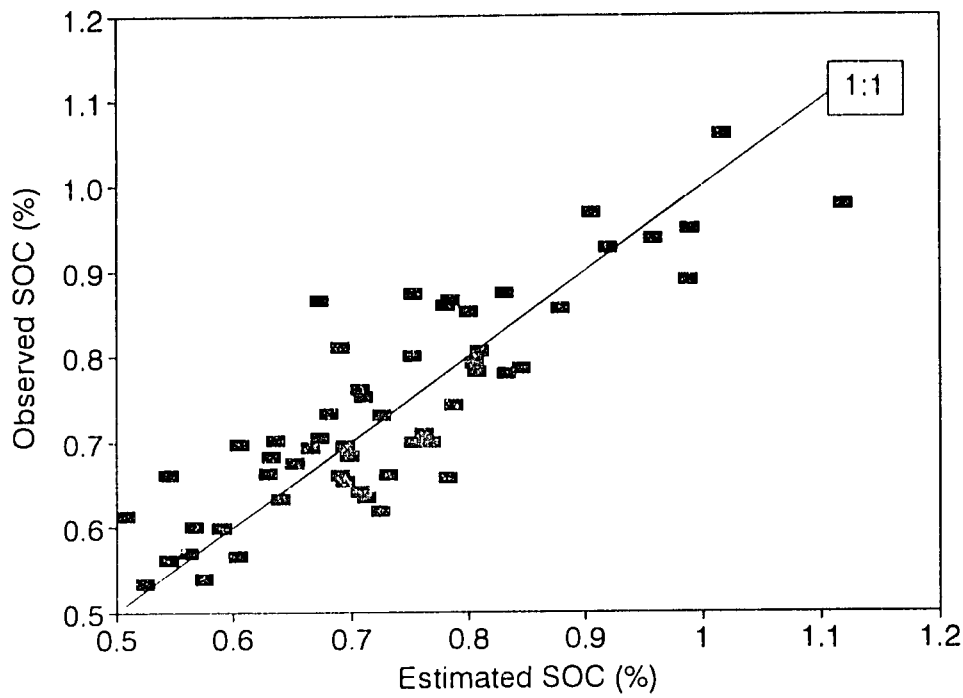


Fig. 15. Observed vs. estimated soil organic C for the five rotations from sequences A and B

Effects of Old and New Compost Applications and Inorganic P and K on Yields, Nutrient Uptake, and Soil Residual Availabilities

Summary

A new compost factor was inserted into the continuous cropping study and vegetable crops were substituted for spring and autumn soybean crops. Likewise, inorganic P and K treatments were revised. Yields and N, P, and K uptake were determined on four crops in the 1994 sequence (onion, tomato, vegetable soybean, and Chinese cabbage). Expected progressions of effects from the old and new compost treatments and from inorganic fertilizer applications were consistent with expectations. New compost and inorganic P and K treatments exhibited stronger effects on yields and uptake with the harvest of each crop. Inorganic K effects on K uptake was evident in tomato and subsequent crops. An inorganic K effect was observed on the yield of the fourth crop (Chinese cabbage).

Introduction

The last continuous soybean crop in the long-term study was harvested on 17 August 1993. Forty-seven-day-old onion seedlings were transplanted on 13 October 1993 to determine if subplot residual effects or interactions of subplots with main plots would be significant before treatments were modified. Only the old compost series treatments were applied to this crop. The onion cultivar Granex (No. 429) and subsubplot inorganic fertilizer applications (252-22-83 kg/ha) were uniform across the experiment.

Materials and Methods

New treatments were fully implemented on the spring 1994 tomato crop. The objectives of the modification were: (1) to determine the effects of SOC accumulations on vegetable yields and nutrient accumulations, and (2) to monitor SOC changes after replacing subplot soybean variety treatments with + and - compost treatments to obtain the following factorial compost treatments in main and subplots:

Old compost series (main plots)	New compost series (subplots) +	New compost series (subplots) -
+	+ +	+ -
-	- +	- -

The factorial combinations of two compost series permitted uninterrupted monitoring of the series implemented in spring 1985 (+ + and - -) as well as monitoring of residual effects from past applications (+ -) and response to fresh applications (- +) to a soil in which the SOC had decreased to less than 0.5%. Another change created a 2x2 factorial of inorganic P and K fertilizer by replacing a treatment with 0-46-75 with x-0-0 kg NPK/ha where x will vary according to crop species. This change at the subsubplot level was made recognizing that responses would be confounded with residual P and K effects for several seasons, but it would also enable assessments of residual inorganic P and K fertilizer contributions.

Full implementation of the new + and - compost series and the P and K factorial modified treatments began when 25-day-old tomato seedlings of FMTT 33 were transplanted on 7 February 1994. Uniform inorganic N applications totaling 90 kg/ha were applied at planting and at 28 days after transplanting. The final harvest was on 17 May. A vegetable soybean cultivar (AGS 292) was planted on 25 May 1994 with a uniform application of 25 kg N/ha. It was harvested on 11 August; 21-day-old seedlings of Chinese cabbage (ASVEG 2) were then transplanted on 25 August. A uniform application of 50 kg/ha of inorganic N was broadcast and incorporated before transplanting; 40 kg N/ha was side-dressed 10 and 20 days after transplanting.

Results

Onion

Mean onion yields of normal + misshaped bulbs, total dry matter yields, and nutrient uptakes are listed in table 4. Without compost 43% of the bulbs were normal shape medium or large in size, 6% were diseased, and 51% were small or misshapen. The corresponding percentages were 38, 15, and 47% when compost was applied.Because the experiment had only two replicates and two compost treatments in main plots, F-tests in the analyses of variance were not sensitive to compost treatments. Interactions of compost treatments with sub- and subsubplot residuals were not detected for any of the attributes in table 4. Standard errors in table 4 were computed from across former subsubplots (n = 16). The slightly higher bulb yields from the compost treatments may be due to sustained N uptake from organic N that mineralized throughout the 103 days of crop growth in the field. Uptake from the N topdressing would be more episodic than uptake from decomposing compost perhaps contributing to a lower utilization efficiency from the inorganic N source.

Table 4. Fresh yield, nutrient uptake, and utilization efficiency of four crops cultivated in the long-term study

Crop	Treatment	Fresh yield	Total dry weight	Total uptake N	Total uptake P	Total uptake K	Utilization efficiency N	Utilization efficiency P	Utilization efficiency K
			t/ha		kg/ha		kg total dry weight/ kg total uptake		
Onion	Without old compost	41.9[a]	5.9[a]	103	14.8	89	57	399	66
	SEx	1.45	0.19	3.1	0.04	2.4	-	-	-
	With old compost	48.3[a]	6.6[a]	102	16.7	108	65	396	61
	SEx	1.45	0.19	3.2	0.64	3.8	-	-	-
Tomato	Without old compost	49.9	7.8	162	18.6	163	48	420	48
	SEx	1.48	0.21	5.0	1.1	7.4	-	-	-
	With old compost	55.8	9.0	186	32.4	225	48	277	40
	SEx	2.18	0.17	3.6	0.6	5.8	-	-	-
Vegetable soybean	Without old compost	12.2	5.4	155	15.9	70	35	343	77
	SEx	0.64	0.39	10.7	1.2	9.0	-	-	-
	With old compost	12.3	6.1	166	24.5	94	36	247	65
	SEx	0.58	0.27	8.1	1.5	8.9	-	-	-
Chinese cabbage	Without old compost	26.8	3.7	125	19.8	133	30	188	28
	SEx	0.86	0.16	4.5	0.78	7.5	-	-	-
	Without old compost	33.2	3.8	128	21.9	132	29	190	28
	SEx	0.67	0.07	2.3	0.51	7.4	-	-	-

[a] Includes all nondiseased onion bulbs

Misshapen bulbs (doubles and thick necks) were more common from the compost treatment, which is additional evidence of sustained N mineralization from this treatment. Abundant N promotes bulb doubling. Loss of many plants to disease, however, resulted in about equal N uptake from the + and - compost treatments. The larger uptakes of P and K and consequent lower utilization efficiency in the + compost treatment were due to the large uptake differences between + and - compost treatments, but with availabilities in neither treatment being limiting. K uptake from the -old compost treatment exceeded the quantity of inorganic K applied. P uptake, however, was much less than that in the inorganic P application.

Sugarcane compost contains Si which can inhibit fungal development and damage by some insects. However, determinations of crude Si in leaf tissue at harvest gave no evidence that compost increased Si availability. On the contrary, the greater loss of plants to disease from the + compost series suggests that the abundant quantities of inorganic and organic nutrients in the combined sources promoted diseases. The mean Si concentration across all treatments was 0.79% ± 0.04.

Tomato

Fresh yields, total dry weights, and nutrient uptakes are given in table 4. Tomato took up more N and K and slightly more P from the soil, the fresh compost, and the inorganic nutrients in 99 days than onion did during a slightly longer field duration. Much more inorganic N, however, was applied to the onion than was taken up by it. Furthermore, virtually no rain to promote leaching occurred during the cultivation of either crop. Therefore, residual N available to tomato was undoubtedly large which may explain the lack of fruit yield response but a modest N uptake yield response to compost.

Compost effects on P and K uptake were large enough to be significant at Prob > F = 0.15 or smaller even though the F-test was insensitive (table 5). The large uptakes of P and K from the + compost series, however, were not used as efficiently as that from the - compost series (table 4).

The inorganic fertilizer effect on total K uptake was significant. Where K applications were suspended since spring 1990, mean total K uptake over all other factors was 168 kg/ha compared to 209 kg/ha when its application was continuous. The crop obtained 191 kg K/ha from the treatment in which continuous P and K applications terminated with this planting, accounting for the significant PxK interaction in table 5. Interactions between inorganic K fertilizer and main or subplot treatments were absent, indicating that K uptake from an inorganic source was largely additive to contributions from the compost. Similarly, no effects of P fertilizer or the new compost series were significant nor did either interact with the old compost series.

Table 4 shows that even without the residual effect of old compost, tomato took up K well in excess of the inorganic K applied. Fig. 16 shows cumulative N, P, and K content of fruit from + and - add compost series as harvest progressed. It is apparent that at final harvest, daily uptake rates had decreased somewhat but remained appreciable.

Of the nutrient determinations made to calculate nutrient uptake, the concentration in leaves plus small stems at harvest was regarded as the most sensitive indicator of nutritional status. An exploratory regression was calculated to determine relationships between total fresh tomato fruit yields and leaf plus small stem nutrient concentrations at harvest. $Y = 51.6 - 18.4\ N\% + 262.0\ P\% - 386.9\%$ (R^2 = 46%). All coefficients were significant at $P < 1\%$. The model showed that for the conditions of this experiment yields would increase by 18 t/ha for every 0.1% decrease in N concentration. Optimum P concentration was 0.34% corresponding to a 63 t/ha yield at 1.9 N%. The N and K concentrations were inversely correlated, however, making model interpretation speculative. The main conclusion from the model is that yields were sensitive to nutrient availabilities within the range found in the study. As the impacts of treatment alterations stabilize, clearer interpretations of treatment effects should be possible.

Table 5. Compost and fertilizer effects and interactions on total fresh yield, total dry weight, and total uptake of nutrients of tomato, soybean, and Chinese cabbage

Source	Fresh economic yield	Total nutrient uptake N	P	K
		Tomato		
Old compost (OC)	NS[a]	+	*	+
P	NS	NS	NS	NS
K	NS	NS	NS	**
P*K	+	NS	NS	*
		Vegetable soybean		
(OC)	NS	NS	*	*
New compost (NC)	NS	NS	NS	NS
OC*NC	+	+	**	NS
P	NS	NS	+	NS
K	NS	NS	NS	**
		Chinese cabbage		
OC	NS	NS	NS	NS
NC	NS	NS	*	*
OC * NC	+	NS	*	+
P	NS	NS	NS	*
K	**	*	NS	**
P * K * + NS +				

[a] NS = not significant, + = P >85%, * = P >95%, ** = P >99%

In this experiment, sunscalding was very common because the trellising geometry was inferior. Many sunscalded fruits exhibited gray wall disorder but relationships between gray wall and treatments were not evident.

Vegetable soybean

Yields of fresh pods and total dry matter and N, P, and K uptake are listed in table 4 for + and - compost treatments. An analysis of variance summary is given in table 5. As noted, the sensitivity of F-tests on both old and new compost was low. Nevertheless, the tests revealed that P and K uptake were significantly affected by old compost and by several interactions between old and new compost treatments as well. Nitrogen uptake and fresh pod and dry matter yields were only weakly influenced by any factor. [The absence of a major effect on N uptake was expected for this crop but not for tomato.] The higher total dry matter yield from the old compost treatment, although not significant, was attributable to greater vegetative growth. The utilization efficiencies for P or K followed the patterns similar to those of onion and tomato. The additional P and K taken up from the compost was not used efficiently, evidence that availabilities of both nutrients in old compost treatments exceeded crop requirements.

Chinese cabbage

Fresh yields of Chinese cabbage were affected by old compost as were total dry weights, but the dry weight yield difference was relatively much smaller. Old compost did not have significant effects on nutrient uptakes (tables 4 and 5). Because total dry weights and nutrient uptakes were not strongly affected by old compost, differences in the utilization efficiencies listed in table 4 for each nutrient were small.

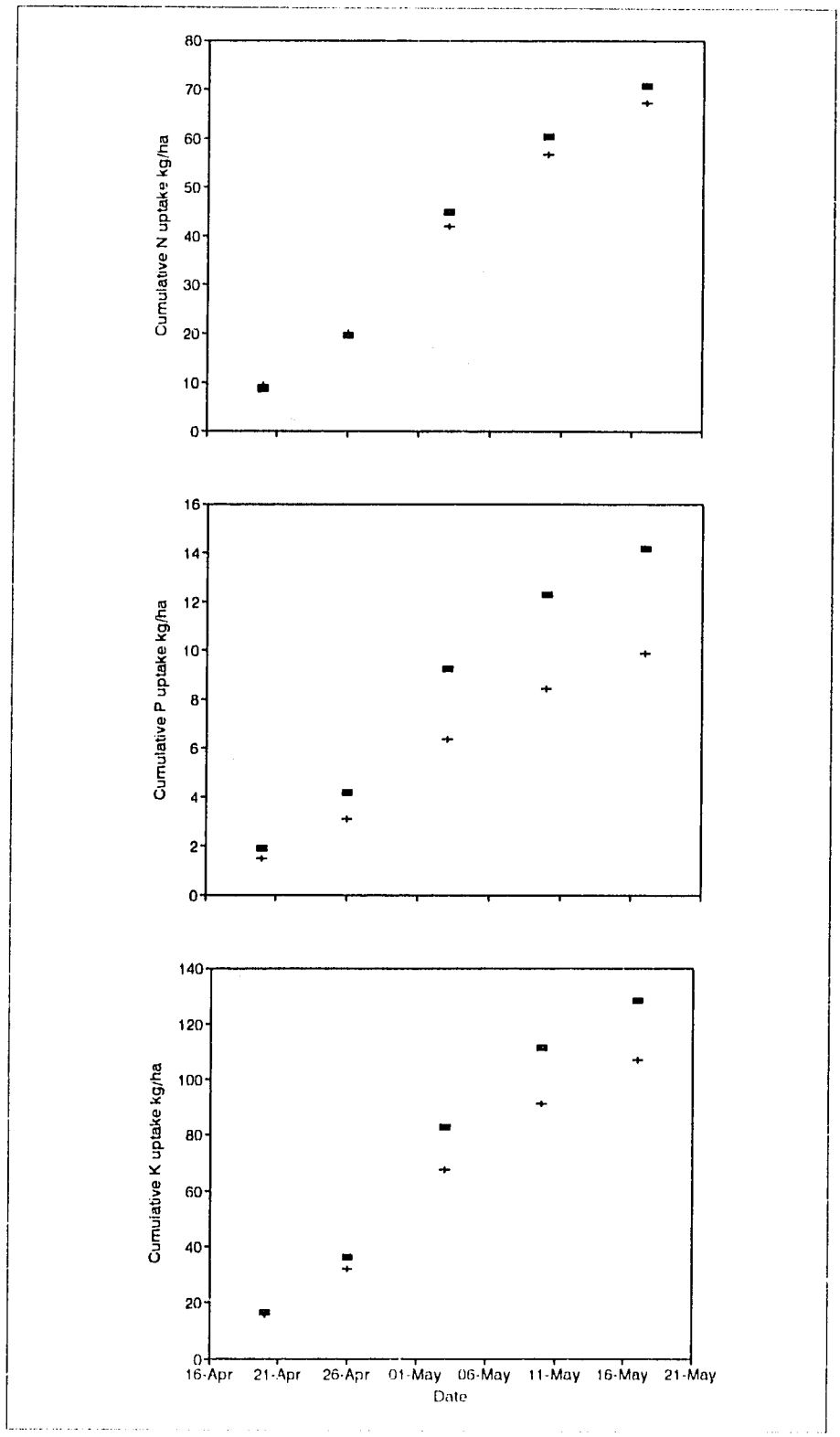


Fig. 16. Cumulative N, P, and K uptake in tomato fruit from the old compost series
Solid boxes are + compost; open boxes are - compost

Potassium affected all attributes in table 5 except P uptake. Potassium uptake ranged from 103 kg/ha when neither new compost nor inorganic K was applied to 160 kg/ha when both were applied. Furthermore, K formed significant interactions with other factors. The contributions of K from the three sources were generally in the order inorganic K > new compost > old compost which suggests that accumulations of available K are less pronounced than accumulations of available P.

The application of 75 kg k/ha increased mean head yield from 28.9 to 31.0 t/ha but K uptake from 112 to 154 kg K/ha. The value of the 2.1 t/ha response was NT$17,400/ha whereas the material cost of the K fertilizer was only NT$560/ha. This illustrates the economics behind a typical farmer's decision to apply fertilizers liberally to high-value crops to ensure that a nutrient deficiency does not limit yields.

Nutrients in the Rotation Experiment

Summary

To use nutrients efficiently as well as to minimize environmental contamination, fertility management practices for intensive vegetable production systems should take into account contributions from crop residues, residual effects of fertilizers, nutrient contributions from composts and straw mulch, and N contributions from fixation by legumes. The effects of these factors were examined on rotations composed of three crops per year. These rotations differed in crop species and residue, straw mulch, and compost applications. The contributions were examined by plotting cumulative net nutrient application (nutrients from all sources minus nutrients removed in harvested crop components) and cumulative changes in available soil nutrients (and total N) for sequences of 11 seasons from 1983 to 1987. Trends showed that returned residues were insufficient to maintain total soil N even though moderate inorganic N applications were applied. When straw was incorporated, the balance between N removals and soil N improved, whereas the addition of compost and straw produced a substantial total soil N increase during the study. In comparison, K was slightly in excess even without straw mulch or compost applications. The K in straw and compost created large excesses over removals but much of the excess was not recovered in the soil. It probably leached or was fixed by clay minerals. The balances of P between applications and removals and soil accumulations were intermediate between those of N and K.

Analyses of seasonal effects showed that available K and NO_3-N concentrations decreased during the summer and peaked at the end of the autumn. Moreover, annual amplitudes were larger than long-term K and NO_3-N trends produced by straw and compost applications. In contrast, total N and soil organic C accumulations from straw and compost exhibited large positive trends with small seasonal amplitudes. The behavior of available P was, again, intermediate between those of K and total N.

Introduction

Strategies for managing soil fertility in three-crop sequences in subtropical and tropical climates, which often occupy a field during 80% of the year, differ from strategies for single crops in temperate climates. Three factors contribute to the differences. First, the quantities of nutrients removed by each crop in a three-crop sequence may equal that of the single crop grown in a temperate climate and, therefore, great demand is placed on the soil to supply nutrients throughout the year. Furthermore, the development rate of the temperate crop may be slower so that daily nutrient uptake rates during maximum growth will often be less than maximum rates of crops grown in warmer climates.

Second, late fall, winter, and early spring precipitation recharges soils for summer crops in temperate environments. Irrigation, where it is practiced in many temperate climates, supplements water from summer rainfall whereas in warm climates irrigation supplies essentially all water during the dry season. Furthermore, rainfall often will be in excess during wet seasons in warm climates. The soil water regimes in the contrasting environments affect nutrient movements and transformations as well as root development.

Third, the effects of low temperatures on nutrient kinetics and on root development during early spring plantings are of little or no concern in warm climates.

Intensive crop sequences in warm environments are often composed of both high-value vegetable crops and low-value staple crops. These two crop groups differ in production costs and possibly in root habit, both of which have implications for fertility management. The generally small root systems of vegetable crops require high soil nutrient concentrations to achieve optimal uptake rates. Furthermore, because production costs and the value of vegetable crops are high, farmers tend to fertilize at rates well above those required for average yields. Therefore fertilizer residuals from vegetable production are large.

Quantitative information on nutrient uptakes and nutrient recoveries by crops in intensive sequences is scarce. Such information is needed to improve fertility management practices. More precisely, management practices should take into account crop needs, returned residues, and soil residuals. The practices must also consider the implications of crop value vs. fertilizer costs.

The objectives of the analyses that follow were to reexamine yields and nutrient uptake of five of the 10 crop sequences in the 1983-87 rotation experiment to determine relationships between crop yields, nutrient applications and removals, and availabilities of soil nutrients. The purpose was to understand better the fates of nutrients applied in the forms of inorganic fertilizers, organic wastes, mulches, and crop residues. Inorganic fertilizers are the easiest and cheapest of the nutrient variables to adjust with respect to amount and time of application. It was our intent to determine how to adjust inorganic fertilizer applications to satisfy crop nutrient requirements taking other nutrient sources, including residual effects, into account.

A general nutrient balance equation can be used to direct the analyses required to meet our objectives. The following equation, modified from Meisinger (1984)[2], includes components relevant to assessing balances between nutrient applications, removals and recycling.

$$M_f + M_{res} - M_{cb} - M_{nh} + M_{misc} - M_e - M_l - M_g - (M_{imm} - M_{min}) + (M_{bimorg} - M_{cimorg}) = 0$$

M_f = inorganic and organic fertilizers

M_{res} = the nutrient content of crop residues

M_{misc} = miscellaneous quantities from atmospheric deposits, symbiotic fixation, etc.

$M_{cb} + M_{nh}$ = nutrients removed in the harvested and nonharvested aboveground plant parts, respectively. M_{nh} is M_{res} for the subsequent crop

M_e = available nutrients carried off by soil erosion

M_l = nutrients lost by leaching

M_g = nutrients transported from the soil as gases

$M_{imm} + M_{min}$ = nutrients immobilized and mineralized (from the soil organic and/or mineral fraction as appropriate for each element) during the cropping period

[2] Meisinger, J.J. 1984. Evaluating plant-available nitrogen in soil-crop systems. p. 391-416. In Hauck, R.D. (ed.-in-chief). Nitrogen in crop production. ASA, CSSA, and SSSA, Madison, WI.

M_{bmorg} - M_{emorg} = the labile or available contents of a nutrient in the effective root zone at planting and at final crop harvest

The rotation experiment was initiated in autumn 1983. Each of six model rotations were planted on 50-m^2 plots. Four rotations were added in summer 1984. The rotations were replicated twice. The total annual applications of inorganic N, P, and K per rotation were limited to nominal rates of 150, 52, and 149 kg/ha, respectively. In spring 1986, however, the total N limitation was increased to 200 kg/ha per year. All crop residues, except that from Chinese cabbage, were soil-incorporated for the following crop. The nutrient concentrations in soil samples taken to 10 cm were determined. However, we have assumed that concentrations in the 0- to 10-cm and 0- to 16-cm depths were nearly equivalent because these were the approximate depths of rotovation used three times each year thereby maintaining a well mixed plow layer. The dry weight of the 0- to 16-cm soil layer was used to compute nutrient mass from concentration.

Nutrient Uptake vs. Yield

The objective of the nutrient uptake component of this study was to quantify the relationships between economic yield and the nutrient uptakes as well as develop a basis for estimating missing nutrient uptakes for the 1984 summer and autumn crops. The relationships between yield and nutrient uptake can be used to estimate M_{ch}, M_{tb}, and M_{tes}. For the analyses, time was regarded as a proxy for nutrient accumulations from inorganic fertilizers, straw mulch incorporation, incorporated crop residues and compost applications. Nutrient concentrations in crop components were determined from spring 1985 until the study was terminated in autumn 1987. Uptake data from Chinese cabbage, rice, soybean, and tomato in all 10 rotations during this period were used to develop relationships between nutrient uptake and economic yields.

Chinese cabbage

Fig. 17 shows N, P, and K uptake in relation to inner head dry matter yield. The curves were hand drawn. Lines in each frame projected from 1.75 t/ha on the X-axis though the curves estimate the nutrient uptake corresponding to that economic yield. Mean water content of inner heads was 94% so that a 1.75 t/ha crop was equivalent to a 29 t/ha crop on a fresh weight basis. Estimated N, P, and K uptake by a crop yielding 1.75 t dry matter/ha was 115, 18, and 110 kg/ha, respectively. The N, P, and K harvest indexes show that about half the N and K and almost 60% of the P was in the inner head. If the residue were returned to the soil, about half of the N and K taken up and 40% of the P would become available for future crops.

The regression coefficients relating each nutrient to dry matter yield are listed in table 6. Whereas the time variable had no impact on N and P uptake, the coefficient for K showed that it accounted for a substantial portion of the uptake. Average uptake increased about 25 kg K/ha in each annual cycle regardless of yield trend. How much of the increase was due to accumulation of K fertilizer residuals and incorporated residues from preceding crops cannot be isolated because the two were confounded. Mean K concentrations in the outer leaves of Chinese cabbage in a rotation with no mulch and no compost (rotation 6) were 1.4, 1.4, and 3.1% in 1985, 1986, and 1987, respectively, evidence that soil K availability was marginal in the first two years but increased with time. The corresponding concentrations from a rotation that received 60 t/ha compost + 20 t/ha rice straw mulch each year (rotation 7) were 2.0, 3.0, and 5.4%, evidence that it increased with time but that deficiencies were unlikely in all years. For common cabbage, a similar heading crop, 1.5 to 3.0% K in wrapper leaves,

is regarded as adequate K (Hochmuth et al. 1991)[3]. Although yields in 1985 and 1986 in rotation 6 probably suffered from a K deficiency, R^2 for the regression of dry matter yield on time overall rotations and years was only 0.08, which showed that yield was largely uninfluenced by increased K uptake with time.

Rice

Fig. 17 shows N, P, and K uptakes in relation to rough rice grain (or paddy) yields. The curve for N was hand drawn for spring crop data. The line from 4.5 t/ha yield on the X-axis projected through the curve intersected the Y-axis at 75 kg N/ha. N uptake was also related to time. Both P and K uptakes were unrelated to grain yield but were related to time (table 6). The relationships between nutrient uptake and rice yield were weaker than those between nutrient uptake and Chinese cabbage yield. The coefficients in table 6 show that the average P and K uptake increased 6 and 33 kg/ha each year, respectively. Mean P and K uptakes over all years were 21 and 80 kg/ha, respectively, which agree approximately with the P and K projections on fig. 17.

More than two-thirds of the N and three-fourths of the P taken up by rice were removed in the grain whereas more than 80% of the K remained in the straw.

Regression of N uptake on rice grain yield and on time separately generated significant regression coefficients. Inclusion of both, however, reduced the significance of each, evidence that grain yield and time were correlated. However, a regression of grain yield on time was not statistically significant (R^2 = 0.23), which suggests that rice yields did not respond strongly to nutrient accumulation. Therefore, both grain yield and time variables were retained in the regression (table 6).

The weakly significant partial regression coefficient for time on N uptake can be attributed to N management. Spring rice crops in rotations without compost were probably deficient in N when only 25 kg N/ha was applied to the crop early in the study. Spring rice received 15 kg N/ha of the extra 50 kg N/ha applied per rotation per year beginning in 1986 and the increase from this extra N probably contributed to modest grain yield and N uptake increases. In 1985, for example, spring rice crops in two rotations (no. 9 and 10) took up an average 61 kg N/ha compared to an average 71 kg N/ha in spring 1986 when 40 kg N/ha was applied. Summer rice received 60 kg/ha throughout the study. The crop took up more N than spring rice did but used it less efficiently (fig. 17).

Soybean

The relationship between N uptake and economic yield is shown by the data and hand-drawn curves in fig. 17. A crop yielding 3.5 t/ha grain took up 275 kg N/ha and 28 kg P/ha. Uptake averaged 98 kg K/ha. Soybean grain (including pods) removed over 70% of N and P and almost 60% of the K. Nevertheless, because soybean took large quantities of N relative to the other species, the quantity returned averaged 74 kg N/ha. Note that the Y-axis scale for soybean on fig. 17 starts at 160 kg N/ha which is where the scales for the other crops end.

The relationship between N uptake and grain economic yield was robust (table 6) without the inclusion of time. However, it was evident in fig. 17 that N uptake increased as yield increased in autumn crops but not in spring crops even though yields of the latter were higher. Time accounted for a significant portion of P uptake variation whereas the time variable alone accounted for 78% of K uptake variation. Soybean is recognized for its ability to take up K in abundance and in proportion to the quantity available but with decreasing utilization efficiency as availability increases.

[3] Hochmuth, G., Maynard, D., Vavrina, C., and Hanlon, E. 1991. Plant tissue analysis and interpretation for vegetable crops in Florida. Booklet SS-VEC-42, Univ. Florida, Gainesville, FL.

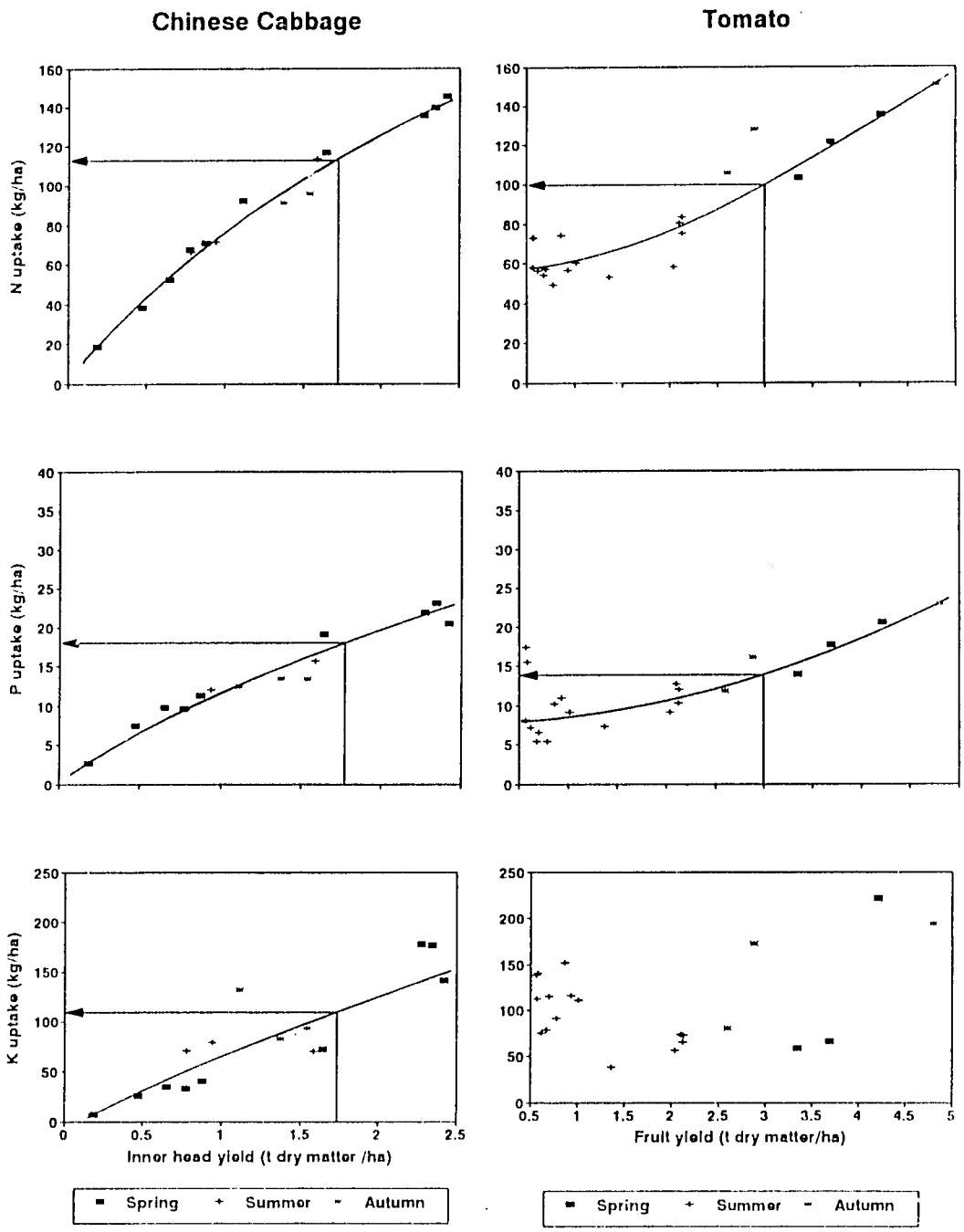


Fig. 17. Total N, P, and K uptake by Chinese cabbage, tomato, rice, and soybean vs. economic yields
Data taken from the 1984–87 Rotation Study

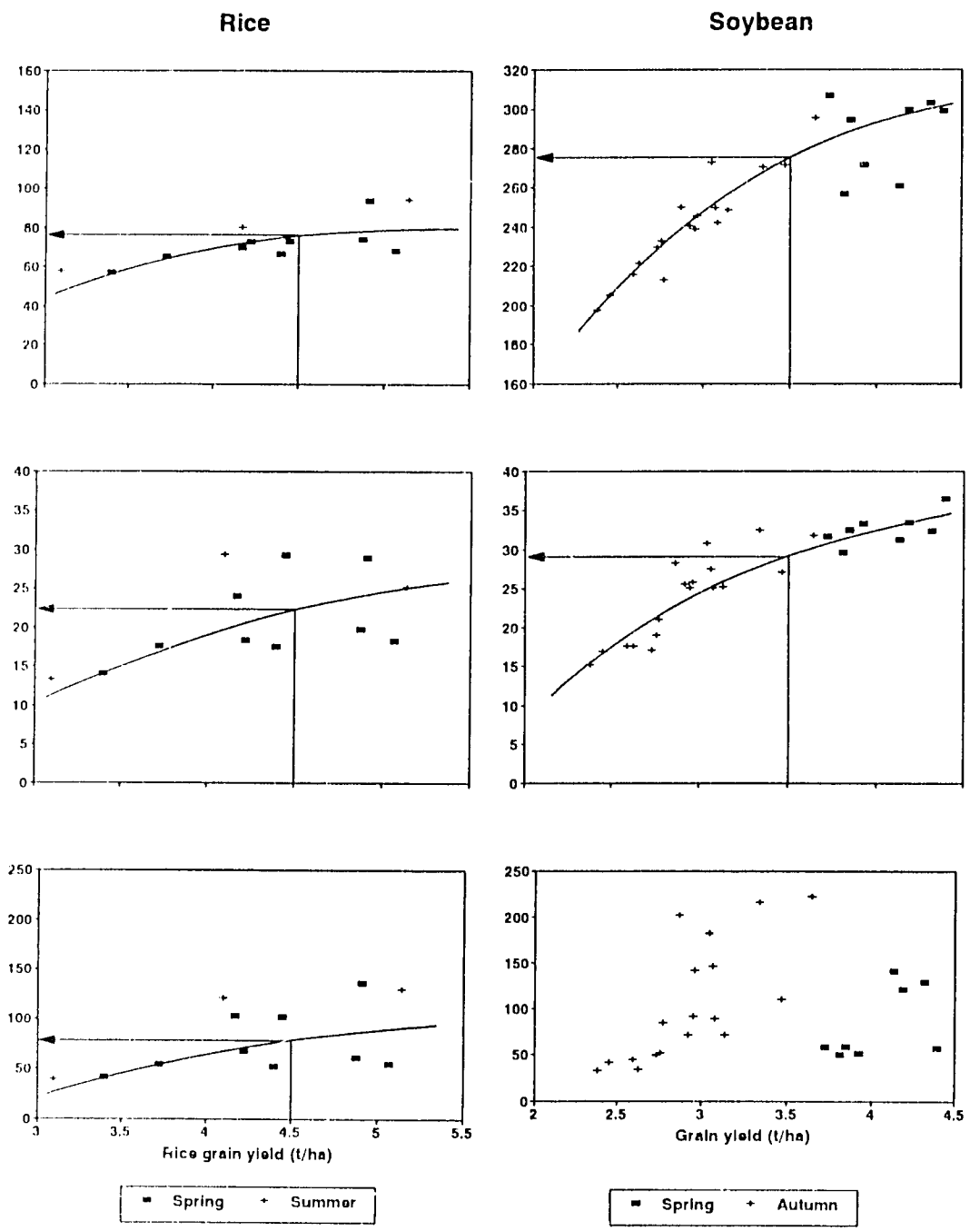

Rice
Soybean
Rice grain yield (t/ha)
Grain yield (t/ha)
Spring
Summer
Spring
Autumn

Table 6. Coefficients from the regressions of nutrient uptake by four species on crop sequence number (spring 1985 = 1) and economic yield

Species	Nutrient	Variable Crop no.	 Economic yield	R^2
Chinese cabbage	N	-	53.01	0.96
(n = 15)			(18.9)[a]	
	P	-	7.93	0.94
			(14.2)	
	K	8.27	58.18	0.93
		(5.2)	(9.5)	
Rice	N	1.47	10.61	0.63
(n = 12)		(1.38)	(2.51)	
	P	2.00	-	0.79
		(6.22)		
	K	11.09	-	0.65
		(4.34)		
Soybean	N	-	46.31	0.78
(n = 26)			(9.11)	
	P	0.68	8.81	0.86
		(3.58)	(10.87)	
	K	20.06	-	0.78
		(9.33)		
Tomato	N	3.84	23.12	0.93
(n = 21)		(4.99)	(15.44)	
	P	0.74	3.16	0.69
		(2.86)	(6.27)	
	K	16.03	18.64	0.74
		(6.83)	(4.08)	

Value in parentheses are t-statistics for partial regression coefficients

Seasonal N and P uptake responses to time were examined by regression. Separate seasonal effects (constant for spring, linear with time for autumn) were significant for N and P but not for K (fig. 18). Moreover, grain yield responses exhibited similar behavior to seasonal spring and autumn time variables. The data, therefore, suggest that increased availability of nutrients affected soybean grain yield of the autumn crops. The response probably arose because the average V4 to R6 duration of an autumn crop, the growth interval during which soybean takes up 80% of its nutrients, was briefer than that of a spring crop.

Tomato

Fig. 17 shows N, P, and K uptakes in relation to tomato yields on a dry weight per hectare basis. The significant time variables in table 6 suggest that nutrient availabilities increased with time. Projecting from a 3 t/ha yield on the X-axis in the N uptake frame through the hand-drawn curve the Y-axis is intersected at 100 kg N/ha. A 3 t/ha crop is equivalent to a 43 t/ha crop at a 93% water content. The corresponding P uptake was 14 kg/ha. Because the effect of time was more significant than that of K, uptake over all seasons averaged 106 kg/na which was taken as a general requirement for a 3 t/ha crop.

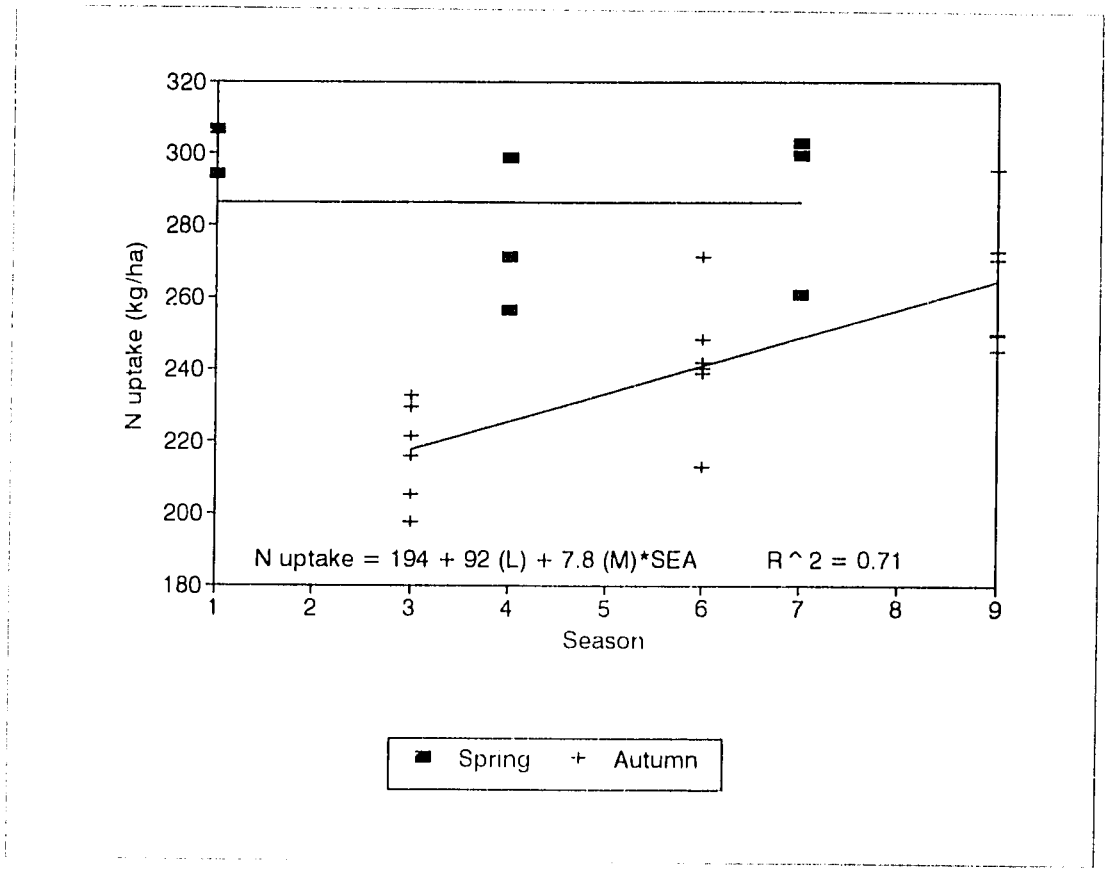


Fig. 18. N uptake by spring and autumn soybean

In the regression model: L = 1 and M = 0 if spring; L = 0 and M = 1 if autumn; SEA = number of season with 1 = spring 1985

Summer tomato yields were low (0.5 to 2.0 t/ha yield range or 7 to 29 t/ha fresh fruit yield) compared to spring or autumn yields. P and K uptakes were similar and in a few instances even higher than those of other seasons. Much of the nutrient taken up by the lower yielding crops was in the vine. The N, P, and K harvest indexes were 0.60, 0.67, and 0.77 for autumn and 0.41, 0.54, and 0.43, respectively. They differed according to season because a larger portion of the nutrients were contained in the fruit of autumn crops. Thus, not only were economic yields larger in these seasons but a greater portion of the nutrients were removed.

Although relationships between the uptake of each nutrient and yield were improved by the inclusion of a time variable, regression of fruit dry matter yield on time was not significant (R^2 = 0.072). The evidence, therefore, suggests that increased nutrient availabilities, while increasing uptake, did not remove or reduce a yield constraint. This statement appears to be valid even when summer observations were excluded.

Conclusions

Sufficient data were available to determine relationships between the quantities of N, P, and K taken up and economic yield. In many cases, relationships were statistically improved by inclusion of a time variable. The regression of economic yield on the time alone, however, was not significant for any of the four crops, suggesting that a nutrient accumulation did not have a broadly based impact on yields. There appear to be three special cases where accumulation was important. In one, inorganic N applications to rice were initially low and the observed grain yield increases with time were probably in response to a 15 kg N/ha increase of inorganic N and to accumulation of N in those rotations to which composts and straw mulches were applied. In the second case, nutrient accumulations probably contributed to autumn soybean yields by increasing N and perhaps P availabilities. In the third case, where straw mulch was not used and compost was not incorporated, K accumulations from returned crop residues probably increased Chinese cabbage yields.

Analyses of nutrient trends

Visual inspection suggested that soil C, N, P, and K trends occurred in the rotation study, but background variation was large. Therefore, regression analysis was used to reveal patterns of changes created by season, flooding (rice cultivation), compost and mulch, and the interactions of time with compost and time with mulch. The 55 observations from five rotations (no. 6 to 10) were used because these contained a mix of compost and mulch treatments applied to a small number of species. The observations were means of soil nutrients in composite samples taken from each of two replicates at the end of each season. Total N was determined by digestion with H_2SO_4 and inorganic N by extraction with KCl. Concentrations in the digests and extracts were determined by ammonia distillation followed by titration. Available P was extracted with 0.5 m $NaHCO_3$ at pH 8.5 and available K was extracted with neutral 1m NH_4OAC. Soil organic C was determined by wet combustion with H_2SO_4 and $K_2Cr_2O_7$.

The partial regression coefficients are listed on fig. 19 where model estimates as functions of time and time x compost interaction (and time x mulch for K) are shown. The analyses confirmed expected response patterns. Time and its interaction with compost had major effects on most dependent variables. The time x compost interaction was greatest for total C, total N, and available P, all of which are comparatively stable soil components. Seasonal effects were important explanatory variables for available P and K and inorganic N, contributing to large seasonal amplitudes for available K and inorganic N. A dummy variable for flooding (spring rice in rotations no. 9 and 10) decreased inorganic N estimates after that crop. A time x straw mulch interaction was detected for available K. The significant interaction was consistent with an estimated 135 kg K/ha contained in the 10 t/ha mulch incorporated after its use.

Available P and K and inorganic N are readily taken up by crops but K is also subject to leaching and inorganic N is subject to both leaching and denitrification. Although it is impossible to apportion the seasonal and the flooding effects to these N processes, the negative flooding coefficient suggests that the effects of water management as well as rainfall contributed significantly to N loss.

The R^2 suggests that large variability was inherent in the experimental units if it is assumed that the regression models contained independent variables sufficient to explain the observed changes. Because the soil data were apparently highly variable and treatment confounding occurred to some degree for crops, seasons and compost and mulch applications, the preceding analyses must be regarded as instructive rather than definitive. Nevertheless, the number of rotations and the 11 seasons of data were substantial and clearly illustrated differences which were consistent with expectations. Regardless of response confoundings, the analyses show that important seasonal effects occur which have implications for fertilizer management and soil sampling practices.

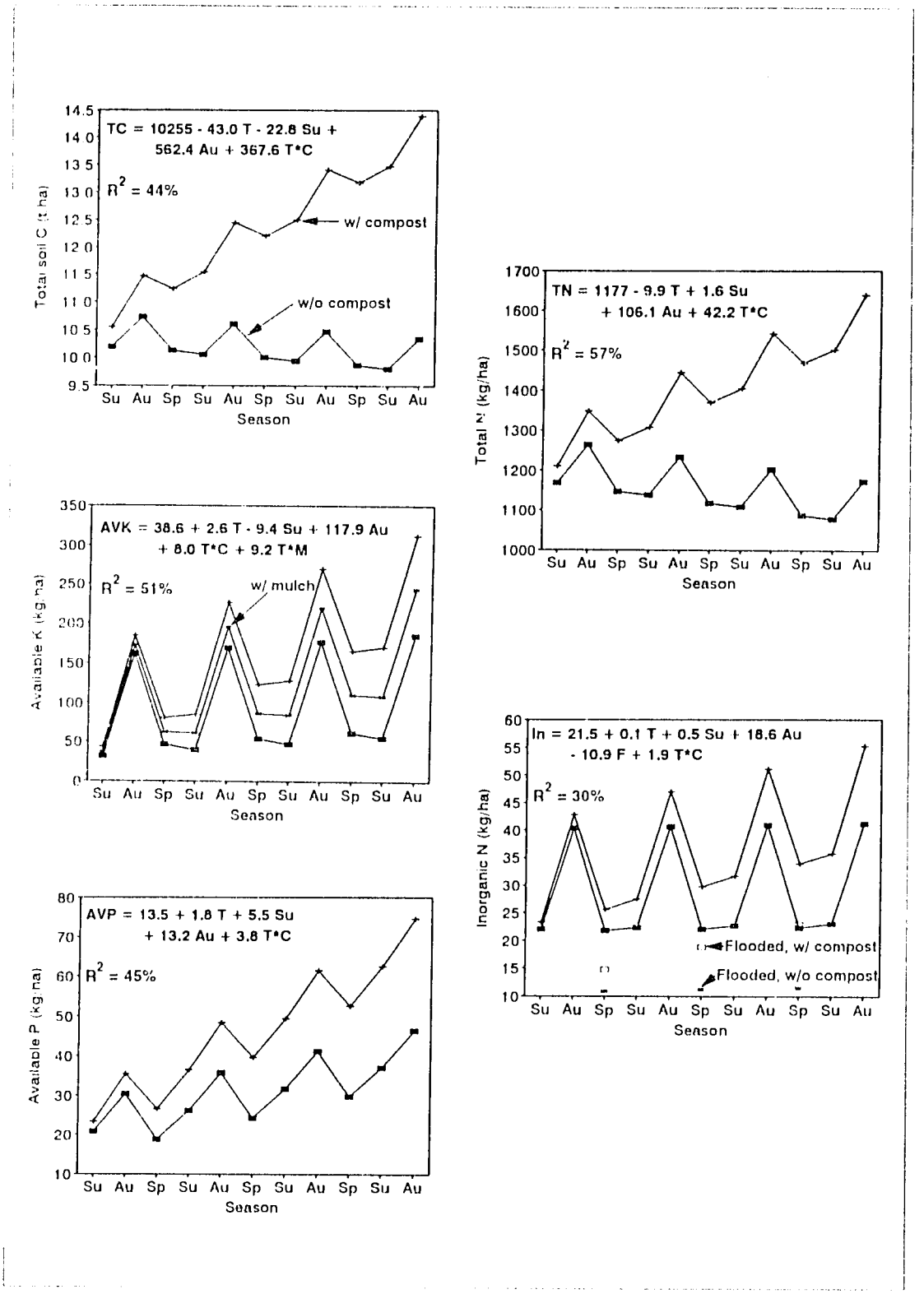


Fig. 19. Seasonal and long-term trend effects of compost, straw mulch, flooding, and time on soil organic C, total N, available K, inorganic N, and available P in the Rotation Study

Nutrient balances

A general nutrient balance equation can be used to visualize accumulated losses of nutrients and accumulations in soil. To compute the balances, the equation described at the outset of this section was rearranged for each nutrient and applied to data from 11 seasons in each of the five rotations. The annual sequences of crops were Chinese cabbage (spring) - tomato (summer) - soybean (autumn) in rotations 6, 7, and 8 and rice - tomato - soybean in rotations 9 and 10.

Nitrogen. The N forms of interest were both organic and inorganic. A rearranged equation was used to compute balances for each rotation with the following assumptions: Miscellaneous additions and erosion losses were small and, furthermore, offset each other. Leaching and gaseous losses (volatilization and denitrification) were unknown and therefore placed on the right. The last term (N_{un}) accounted for overlooked additions and removals and measurement error.

$$(N_I + N_{leg} - N_{ch}) - (N_{imm} - N_{min}) + (N_{inorg} - N_{inorg}) = N_l + N_g + N_{un}$$

The three terms in the first set of parentheses represent N applications minus crop removals. The N_{leg} term represents a biological N fixation contribution from legumes. On the basis of research reported in the AVRDC 1984 Progress Report, we assumed that 70, 50, and 30% of total N uptake was fixed biologically in rotations 6, 8, and 7, respectively. The two terms in the second set represent net mineralization or immobilization and the two terms in the third set represent the net change in inorganic N.

The progressions over time of the three components on the left-hand side of the N balance equation are displayed in fig. 20 for rotations 6, 7, and 8 and in fig. 21 for rotations 9 and 10. The trends on these figures indicate cumulative applications in excess of removals (+), immobilization less mineralization (i.e., change in total soil N) (*), and inorganic N accumulation or loss (■). As evident in the previous section, inorganic N quantities were almost imperceptible when shown at the same scale as total N. Because inorganic N increases and decreases were negligible, the right-hand side of the equation is approximated by the difference between the + and * points. Without incorporation of either mulch or compost and mulch, net total N mineralization occurred in rotation 6 even though N was applied in excess of removal. The excess was about 200 kg N/ha and net mineralization was about 500 kg N/ha. Therefore, after 11 seasons the right-hand side equaled about 700 kg N/ha. Only slightly less was lost where straw mulch was incorporated in rotation 8 even though the mulch undoubtedly immobilized N for short periods after incorporation. In rotation 7, net immobilization occurred, or more correctly, a large portion of the inorganic N in the compost did not mineralize. Although much more N was applied to rotation 7 than to either rotation 6 or 8, the quantity of N unaccounted for after 11 seasons was similar to that in the latter two rotations. Rotation 9 received compost and straw mulch and rotation 10 received straw mulch. Although the cumulative N loss after 11 crops was about 800 kg N/ha after 11 seasons in rotation 10, the loss from rotation 9 was about 2000 kg N/ha over the same period. The large loss may be due to the abundant N available from the compost which leached and/or denitrified during spring rice cultivation in this rotation.

The contrast between rotations 7 and 9, in which compost contributed N in surplus of crop uptake, is worth contemplation. In rotation 9, most of the surplus was lost from the system (at least from the soil surface) whereas in rotation 7, the soil retained much. Another comparison (rotation 6 vs. 8) suggests that the N in the rice straw contributed N to the system and thereby reduced mineralization of soil N.

Phosphorus. Labile inorganic P, of which available P in this study was an index, was of interest. Assumptions to simplify the equation were that miscellaneous deposits and erosion losses were small and were mutually offsetting and that leaching and gaseous losses were negligible. The term for net immobilization of P (organic as well as mineral forms) was moved to the right. A term to account for overlooked changes and measurement error was also placed on the right.

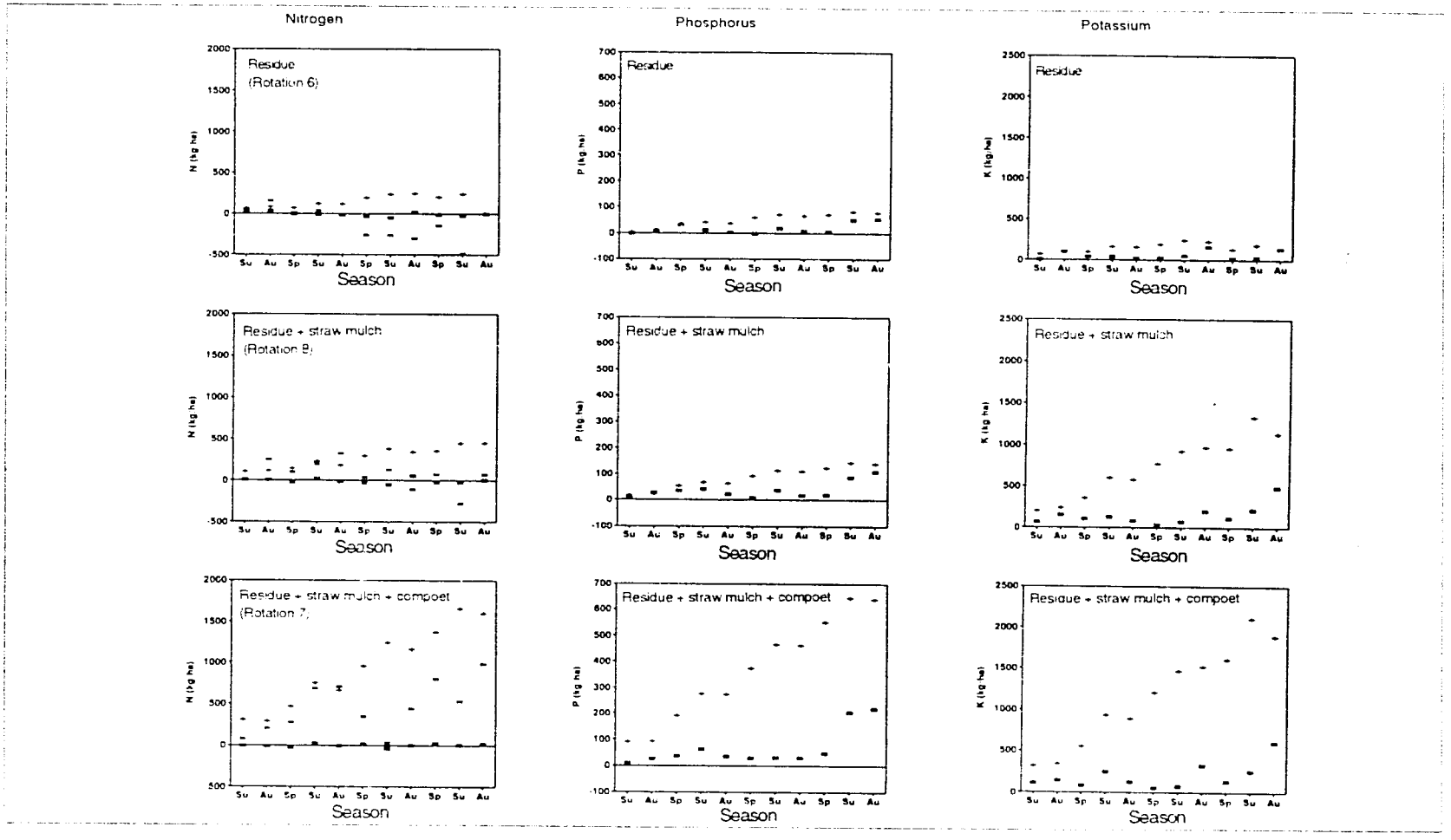


Fig. 20. Plotted components of left-hand sides of N, P, and K balances for tomato (summer), soybean (autumn), and Chinese cabbage (spring) rotations, 1984–87

Plus = applied - removed (M_t - M_{ch}); Box = ending - beginning inorganic (M_{einorg} - M_{binorg}); Asterisk = immobilized - mineralized (M_{imm} - M_{min})

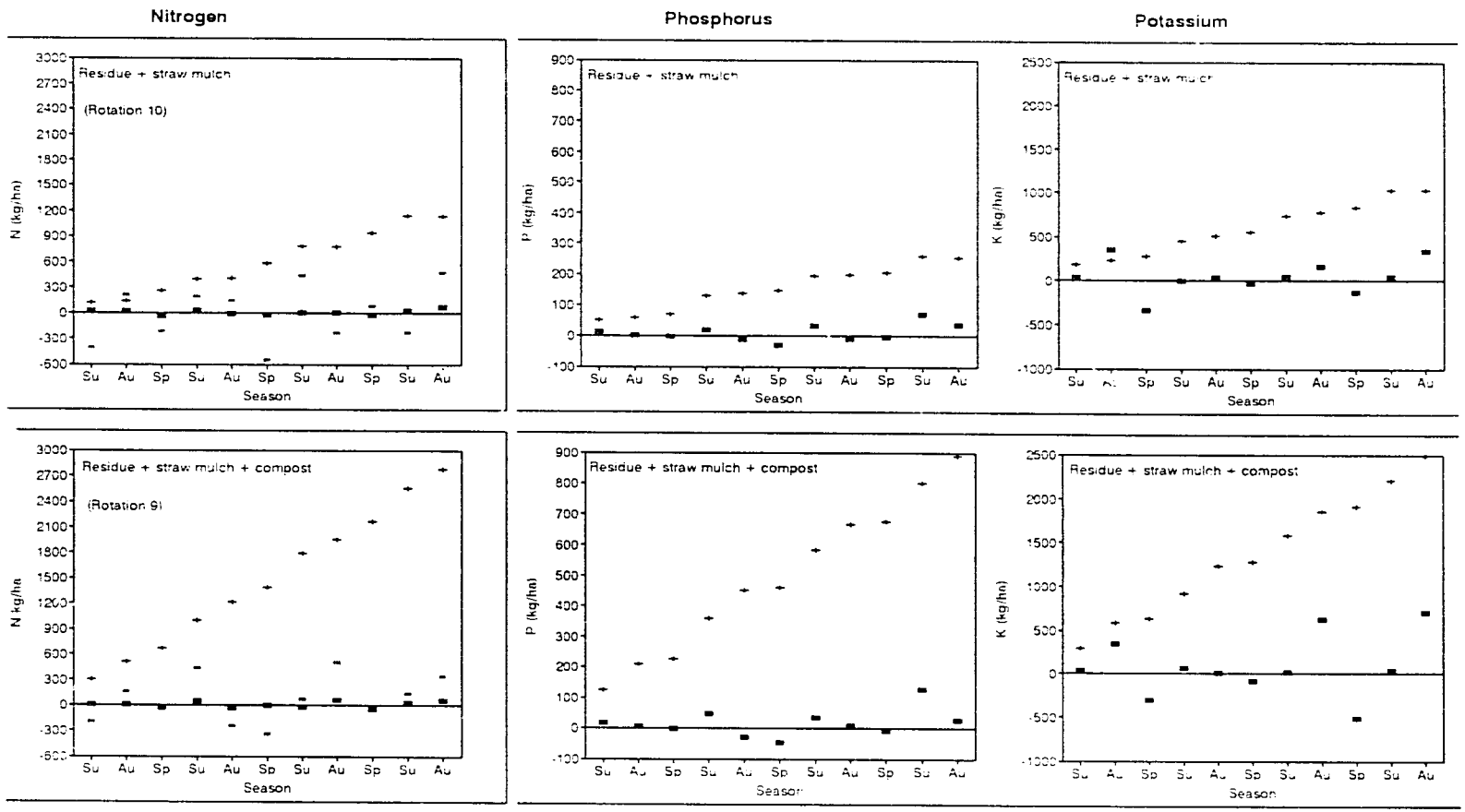


Fig. 21. Plotted components of left-hand sides of N, P, and K balances for tomato (summer), soybean (autumn), and rice (spring) rotations, 1984–87

Plus = applied - removed (M_f - M_{ch}); Box = ending - beginning inorganic (M_{einorg} - M_{binorg}); Asterisk = immobilized - mineralized (M_{imm} - M_{min}

$$(F_i - P_{ch}) + (P_{bmorg} - P_{emorg}) = (P_{imin} - P_{imm}) + P_{im}$$

The two components on the left side of the P balance equation are displayed in fig. 20 and 21. Even though P was applied in only slight excess of crop removals in rotation 6, the excess was sufficient to cause a modest accumulation of available P after 11 crops. The P in the compost was in large excess over removals (650 and 900 in rotations 7 and 9, respectively). However, relative to the excess applications, comparatively modest accumulations of available P occurred in rotation 7 and even smaller accumulations occurred in rotation 9. The P balance equation suggests that the excess was immobilized. In the rotations being analyzed, abundant calcium was applied through irrigation, and therefore insoluble $Ca\text{-}PO_4$ compounds undoubtedly formed (AVRDC 1993 Progress Report). Spring rice in rotations 9 and 10 probably contributed more Ca to the soil because rice is both flooded and of longer duration than the spring Chinese cabbage in corresponding rotations (no. 7 and 8). Where mulch and compost were applied, however, some excess P probably remained in an organic form not hydrolyzed by the chemical extractant.

Potassium. Exchangeable K was the soil form of interest. Assumptions to simplify the equation were the same as those for the P equation except that leaching losses were not regarded as zero but net immobilization or mineralization was negligible.

$$(K_i - K_{ch}) + (K_{cmorg} - K_{emorg}) = K_f + K_{im}$$

The two components on the left side of the K balance equation are displayed in fig. 20 and 21. After 11 seasons of rotation 6, where only residues and inorganic fertilizers were applied, the excess of applied K above removal was about 700 kg/ha even though Chinese cabbage residues were not recycled. The excess of applied K over removal exceeded 1000 kg/ha when mulch was incorporated and 2000 kg/ha when mulch and compost were incorporated. The contribution of K in rice straw mulch was evident in rotations 8 and 10.

In the rotations to which both mulch and compost were applied, accumulations of exchangeable K were small relative to the amounts in excess of removals. Moreover, in rotations 9 and 10, rice cultivation appeared to reduce soil K accumulation relative to accumulations in rotations 8 and 7 even though rice residue was returned and Chinese cabbage residue was removed. Flooding during rice cultivation probably promoted leaching, particularly in the spring when the Ca content of water is high.

These balances can be used to examine additions and removals in the five rotations, the cycling of nutrients, nutrient recoveries and efficiencies, and the likely fates of unaccounted quantities.

The effects of compost were pronounced for all nutrients (rotations 7 and 9 vs. rotations 6, 8, and 10). Although there was evidence that mulch contributed N and P, contributions were small compared to those from compost. For K, however, contributions from mulch as well as from compost were significant.

The cumulative contributions of mulch and of compost to the nutrient status of the crops and soil in these rotations during the 11 crops was evident. A comparison of total economic yields expressed in dry matter from rotation 8 vs. that of rotation 6 showed that contribution of the mulch increased output by 10%. The marginal increase due to compost increased output from rotation 7 by 5% over that of rotation 8. The corresponding increase from rotation 9 over that in rotation 10 was 6%. The large addition of C and nutrients from the compost had a comparatively small effect. Compost was applied in addition to nutrients in the inorganic fertilizers and straw mulch and crop residues, which in most cases were ample, especially after the first annual cycle.

Direct and Residual Yield Responses of Leafy Vegetables to and Nutrient Recoveries from Agricultural Wastes

Summary

The direct and residual nutrient contributions from agricultural wastes are not well established. To estimate the contributions from three representative wastes, leafy vegetable crops were grown in sequence and yield responses and NPK recoveries on each were determined. The materials tested were raw poultry manure (PM), composted pig manure (PG), and composted sugar mill waste (SC). Inorganic N was included for comparative purposes. Dry matter yield response was proportional to the quantity of N in the wastes and to the quantities of inorganic N applied. Regression analyses showed that more N was recovered from inorganic N than from the wastes but utilization of N from the wastes was higher. Apparent N recovery over three crops totaled 46% for inorganic N and 13% for N in the wastes. Residual effects on the third crop were small and not significant. Uptake of both P and K were probably promoted by the responses to N in the wastes. Potassium uptake was especially boosted by N. Whereas only 5 to 7% of P in the waste was recovered by the three crops, 25 to 35% of the K was recovered. Recovery of P and K decreased with each successive crop but rates of decrease were much less than observed for N.

Introduction

Agricultural wastes are frequently used in conjunction with inorganic fertilizers to produce vegetables. Kangkong, a common leafy vegetable in Asia and one that tolerates waterlogging was used to determine the N, P, and K contributions from three agriculture wastes to nutrient uptake. After a second kangkong crop was cultivated to determine residual effects from waste and inorganic N applications to the first kangkong crop, a third crop, amaranth, was cultivated to test for additional residual effects.

Materials and Methods

Waste characterization

Poultry manure and pig manure compost were supplied by the Taiwan Livestock Research Institute in Hsinhua, Tainan. Poultry manure was a week-old litter from layers. Pig manure was composted with rice hulls for 1 month. The sugarcane compost was a mixture of filter slurry from sugar press-extraction process and finely chopped sugarcane bagasse from Shanhua Sugar Mill Corporation. It had been composted for 4 months. Total N in each waste was determined by acid digestion followed by ammonia distillation. Total P and K in the wastes were determined from the solution obtained from the digest of the same sample. Total C was determined by wet digestion.

Treatment design

The treatments were combinations of two N levels (0 and 60 kg/ha) and three agricultural wastes, plus 0, 60, and 120 kg N/ha controls, bringing the total number of treatments to nine (listed in table 7). Plots (40-cm-high beds of 1.0 x 2.5 m with 0.5 m furrows between beds) were arranged in three randomized complete blocks.

Table 7. Inorganic N and agricultural waste applications in nine treatment combinations

Inorganic N[a]	Source	Agricultural waste Dry weight PM	PG	SC	Total N[b]	P	K
kg/ha		t/ha			kg/ha		
0	-	0	0	0	0	0	0
60	-	0	0	0	0	0	0
120	-	0	0	0	0	0	0
0	Poultry manure (PM)	3.8	0	0	100	107	168
0	Pig manure compost (PG)	0	3.4	0	134	54	39
0	Sugarcane compost (SC)	0	0	5.0	58	24	71
60	PM/2	1.9	0	0	50	53	84
60	PG/2	0	1.7	0	67	27	19
60	SC/2	0	0	2.5	30	12	36

[a] Coded as IN in text and in regression analyses

[b] Coded as ON in text and in regression analyses

First crop. Waste and inorganic N treatments were incorporated into the top 5 cm of the beds. Seeds were planted in rows on 20 July 1994 (10 cm between rows and 5 cm between hills). For treatments requiring inorganic N [as $(NH_4)_2SO_4$] fertilizer, half was applied before planting and the remainder was topdressed 11 DAE. Because the first crop was cultivated during a period of seasonally intense rainfall, the beds were covered with net tunnels to prevent erosion of soil and the incorporated wastes.

Plant sampling of the first crop started 12 DAE with subsequent harvests at 18, 26, and 30 DAE. Twelve plants were harvested from two rows on each sampling date, leaving one row between the current harvest and the previous harvest. Plants were washed with distilled water to removed adhering debris. The samples were air-dried for 1 day before being dried at 65°C in a forced-air oven. Digested samples were analyzed for total N by distillation and titration, total P by spectrophotometry, and total K by flamephotometry.

Second and third crop. The second and third crops were seeded on 24 August and 14 October, respectively, after residues remaining from the previous crop were removed and the soil was tilled to 5 cm with a hand hoe. Plant densities were approximately the same as those of the first crop. Uniform applications of inorganic N fertilizer were given to the second and third crops (45 and 20 kg N/ha, respectively). Dry matter yields and nutrient concentrations were determined by the same procedures applied to the first crop. These crops were harvested on 29 September and 25 November, respectively. Neither soil nor plants were sampled during growth of the second and third crops.

Results and Discussion

Soil and manure characteristics

Before treatments were applied, the soil contained 0.84% organic C, 0.098% total N, 35 ppm available P (Bray P$_1$), 118 ppm exchangeable K, and its pH was 8.0. Among the wastes, pig manure compost and poultry manure contained more total N and C on a unit dry weight basis than sugarcane compost (SC) (table 8).

Table 8. Total C, N, P, and K in agricultural wastes (% dry matter basis)

Characteristics	Poultry manure	Pig manure compost	Sugarcane compost
Total C (%)	24.1	36.1	7.53
Total N (%)	2.62	3.93	1.18
C: N Ratio	9.2	9.2	6.4
Total P (%)	2.80	1.60	0.48
Total K (%)	4.41	1.14	1.44

Yield and N uptake

Dry matter yields of the first crop differed among waste, inorganic N, and inorganic N + waste treatments (fig. 22). The effects of treatments decreased with each crop. In the third crop, the only significant contribution to the treatment mean square was the linear response to 0, 60, and 120 kg inorganic N/ha.

The overall effect of inorganic and organic N on dry matter yields is evident in fig. 22. Fresh yield responses were little different from dry matter yield responses because dry matter percentages did not differ within crops (8.1, 18.2, and 7.9% for the first, second, and third crops, respectively).

Dry matter accumulations from all treatments in the first crop were initially slow but increased rapidly after 18 days (fig. 23). The accumulation pattern from PG + 0 IN was between those of 60 and 120 IN whereas 1/2PG + 60 IN (not shown) was similar to that of 120 IN. Accumulations from PM + 0 IN and SC + 0 IN alone were clearly lower than that of PG + 0 IN. However, the addition of 60 IN to 1/2 applications of PM and SC increased dry matter accumulation rates (not shown in fig. 23 but evident from fig. 22).

Although the response to inorganic N was essentially linear, tissue N concentrations increased as inorganic N increased from 0 to 120 kg/ha. As inorganic N increased, P and K tissue concentrations decreased from 0.46 to 0.27% and from 4.7 to 2.3%, respectively. Nutrient dilution would be expected even though soil tests indicated that both nutrients were abundantly available. These data are evidence that kangkong growth was limited by N deficiency.

A regression model of the form $DMY = a + REP + CROP + b_1ON \times CROP + b_2IN \times CROP$ was formulated to estimate the contributions of N in agricultural wastes (ON) and inorganic N (IN) to each of the three crops. The highest inorganic N treatment was excluded from the analysis. In this model, REP and CROP were classification variables, ON and IN were the quantities of N in the agricultural wastes and inorganic N applied to the first crop, respectively. Model coefficients are listed in table 9 (the mean REP effect was added to the intercept) and the observed and estimated means (over replicates) for treatments are shown in (fig. 24) for the first and second crops.

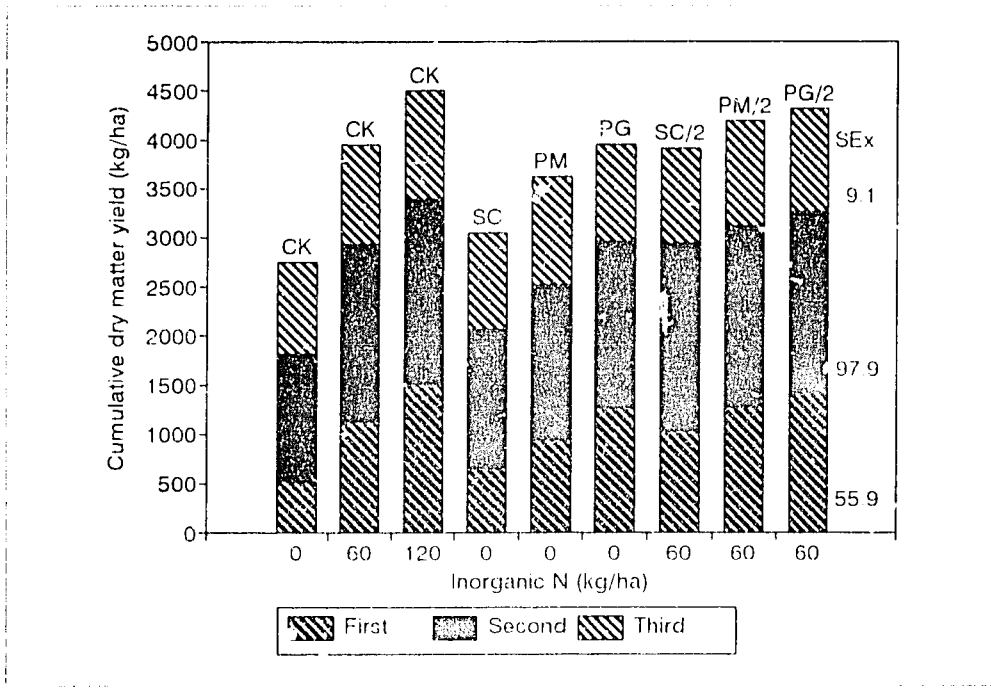


Fig. 22. Cumulative dry matter yields from inorganic N control (CK) and organic wastes (SC = sugarcane, PM = fresh poultry manure, PG = composted pig manure) applied without and with 60 kg inorganic N/ha to the first three leafy vegetable crops (SEx is on a treatment within crop basis)

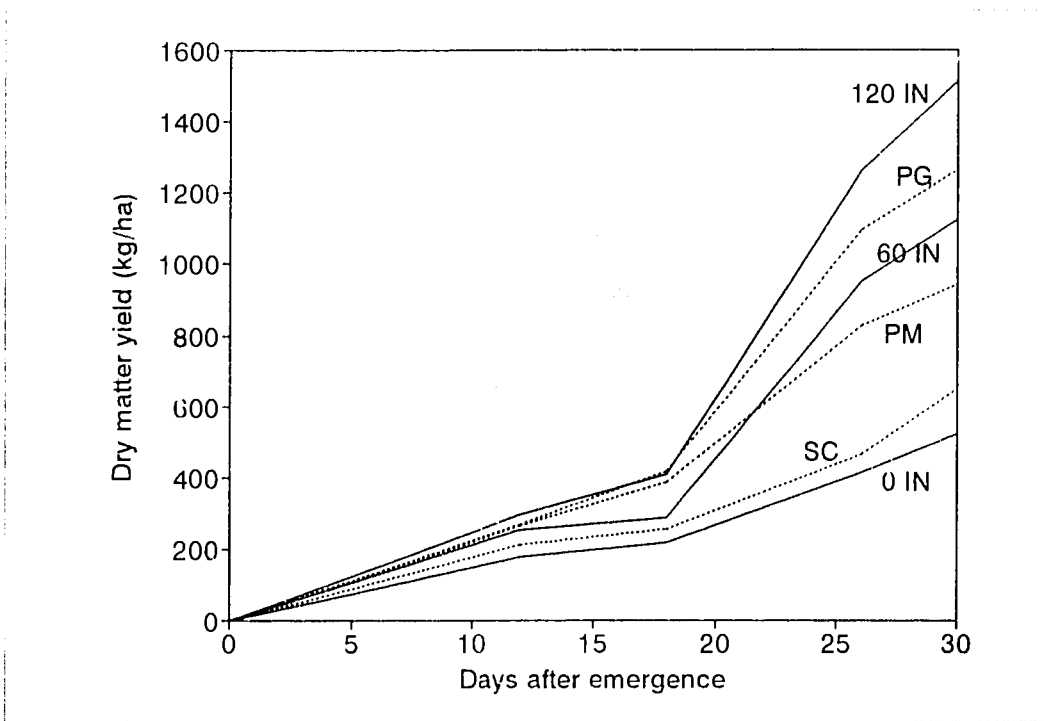


Fig. 23. Dry matter accumulations from three inorganic N and three agricultural waste treatments Total N in the PG, PM, and SC treatments were 134, 100, and 67 kg/ha, respectively

Table 9. Partial regression coefficients and statistics from models used to examine the effects of organic and inorganic N applications on dry matter yield and N uptake by three crops

	Dry matter yield		N uptake	
	Partial regression coefficient	$P_t > /t/$	Partial regression coefficient	$P_t > /t/$
Intercept[a]	945.9		41.2	
CROP 1	-480.4	0.001	-33.1	0.001
CROP 2	408.4	0.001	-7.3	0.015
CROP 3	0			
ON*CROP 1	5.29	0.001	0.072	0.002
*CROP 2	2.48	0.001	0.052	0.024
*CROP 3	0.76	0.250	0.010	0.667
IN*CROP 1	8.96	0.001	0.200	0.001
*CROP 2	5.63	0.001	0.219	0.001
*CROP 3	1.19	0.107	0.045	0.168
R^2	90.1		90.7	

[a] Adjusted for mean replicate effects

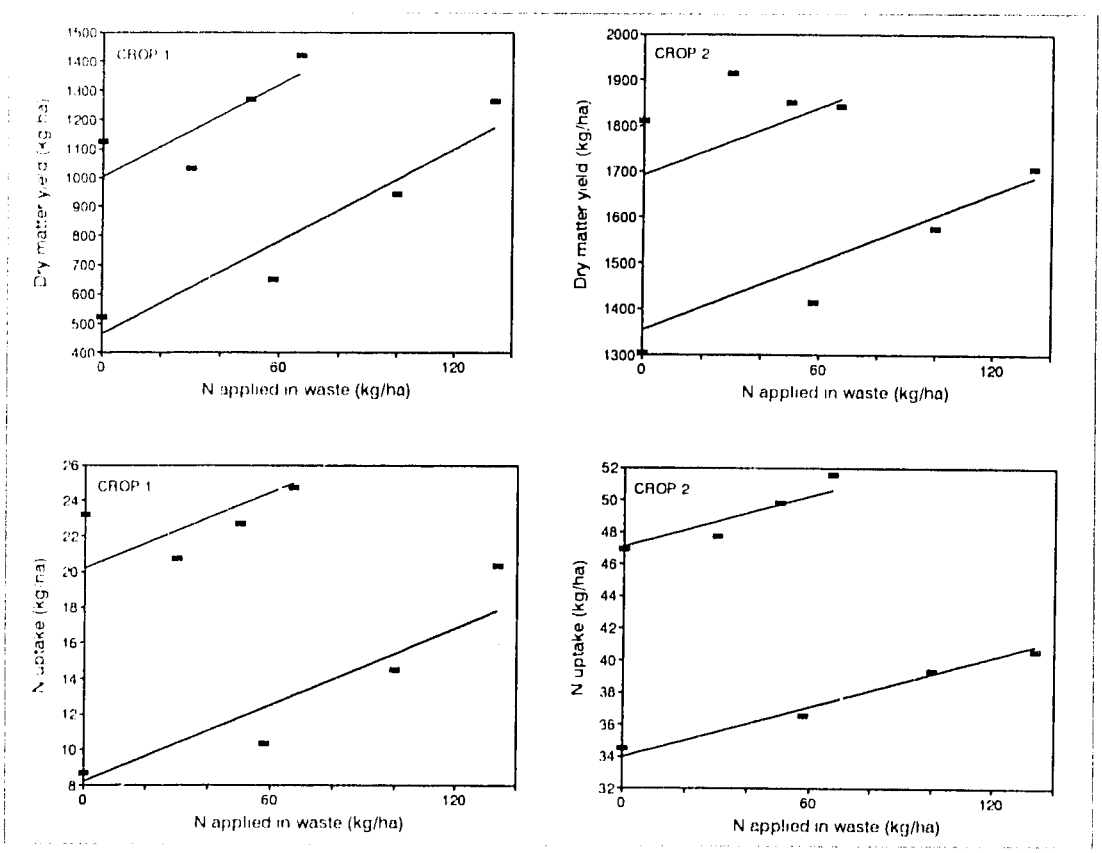


Fig. 24. Dry matter yield and N uptake by CROPS 1 and 2 as functions of N in agricultural wastes and inorganic N

The direct effects of both ON and IN on dry matter yield of the first crop are apparent in fig. 24. Similarly, the residual ON and IN effects were evident on the second crop. Evidence for residual effects on the third crop was weak. The response to N in the agricultural waste averaged 56% of the response to inorganic N (mean of direct and residual responses). An analysis of variance on residuals from the regression revealed no remaining variation attributable to waste or inorganic N treatments.

As expected, in the regression of dry matter on N sources, the coefficients for both inorganic and organic sources decreased with each crop. To expose more clearly the effects of N applications on yields, responses were resolved as follows:

DMY = Apparent recovery x Utilization efficiency

Apparent recovery = N uptake/N applied

Utilization efficiency = DMY/N uptake

To examine the effects of treatments on apparent recovery, the model used to estimate DMY from the variables REP, CROP, ON and IN was also used to estimate N uptake (NUP) from the same variables. Coefficients for the N uptake model are shown in table 10. Fig. 24 shows responses to applied N from the first two crops. As in DMY responses to N, the effects deteriorated with time and by the third crop agreement between estimates and observations was weak.

Table 10. Partial regression coefficients and statistics from models used to estimate effects of agricultural wastes on N utilization efficiency

	Regression coefficients for CROP 1	$P_r > \|t\|$	Regression coefficients for CROP 2	$P_r > \|t\|$	Regression coefficients for CROP 3	$P_r > \|t\|$
Intercept	218.7		-1709.5		28.6	
NUP1	39.0	0.001	-26.1	0.001[a]	21.9	0.001
$NUP^{1/2}$			694.4			
D[b]	203.2	0.001	60.8	0.31	53.5	0.024
R^2	96.1		81.9		86.4	

[a] For CROP 2, the response to N uptake was significant but the linear and square root regression coefficients separately were not significant when included jointly

[b] For CROP 1, D = 1 for all PG and PM treatments and D = 0 for others; for CROPS 2 and 3, D = 1 for all agricultural waste treatments

The coefficients for IN in crops 1 and 2 were of similar size, indicating that apparent recoveries by each crop were about 20%. The similarities suggest that much of the inorganic N, which was applied in ammonium form, was retained in the soil through the period of heavy rainfall during the first crop. Because it was not extracted by KCl from the top 10 cm, it either was held in a nonextractable form (mineral or organic) or leached but remained in the root zone. In either event, it became available to the second crop. Apparent inorganic N recovery summed over the three crops was 46%. By contrast, apparent recovery of N in the wastes was only 13%. Furthermore, the ON coefficients exhibited decreasing recoveries. Expressed in terms of inorganic fertilizer equivalents using a 20% recovery rate as a base, 1 kg N in waste would be equivalent to 0.36, 0.26, and 0.05 kg of freshly applied inorganic N freshly applied to each successive crop. For example, if 60 kg N were needed, and a waste application contained 75 kg N, 33 kg from an inorganic source would be required to obtain an equivalent of 60 kg N/ha for the first crop. For the second crop, 40 kg inorganic N would be required. For the third crop, almost the full amount would have to come from inorganic N unless N fertility were built up by successive waste applications. Note that these estimates are based only on N recovery.

Comparisons of the ratios of IN to ON coefficients for DMY and N uptake in table 9 show that DMY responses to N in wastes were not as unfavorable when expressed in inorganic N fertilizer equivalents, as N recovery was. To quantify utilization efficiency, a regression model of the form $DMY = a + C_{1i}REP + C_{2j}D + b_1NUP + b_2NUP_{1/2}$, where REP was classification variable for i = 3 replicates, D was a classification variable for j = 2 dummy variables (for crop 1, D = 1 if either PG or PM were applied and 0 otherwise; for crops 2 and 3, D = 1 for the application of any waste), and NUP was N uptake. This model was applied separately to data from each crop. The $NUP_{1/2}$ variable was retained only for the second crop. The N utilization efficiency differences are evident, especially in the first and third crops in fig. 25. The coefficients for D show that the N taken up from the waste sources was used more efficiently than that from inorganic N. Moreover, in crop 1, utilization efficiency from SC was not different from that of inorganic N.

The beneficial contributions of agricultural wastes to utilization efficiencies may have been derived from slow but sustained N mineralization from the incorporated materials. To achieve the yield equal to that which would be obtained from a 60 kg N/ha fresh inorganic N application from a mixture of fresh inorganic N and the 75 kg N/ha in waste applied to the first crop, the utilization effect shows that inorganic N application would be reduced from 33 to 19 kg N/ha in the first crop. Without taking residual effects from inorganic N into account, the inorganic N application to the second crop would be reduced from 40 to 29 kg/ha. The utilization efficiency effect on the third crop appears to be more substantial than the recovery effect, contributing an equivalent of 13 kg N/ha. These estimates assume 20% recovery and the utilization efficiency equations in fig. 25.

Phosphorus and potassium availabilities

Determinations of P and K availabilities from agricultural wastes are complicated by two factors. First, the wastes contain N to which crops respond. The response to N in the wastes makes it difficult to separate out direct effects of P and K because the uptake of N promotes P and K uptake even when the latter are not deficient. Second, in this study, soil P and K were abundantly available. Therefore, crop growth responses were unaffected by P and K availability, but were affected by N availability. Inorganic N was included to determine its effect on P and K uptake independent of the effect of N in the wastes. To adjust for the N contribution from agricultural wastes, N uptake by each waste treatment in each crop was estimated from the coefficients in the N uptake model (table 9) and the quantity of N applied (table 7). Nitrogen uptake was designated ONUP (kg/ha) in the regression models formulated to estimate contributions to P and K uptake from agricultural wastes. A corresponding N uptake estimate from the inorganic source, designated INUP (kg/ha), was estimated from regression coefficients and the quantities applied. The model for P uptake (PUP) was $PUP = a + REP + CROP + b_{1i}APP \times CROP + b_2ONUP + b_3INUP$ where REP and CROP were classification variables for replicates and the three crops, respectively, and APP was P in the wastes (kg/ha) applied to the first crop. The same model formulation, but with KUP (K uptake) replacing PUP and APK (K in the wastes) replacing APP, was used to estimate the contribution of K in the waste to uptake. Coefficients for full models are listed in table 11. Table 11 also contains coefficients for a model from which ONUP was excluded.

A comparison of APP x CROP interaction coefficients from the two P models suggested that, when ONUP was included on the assumption that N from the waste promoted P uptake, essentially no P uptake over the three crops was attributable to the application P per se. That is, the sum of the coefficients APP x CROP coefficients was zero. Even in the second P model, in which the uptake promoting effect of N was disregarded, total P uptake by the three crops from the wastes amounted to only 6% of that applied. The ONUP of the first model suggests that for each kilogram of N taken up from the wastes, the crop took up 0.39 kg P. The promotion effect of ONUP was four times that of N taken up from the inorganic source.

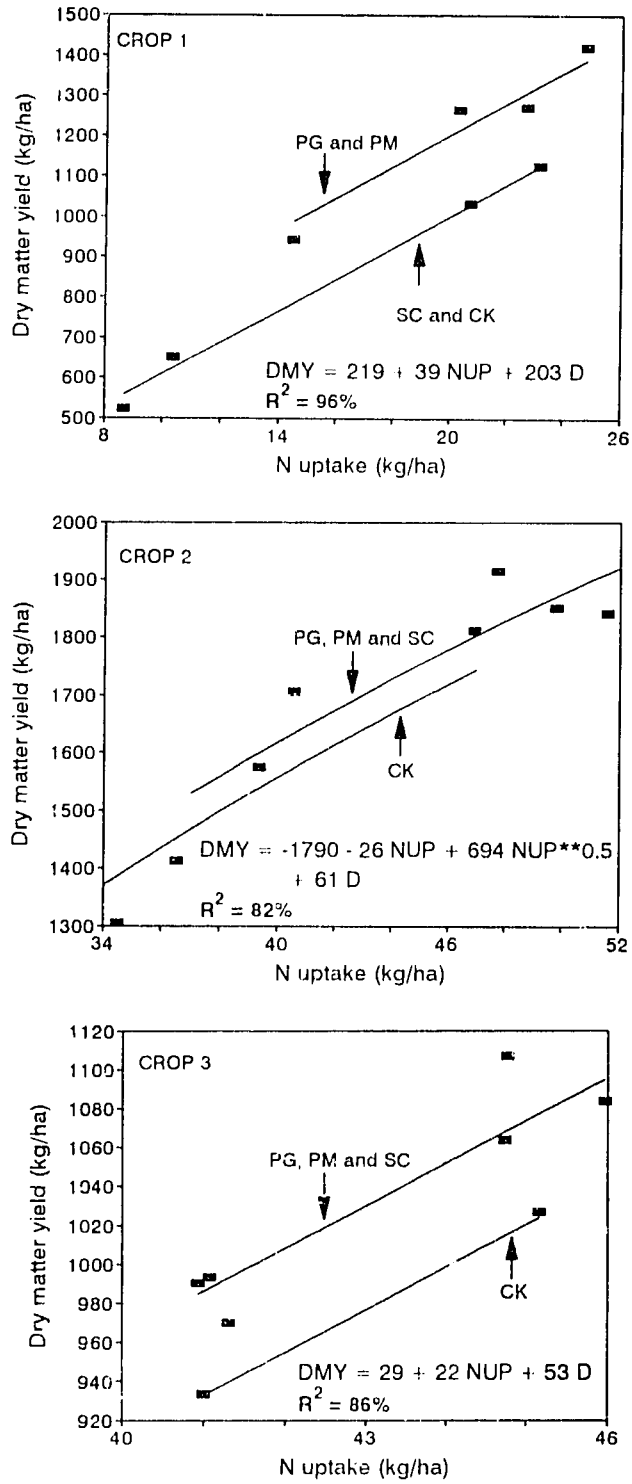


Fig. 25. Utilization efficiencies of N from inorganic and agricultural waste sources from three crops (PG = pig manure compost, PM = poultry manure, SC = sugarcane compost, CK = inorganic N or no applied N)

Table 11. Partial regression coefficients and statistics from models used to examine the effects of N uptake and P and K in the wastes on uptake of P and K by three crops

	P uptake				K uptake			
	Regression coefficient	$P_t > \|t\|$	Regression coefficient	$P_t > \|t\|$	Regression coefficient	$P_t > \|t\|$	Regression coefficient	$P_t > \|t\|$
Intecept[a]	4.95		5.08		48.3		49.6	
CROP 1	-2.98	0.001	-2.30	0.001	-25.3	0.001	-18.1	0.001
CROP 2	-0.58	0.046	-0.05	0.897	-39.9	0.001	-34.2	0.001
CROP 3	0		0		0		0	
APx[b]*CROP 1	0.004	0.419	0.030	0.001	0.070	0.029	0.146	0.001
*CROP 2	-0.011	0.017	0.027	0.196	-0.001	0.987	0.050	0.160
*CROP 3	0.009	0.024	0.012	0.020	0.044	0.122	0.054	0.123
INUP	0.096	0.001	0.068	0.001	0.586	0.002	0.281	0.188
ONUP	0.389	0.001	-		2.70	0.001	-	
R^2	78.2		58.6		84.3		75.7	

[a] The intercept was adjusted by the mean of the replicate effects

[b] Phosphorus in the agricultual waste treatments for the P regression model and K in the treatments for the K regression model

An examination of the K models produced a parallel interpretation. Potassium contributions from the wastes, however, were large whether promotion by N uptake from wastes was regarded as a valid or invalid assumption (about 11% vs. 25% recovery over the three crops). Moreover, the ONUP coefficient suggested that 2.7 kg K was taken up for every kg of N taken up from the wastes. Even though the total recovery of N from the waste was small (13%), the N that was taken up from it promoted K uptake to a much greater degree than inorganic N did.

Whereas the coefficients for successive crops in table 9 for N uptake give a ratio of only 0.14 kg N/kg N for ON x CROP 3 vs. ON CROP 1, the corresponding ratios from table 10 for P and K were 0.40 kg P/kg P and 0.37 kg K/kg K. These high ratios are evidence that P and K availabilities were sustained longer than that of the N contained in the wastes.

For this study, neither P nor K was sufficiently deficient to limit growth and as a corollary, the uptake promoting effects of N decreased the utilization efficiency of P and of K.

Soil inorganic N during the first crop

Inorganic N in the soil decreased sharply from an initially high concentration 5 days after incorporation until the final harvest (fig. 26). Although treatments affected concentrations, all diminished approximately proportionately as sampling progressed from emergence to 8 DAE to 26 DAE as the Y-axis scale changes show. Rainfall during the first crop totaled 1499 mm.

The first three sets of samples were taken after periods of no or low rainfall and, therefore, nitrate made up a large portion of total inorganic N. Sampling at harvest occurred 4 days after the third sampling. A high rate of uptake during this period as well as denitrification from 15 mm of rain 2 days before harvest probably contributed to the marked nitrate reduction. The absolute reduction was most pronounced in the 120 kg inorganic N treatment.

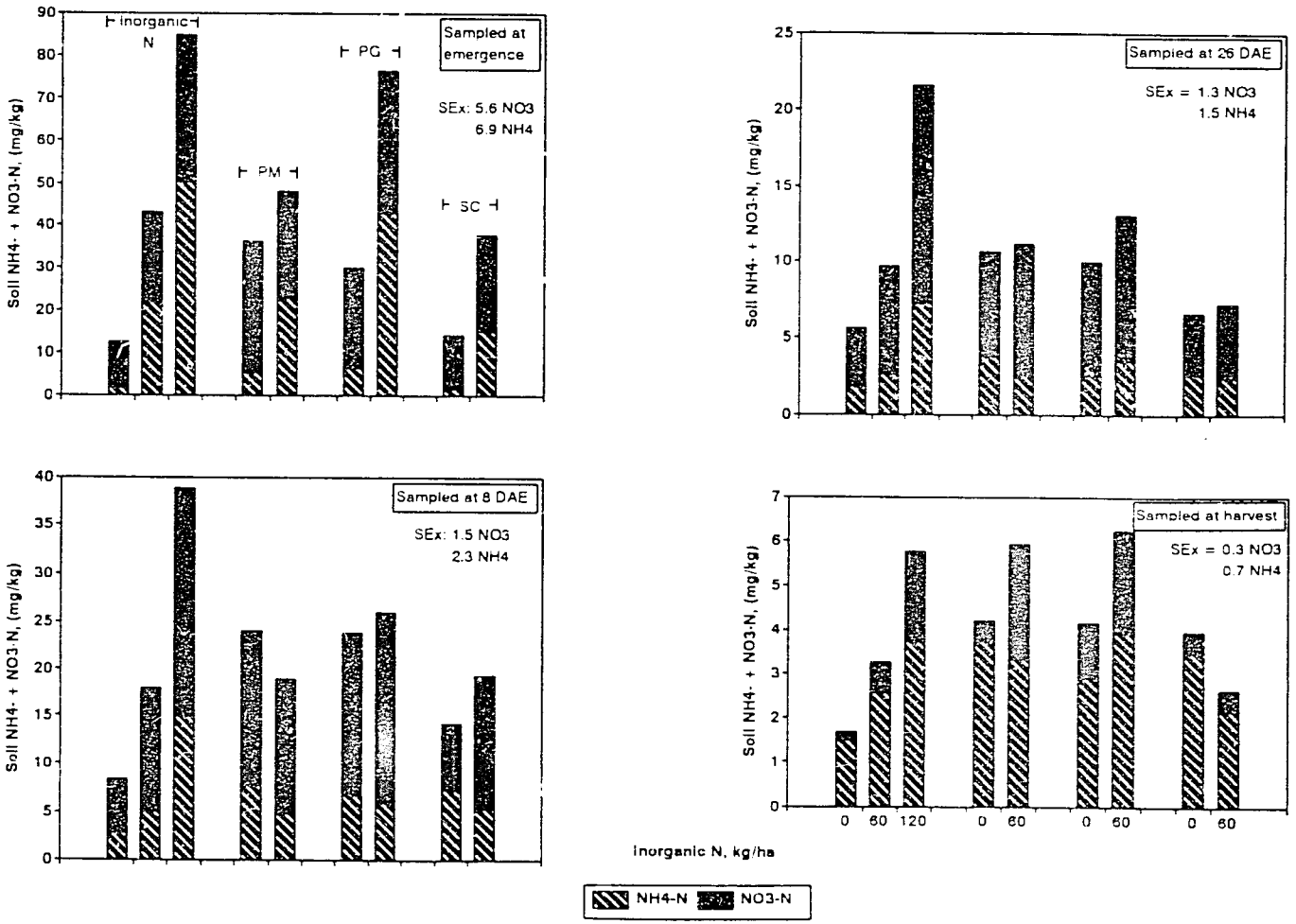


Fig. 26. Soil concentrations of nitrate and ammonium on four dates after emergence of the first crop

International Cooperation Program

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Training

Summary

AVRDC had 55 training scholars from nine countries. Two had degree-oriented training, while others undertook nondegree training. Forty-two percent of the scholars had special skills research training. Fifty-five percent of the trainees were women. The Crop Improvement Program (CIP) had 67% of the scholars, of which 62% were trained in plant pathology and plant physiology. The Production Systems Program (PSP) and the International Cooperation Program (ICP) had 16 and 7% of the scholars, respectively. In 1994, AVRDC provided a total of 163 person-months or 13.6 person-years of training. Ten other donors supported the training program. The other major donors were AVNET, Japanese Special Project, District Agricultural Improvement Station/Tainan, and Taiwan Agricultural Research Institute.

Fourteen scholars from AVRDC and other institutes in Taiwan underwent training on the application of agricultural biotechnology. Through the JSIF project, the Directory of AVRDC Training Alumni was produced. In 1994, a total of 298 slide sets with scripts and 15 videos were distributed.

Enrollment

In 1994, AVRDC had 55 training scholars from nine countries. Two had degree-oriented training; the others had nondegree training under four different categories (table 1).

Table 1. Distribution of training scholars by country and by category

Country	PF	RF	RS	RI	SPT	VS	UST	Total
Germany			1					1
India		1			1	1		3
Korea				1	1			2
Malaysia					3		1	4
Nepal		1						1
Netherlands			1					1
Philippines	1	5				2		8
Taiwan					15		17	32
Thailand					3			3
Total	1	7	2	1	23	3	18	55

PF = Postdoctoral Fellow
RF = Research Fellow
RS = Research Scholar
RI = Research Intern
SPT = Special Purpose Trainee
VS = Visiting Scientist
UST = Undergraduate Student Trainee

In line with the policy of the center, 42% of the training scholars underwent special research skills training. Fifty-five percent of the training scholars were women.

Program and Discipline Specialization

The distribution of training scholars within AVRDC programs and disciplines is shown in table 2. CIP had the major share of scholars with 67%. Nearly 62% of this group pursued training in plant pathology and plant physiology. PSP and ICP had 16 and 7% of the total training scholars, respectively. In 1994, AVRDC provided 163.0 person-months or 13.6 person-years of training (table 3).

Special Research Skills Training Course

Fourteen training scholars, eight from AVRDC, participated in the special purpose training on the application of agricultural biotechnology from 21 February to 22 March 1994.

The course aimed to provide hands-on experience on basic and newly developed plant molecular methods and technologies in plant gene identification, organization, expression, cloning and analysis, and gene transfer. The training course not only provided the participants with basic theoretical knowledge, but also training in laboratory techniques.

Training Materials Production

In 1994, 1300 copies of the Directory of AVRDC Training Alumni were produced with JSIF assistance and distributed to alumni in CTA-member countries, AVNET, and SAVERNET countries. Some were distributed through AVRDC scientists when they visited these countries. The directory lists all alumni and participants of AVRDC's human resource development offerings since the center started its training program at its headquarters in Taiwan in 1974. A total of 646 copies have already been distributed.

During the year, a total of 298 slide sets with scripts and 15 videos were distributed to 43 training scholars.

Table 2. Distribution of trainees by program/discipline and training period

Program/Discipline	PF	RF	RS	RI	SPT	VS	UST	Total	Person-month
Crop Improvement Program									
Plant breeding						1	2	3	16
Plant pathology					2	1	5	8	12.8
Plant physiology					14		1	15	16
Entomology					4		4	8	14
Genetic resources management		1				1	1	3	4
Subtotal		1			20	3	13	37	62.8
Production Systems Program									
Agricultural economics		1			3		1	5	15.2
Soil science	1		1					2	11
Crop management		1	1					2	17
Analytical laboratory				1			4	5	13
Subtotal		2	2	1	3		5	14	56.2
International Cooperation Program									
Publications & documentation		4						4	44
Subtotal		4						4	44
Grand total	1	7	2	1	23	3	18	55	163.00

Table 3. Distribution of grantees in each training category by sponsor

Sponsor	PF	RF	RS	RI	SPT	VS	UST	Total	Person-month
AVRDC	1	6		1	12	1	18	39	127.2
SAVERNET					1			1	0.8
AVNET					3			3	4
UNDP/RDA					1			1	2
JSP		1				1		2	2
ACIAR						1		1	1
DAIS/Tainan					2			2	2
TARI					2			2	2
AS					1			1	1
GTZ			1					1	12
FSC					1			1	1
Self-sponsored			1					1	8
Total	1	7	2	1	23	3	18	55	163

ACIAR – Australian Centre for International Agricultural Research
AVRDC – Asian Vegetable Research and Development Center
AVNET – Collaborative Vegetable Research Program for Southeast Asia, Asian Development Bank
SAVERNET – South Asian Vegetable Research Network, Asian Development Bank
AS – Academia Sinica, Taipei, Taiwan
FSC – Fulan Seeds Co., Ltd.
GTZ – German Agency for Technical Cooperation
JSP – Japanese Special Project
TARI – Taiwan Agricultural Research Institute, Wufeng, Taiwan
UNDP/RDA – United Nations Development Program/Food and Agriculture Organization of the United Nations
DAIS – District Agricultural Improvement Station, Taiwan

Information and Documentation

Summary

In 1994, a total of 2530 titles of books and crop documents, and 62 new serial publications were added to the library collection. Library holdings totaled 15,924 books, 1594 serial titles, and 35,484 entries of crop documents. Both the in-house and external databases on CD-ROM were well used. Reader services reported an increase in both library loans and photocopy/document delivery services and a drop in literature searches.

Information Collection

Acquisitions and exchanges

A total of 295 titles of books and photocopies were ordered, and subscription to 130 serial publications was continued. The average cost was US$43.66 per book and US$191.73 per serial title. Some 289 titles of books, 85 titles of photocopies, and 483 titles of serial publications were acquired through exchange/gift from 235 institutes/organizations in 69 countries (table 1).

Table 1. Acquisitions in 1994

	Request for order	Ordered	Paid	Exchange
Book	133	108	70	289
Photocopy	200	187	178	85
Serial publication				
Subscription		130	130	483
CD-ROM		3	3	1
Diskette		2	2	

Documentation and In-house Database

A total of 2530 titles of books and crop documents, and 62 new serial titles were indexed and added to the Tropical Vegetable Information Service (TVIS) databases which now holds 23,717 bibliographic records and 1594 journal records. DAI CD-ROM (Development Activity Information on CD-ROM), produced by the International Development Research Centre (IDRC) of Canada, was added to the collection of databases on CD-ROM. Additions to the library collection and in-house databases are given in table 2.

Table 2. Status of library collection and retrieval system

	1994 additions	Total holdings	Retrieval system: Card catalog	Retrieval system: Online database
Books	514	15,925	9,655	6,269
Serial publications (title)	62	1,594		1,594
Back volumes	281	13,156		
Cassettes		52		52
CD-ROM (disc)	1	36		
Diskettes	80	336		
Maps		84	77	7
Microforms		11		11
Documents	(title)	(title)	(title)	(title)
Amaranth	176	176	176	
Chinese cabbage	24	1,464		1,464
Common cabbage	5	131		131
Diamondback moth	24	618	314	304
Eggplant	12	85		85
Garlic	49	279		279
Mungbean	59	4,607	2,352	2,252
Okra	518	535		535
Onion	70	924		924
Peppers	176	1,459		1,459
Shallot	1	45		45
Snap bean	205	286		286
Soybean rust	1	445		445
Sweet potato	2	3,887	2,517	1,370
Tomato	217	7,943	5,237	2,706
Others	301	2,266		2,266

Information Dissemination

Bibliography services

Six bibliographies retrieved from in-house databases and CD-ROM were provided to ARC-AVRDC and the Workshop on Vegetable Research and Development in Cambodia, Lao PDR, and Vietnam (table 3).

Table 3. Bibiographic entries

Crops	Total entries (title)
Amaranth	562
Okra	2,275
Snap bean	485
Vegetables in Cambodia, Lao PDR, and Vietnam	106
Water convolvulus	145
Yard-long bean	120

Current awareness and SDI services

SDI services recorded an additional 42 users from 22 countries. There are now 236 users in 54 countries. Four issues of Library List (new acquisitions) were provided for internal users. Ten issues of Tropical Vegetable Information SDI Bulletin were published and distributed to 167 users and 8 libraries in 46 countries (table 4).

Table 4. Library list and TVIS SDI Bulletin distribution

Topics	Issue	Entries	User	Library	Country
Library list	4	497	(for internal user)		
Chinese cabbage	1	70	49	3	40
Garlic	1	58	20		13
Mungbean	2	257	85	6	36
Pepper	2	165	73	1	40
Soybean	2	185	83	7	38
Tomato	2	264	76	1	42

Reader Services

Literature searches

Some 48 requests for literature searches were processed in-house and externally on CD-ROM (table 5).

Table 5. Literature searches

Databases	Internal user	External user
AVLIB	17	3
AGRICOLA	15	1
AGRIS	13	1
CAB	15	2

Library loan and photocopy/document delivery services

Total book/crop document loans were 5840. A total of 1316 document titles were photocopied and sent to 48 external users and 76 libraries in 19 countries (table 6).

Table 6. Photocopy/document delivery services

	User	Library	No. of titles delivered
Australia	1		5
Belgium		1	1
Cuba		1	4
Germany	1		1
India	1	2	18
Iran	1		2
Kenya		1	8
Korea	1		1
Mauritius		1	11
New Zealand		1	1
Papua New Guinea		1	9
Peru	1	1	11
Philippines		1	5
Singapore	1		2
Syria		1	9
Taiwan, ROC	38	65	1,190
Thailand	1		8
Venezuela	1		3
Vietnam	1		27

Publications and Communications

Summary

OPC provides publication production and information support services to the center. These include artistic, graphic, audiovisual, desktop publishing, editorial, photographic, printing, public awareness, and writing services. Publication distribution is also managed by OPC.

Publications

See the section on Publications (p. 502)for the list of publications produced in 1994. In addition to these publications, OPC also produced new brochures about the center, the AVRDC-Africa Regional Program, GRSU, ARP training programs, and Diamondback Moth International Workshop. An updated publications brochure was also prepared.

Public Awareness

This year, OPC facilitated and coordinated the following public awareness activities: visiting journalist program (20 Taiwanese, 1 Japanese, 1 American); media linkages (2 tv programs produced by local television stations, Radio Taiwan interview on IPM, and BBC "Farming World" interview on SAVERNET); press releases (international publications such as CTA Spore, FAO Ceres, African newspapers; local press/magazines, including Free China Journal and China Post) on AVRDC, grafting, peri-urban farming, fertilizer use, summer tomato production, ARP, and other newsworthy items.

Collaborative Vegetable Research Program for Southeast Asia

Summary

The building up of agricultural development resources and skills in the national agricultural research systems (NARS) in recent years has given birth to the networking approach. With research collaboration as the key, this strategy banks on the sharing of resources and improved vegetable germplasm and technology among participating countries.

The first of AVRDC's network agreements, implemented in May 1989 and supported by the Asian Development Bank (ADB), is the Collaborative Vegetable Research Program for Southeast Asia (AVNET). Through the network, partner countries, namely, Indonesia, Malaysia, the Philippines, and Thailand, cooperate on planning and monitoring research activities; share research responsibilities; and complement each others' infrastructure and manpower development, with AVRDC as the coordinating body and an active partner in this endeavor.

The first phase of AVNET was completed in September 1992. Its achievements in just 3 years of implementation have been so significant that a second phase has been set up to sustain and strengthen the network. The joint planning meeting for the implementation of AVNET II was held on 24-26 September 1992, in conjunction with the final workshop of the first phase. A jointly prepared proposal for AVNET II was developed and submitted to ADB for financial support which was approved in March 1993, signaling the implementation of the second phase.

Two subnetworks were created to achieve AVNET II's goal: subnetwork I - field verification and technology packaging for selected vegetables; and subnetwork II - disease and pest management.

Introduction

Research collaboration is the key to AVNET's success. The participating countries, in cooperation with AVRDC, collaborate on the collection, exchange, and evaluation of vegetable germplasm for regionally important species. Varieties that are high yielding and those resistant to pests and diseases and other stresses in the hot, humid tropics are identified. The presence and characteristics of bacterial and virus diseases in vegetables are likewise explored in an attempt to identify and develop disease-resistant varieties to counter disease losses. The ultimate goal of these activities is to encourage farmers to grow more vegetables by making available to them varieties that will guarantee improved yield, thereby, improving their income, as well as their nutrition. AVNET also addresses pesticide overuse by introducing the integrated pest management (IPM) approach, with emphasis on the control of crucifer pests using natural enemies.

Project Management

The program coordinating committee consists of the technical coordinators from each country and the AVNET coordinator.

Indonesia	Dr. Farid Bahar
Malaysia	Dr. Saharan Haji Anang
Philippines	Dr. Joycelyn Eusebio
Thailand	Dr. Sutevee Sukprakarn
AVRDC	Dr. S. Shanmugasundaram

At AVRDC, the following senior scientists provide support for the network:

Dr. I.M. Engle	Head, Genetic Resources and Seed Unit
Dr. S.K. Green	Virologist
Dr. N.S. Talekar	Entomologist
Dr. J.F. Wang	Bacteriologist

In addition, on an ad hoc basis, Drs. J.M. Poulos (pepper breeder), Peter Hanson (tomato breeder), C.S. Pathak (allium breeder), and L.L. Black (plant pathologist) provide support to the network.

Subnetwork I
Field Verification and Technology Packaging for Selected Vegetables

A persistent problem in farmers' efforts to increase vegetable production is the lack of improved vegetable varieties with desirable traits such as heat tolerance, high yield, and resistance to pests and diseases. Hence, promising varieties of priority vegetables (yard-long bean, cucumber, pepper, and tomato) are identified, selected, and evaluated under farmers' field conditions in different seasons and locations using appropriate technology.

Indonesia. Tomato variety PT 4225 produced 37 t/ha, while ANT 23 and ANT 6 produced 27 and 23 t/ha, respectively. Among the hot pepper varieties, Jatilaba and Prembun each yielded 15 t/ha fresh fruit.

Medium elevation location (668 m asl) appears to be better for yard-long bean production. Varieties DOA from Malaysia and LV 2316 from Indonesia produced 24 and 21.7 t/ha, respectively, in medium elevation (Garut), while variety CSL 19 from the Philippines produced 17.5 t/ha in the lowlands (8 m asl). Pod length was longer in those grown in the lowlands than in the highlands.

Malaysia. Trials have shown that yard-long bean cultivars KU 8 from Thailand; CSD 5, CSD 4, and CSD 19 from the Philippines; and KP 5 and DOA from Malaysia are the highest yielders.

A total of 30 accessions of hot pepper from the International Hot Pepper Trial Network (INTHOPE) member countries were evaluated in a second season trial. PBC 375 LV 1583 (17) from Indonesia gave the highest yield of about 20 t/ha, followed by PBC Hot Beauty and PBC 373 Keriting, each with 13 t/ha (table 1).

Philippines. Traditional asexual propagation of garlic and shallot is slow, seasonal, and promotes rapid transmission of virus diseases resulting in yield reduction of 3-45%, while the use of seeds in shallot results in a wide variation in most characters. In response to this concern, the micropropagation of garlic and shallot is dealt with under subnetwork I. This activity is mainly the responsibility of the Philippines. Basic studies on in vitro shoot vitrification and its control are ongoing, vitrification being a crucial problem in micropropagation. Multiplication of shallot shoots up to the 25th passage and beyond was achieved. Plantlets from tissue-cultured materials grew vigorously in the greenhouse and first generation (G_1) bulbs have been harvested.

Table 1. Yield performance of 30 accessions of chili in the INTHOPE trials

Variety	Yield (t/ha) 2nd trial	Yield (t/ha) 1st trial	Fruit size (g)	Plant height (cm)
PBC 510 Long Fruit	2.2 e-g	1.8 de	3.1 d-i	32.1 c-j
PBC 156 KKU Cluster	6.4 d-g	6.0 c-e	1.8 f-k	29.5 d-j
PBC 155 Huaruar	6.2 d-g	5.3 c-e	1.2 i-k	34.2 c-g
PBC 157 Huay Sithon	2.5 e-g	2.2 d-e	1.3 i-k	46.4 ab
PBC 405 Var. PL 2289	0.5 fg	0.4 e	0.8 k	22.4 i-k
PBC 402 Var. P. Sakaraho	0.1 g	0.1 e	0.7 k	23.4 h-k
PBC 186 Cheongryong	2.9 e-g	1.8 de	4.8 b-d	35.0 c-g
PBC 266 Twist Green (F_1)	1.5 e-g	1.0 de	1.9 f-k	16.5 kl
PBC 270 Hot Long F_1	3.3 d-g	2.2 de	5.2 bc	27.9 f-k
PBC 241 Magic Scarlet F_1	6.4 d-g	5.5 c-e	3.1 d-i	36.9 a-f
PBC 076 Szechuan	4.5 d-g	3.7 c-e	3.4 c-h	38.3 a-e
PBC 210 Long Chilli F_1	5.6 d-g	3.2 c-e	4.7 b-d	27.9 e-j
PBC 207 Chain pair F_1	5.2 d-g	4.0 c-e	4.5 b-d	41.9 a-c
PBC 233 Hot Beauty F_1	13.6 ab	13.2 b	3.2 c-i	41.0 a-c
PBC 151 IAC Ubatuba Cambuci	0.2 g	0.2 e	15.0 a	36.2 b-g
PBC 373 Keriting	17.1 ab	13.2 b	0.9 jk	39.2 a-d
PBC 375 LV 1583	20.1 a	19.8 a	3.9 b-f	36.3 b-g
PBC 376 LV 2319	4.0 d-g	2.3 de	4.3 b-e	29.1 d-j
PBC 377 LV 1092	9.6 cd	9.0 bc	3.6 b-g	33.6 c-h
PBC 378 Cipanas	7.4 de	4.9 c-e	3.3 c-i	26.9 f-k
PBC 374 LV 2323	7.1 d-f	6.9 cd	3.5 b-g	33.1 c-i
PBC 140 Jawahar	2.1 e-g	1.4 de	1.6 g-k	23.6 h-k
PBC 146 Ludliana 1/Slim	1.9 e-g	1.3 de	3.1 d-i	25.8 h-k
PBC 147 Extra Long Slim	1.2 e-g	0.7 de	1.9 f-k	22.0 j-l
PBC 148 Punjab Lal	3.3 d-g	1.6 cd	1.3 h-k	27.6 e-j
PBC 398 KA 2 (t-6)	3.4 d-g	2.7de	2.4 e-k	33.8 c-h
PBC 401 KA 11	3.8 d-g	2.9 c-e	1.7 g-k	26.7 f-k
PBC 171 Atanada	n. d.	.00 e	n.d	12.8 l
PBC MC 4	3.6 d-g	2.9 cde	5.6 b	46.8 a
PBC MC 5	1.8 e-g	1.5 de	2.8 e-k	39.7 a-d
CV (%)	78.6	91.2	36.17	20.1

Note: Values with the same letters are not significantly different at 5% level (DMRT)

Seeds of tomato, cucumber, hot pepper, and yard-long bean have been multiplied. The trials are in progress in the Department of Agriculture (DA) Region 5, San Augustin, Pili, Camarines Sur; DA Region 10, Lanao Del Norte; Central Luzon State University, Muñoz, Nueva Ecija; and Upper Manama, Naic, Julugan, and Tanga, Cavite. The following entries have been selected and included in the technology adaptation trials:

Yard-long bean	Cucumber	Chili
(1) KU 8	(1) Jed Bai	(1) Jatilaba
(2) KP 5	(2) LV 1723	(2) LC Serdang
(3) CSL 19	(3) BPI-Cu22	(3) Szechuan 10
(4) BPI-PS3 (national check)	(4) UPL-Cu 6 (national check)	(4) Hot Shot (national check)
(5) EG PS1 (regional check)	(5) White (regional check)	(5) Kawit (regional check)

Tomato

Determinate	Indeterminate
(1) CL 5915-93-4D	(1) FMTT 138
(2) BL 695	(2) CL 6043 BC 3F2-51
(3) BL 699	(3) CL 5915-206 D_4
(4) Maigaya (national check)	(4) Pope (national check)
(5) Pope (regional check)	(5) Bonanza (regional check)

Yard-long bean cultivars were evaluated in eight locations in wet and dry seasons through National Cooperative Testing.

"Field Verification Trials of Promising AVNET Elite Cultivars of Pole Sitao" was selected the best technical paper by the Philippine Bureau of Plant Industry.

The JICA irrigation project and PhilRice are now AVNET trial sites.

Thailand. Cucumber, yard-long bean, hot pepper, and tomato trials are being conducted both in the experimental field at Kamphaengsaen and in farmers' field at Ratchaburi province. Data collection in all trials is still ongoing. The following entries are included in the trials:

Cucumber	Yard-long bean
(1) LV-1043	(1) CSD 4
(2) MFi 1	(2) KP 5
(3) UPL-CU 6	(3) CSL 19
(4) Jed Bai (local check)	(4) LV 801
(5) Taeng-ton (national check)	(5) KU 8 (national check)
	(6) Rajburi (local check)
	(7) RW 24
	(8) White Bean
	(9) VU 408

Hot pepper

Large fruited	Small fruited
(1) Yangiao	(1) Huey Sithon
(2) Tit	(2) Huarea
(3) Paris	(3) Huarea Ubol
(4) Bangchang	(4) Punjab Lal

Tomato

Large fruited	Small fruited
(1) CLN 5915-206 D4-2-2-0	(1) Seedathip 2
(2) CLN 657 BC1 F2-274-0-15-7	(2) Seedathip 3
(3) CLN 399 BC1 F2-2-6-0	(3) Seedathip 91
(4) PT 4225	(4) CL 6046 BC3 F2-51-0-20-0
(5) PT 3027	

Subnetwork II
Disease and Pest Management

Subnetwork II has been divided into three major activities: (1) bacterial wilt disease management; (2) management of major viruses of peppers; and (3) integrated pest management (IPM) of crucifers.

Bacterial Wilt Disease Management

Bacterial wilt, caused by *Pseudomonas solanacearum*, is one of the most destructive diseases of tomato in the lowland tropics and subtropics, causing yield losses ranging from 15 to 95% worth about US$25-158 million. The destructiveness of this pathogen is attributed to its widespread occurrence, broad host range, exceptional ability to survive in the soil and in the roots of nonhost plants, and the existence of different races, biovars, or strains. Results of extensive bacterial wilt surveys of major tomato-growing areas in the participating countries confirmed the presence of different bacterial wilt causal pathogens, partially explaining the differences in resistance of cultivars in different environments. Bacterial wilt materials collected from these surveys have been isolated, and differences in virulence and characteristics among the isolates are being studied.

In Malaysia, a method for quick detection of *Pseudomonas* infection in tomato using PCR (polymerase chain reaction) is being developed. In the Philippines, a total of 80 pure culture tomato isolates are now being maintained. In Thailand, a membrane protein complex (MPC) antigen of two strains of *P. solanacearum* has been prepared for use in further identification and detection of the pathogen. In the International Bacterial Wilt Disease Nurseries (IBWDN) established under AVNET I, tomato accessions and local varieties are being screened for disease reaction under local environmental field conditions.

In Indonesia, bacterial wilt screening in tomato identified the following as resistant: L 285, L 95, L 96, L 12, L 155486, L 274, BL 341, and BL 437.

Preparation of a polyclonal antisera against *P. solanacearum* isolate TOM 2 production is in progress.

In Malaysia, virulence of the bacterial isolates collected is being studied by DNA probes. A rapid method of detecting *P. solanacearum* in tomato using PCR is also being explored. Fluorescent pseudomonads have been isolated from the rhizosphere of the tomato plant and their role in protecting tomato plants from infection by *P. solanacearum* is being studied.

In Thailand, 13 promising entries are being evaluated for bacterial wilt resistance.

Management of Major Viruses of Pepper

The virus complex is considered as the biggest production constraint among the numerous diseases affecting pepper. Yield losses due to viruses, such as cucumber mosaic virus (CMV) can be as high as 97% in tropical peppers, whereas tobacco mosaic virus (TMV) and/or potato virus Y (PVY) can reduce yield by 45-75%.

AVNET II facilitates the conduct of pepper virus studies in all participating countries. Extensive surveys have been conducted to collect diseased samples for identification and isolation. In a survey of chili viruses on weed hosts in Malaysia, chili veinal mottle virus (CVMV) was detected in *Physalis minima*, while CMV, an aggressive virus with a wide host range, was found in *Amaranthus hybridus*, *Datura metel*, *Solanum nigrum*, and *Physalis minima*. No leaf curl symptom was observed in any of the areas surveyed in the Philippines, while 25 out of 70 samples collected showed the presence of TMV and TMV-PVY mixture. In Thailand, a total of 109 samples from pepper plants showing

viruslike symptoms were collected and tested for the presence of leaf curl or geminivirus. Among 91 samples collected for the detection of six other viruses, the most prevalent was CMV, followed by CVMV (table 2).

In Indonesia, 15 pepper cultivars received from AVRDC were screened for CMV and CVMV. All showed CMV symptoms, but by ELISA test, several plants of the following entries were negative (did not react with CMV antiserum and classified as healthy): Taiwan 83-168, Punjab Lal, Lek Haeng, VC 16a, and Jatilaba.

Out of 21 pepper cultivars evaluated for CVMV resistance, Toom, VC 58a, Taiwan 83-168, VC 16a, and LV 3633 were symptomless.

In Thailand, a nonradioactive DNA probe was produced from tomato yellow leaf curl virus DNA (TYLCV-A) and mungbean yellow mosaic virus DNA1 (MYMV-1). TYLCV-A or MYMV-1 could be incorporated into the leaf curl of pepper. The following plants also reacted with the probe used: false daisy, potato tree (*Solanum wrightii*), vervain (*Stachytarpheta jamaicensis*), node weed (*Synedrella nodiflora*), cucumber (*Cucumis sativus*), loofah (*Luffa acutangula*), pumpkin (*Cucurbita moschata*), and wax gourd (*Benincasa hispida*), suggesting that they may be alternative or natural hosts of the geminivirus (table 3). Pure isolates of CMV and CVMV are maintained for strain differentiation.

Table 2. Distribution of viruses infecting peppers in Thailand identified by direct antigen coating-indirect enzyme-linked immunosorbent assay (DAC-ELISA) in July-September 1993

Provinces	Type of pepper	No. of samples	No. of samples infected with viruses					
			CMV	CVMV	PeMV	TEV	AMV	TSWV
Chiangmai	hot pepper	7	2	2	0	2	0	1
	bell pepper	22	7	12	3	3	2	5
Kanchanaburi	hot pepper	12	7	0	1	2	1	0
	bell pepper	30	13	0	3	0	4	0
Nakorn Ratchasima	hot pepper	5	0	3	0	1	0	0
Songkla	hot pepper	11	1	3	1	0	0	1
	bell pepper	4	0	1	0	0	0	1
Total		91	30	21	8	8	8	8
detection (%)			93.0	23.1	8.8	8.8	8.8	8.8

Table 3. Weed plants showing potential as alternative or natural hosts of geminivirus

Plant name	Symptoms
False daisy (*Eclipta prostrata*)	yellow mosaic, yellow vein netting
Potato tree (*Solanum wrightii*)	bright yellow mosaic
Vervain (*Stachytarpheta jamaicensis*)	mild mottle
Node weed (*Synedrella nodiflora*)	yellow mosaic, leaf curling
Cucumber (*Cucumis sativus*)	yellow mottling or spotting, leaf curling
Loofah (*Luffa acutangula*)	chlorotic or yellow mottling, reduced size of leaf, leaf curling, stunting
Pumpkin (*Cucurbita moschata*)	yellow mottling venation, leaf puckering and curling
Wax gourd (*Benincasa hispida*)	yellow mosaic, leaf curling

Integrated Pest Management in Crucifers

Pesticide overuse is very evident in developing countries where crucifers (cabbage, cauliflower, broccoli, radish, kohlrabi, kale, and mustard) are economically important and are highly susceptible to insect pests, especially diamondback moth (DBM) (*Plutella xylostella*), *Hellula undalis*, *Crocidolomia*, and *Spodoptera*. To address the insect pest problem, IPM of crucifers has become an important AVNET activity. It involves the introduction, establishment, and adoption of the technology of using parasitoids and other nonchemical measures to control pests in both the highlands and lowlands where crucifers are cultivated.

After 3 years of implementation under AVNET I, the IPM technology reduced and minimized the use of pesticides in most cooperating farms in the participating countries. Environmental hazards of pest control were reduced, while consumer safety was ensured. With the continuous adoption of the technology, farmers' income and the quality of produce are, thus, expected to improve. And with the construction of new facilities for mass rearing of parasites through AVNET and manpower development through training, the research capabilities of the NARS have been greatly enhanced. Hence, to sustain the gains of IPM under AVNET I, IPM was included as a critical component of AVNET II. Under the second phase, the control of other important crucifer pests such as *Hellula undalis*, *Crocidolomia*, and *Spodoptera* is also dealt with.

In the Cameron Highlands, Malaysia, production of parasitoid *Diadegma semiclausum* is approximately 5000-8000 pupae per week, while that of *Diadromus collaris* is about 2000 per week. *Cotesia plutellae* production in 1993 is approximately 50,000 adults, while *Trichogrammatoidea bactrae* production is about 2-4 cc *Corcyra* eggs per day. These parasitoids are being released in farmer-cooperators' farms. The most effective IPM package for managing DBM based on five trials was IPM II which consisted of the following measures:

Crop at 0-5 weeks

No spray	-	DBM < 4 per plant
No spray	-	DBM > 4<7, parasitization > 40%
Spray *Bacillus thuringiensis* (*Bt*)	-	DBM > 4<7, parasitization < 40%
Spray insecticides	-	DBM >7

Crop at 5 weeks to harvest

No spray	-	DBM<8 per plant
No spray	-	DBM>8<14, parasitization >40%
Spray *Bt*	-	DBM>8<14, parasitization<40%
Spray insecticides	-	DBM>14

Indian mustard as a trap crop was not effective in controlling DBM and *Hellula* spp. in crucifers. Water extracts of the neem seed kernel at 1.25, 2.5, and 5% gave good protection against shoot damage by *Hellula*.

In the Philippine highlands, approximately 161,500 cocoons of *D. semiclausum* were produced in the rearing house from January to June 1993, and 55,000 cocoons from July to October 1993. Parasitoid releases have become a team activity under the National IPM of DBM program involving several government agencies. The use of *D. semiclausum*, together with a selective insecticide, *Bt*, did not show significant results. Meanwhile, in the lowlands 2 to 3 years after initial releases, *C. plutellae* has been observed in cooperators' farms. Preliminary field evaluation indicates that cabbage moth

(*Crocidolomia binotalis*) and other insect pests of cabbage were attracted to Indian mustard. The presence of *Hellula* was high during the latter stages of cabbage growth, and most destructive during the seedling and vegetative stages where the larva feeds on the developing shoots, adversely affecting the production of marketable heads.

The key activities of IPM of crucifers in Thailand have been the development of a proposal for an in-country training and the construction and renovation of laboratory facilities. Training on the Management of the Diamondback Moth on Crucifers was held on 14-16 December 1993 at Kasetsart University.

In Indonesia, parasitoid rearing continues at the screenhouse in Segunung. The parasitoid *Cotesia plutellae* has been released in a pesticide-free field.

A nematode is being reared as a parasitoid for cabbage pest.

Training

A bacterial wilt training course was held at AVRDC from 25 April to 23 May 1993. One researcher from each of the four collaborating NARS participated in the training.

A training course on nonradioactive DNA probe-diagnosis of leaf curl virus was held at Kasetsart University on 9-16 June 1993. One participant each from Malaysia, the Philippines, and Indonesia and five participants from Thailand attended the training.

Other Activities

AVRDC also organized a workshop on Agricultural Economics Research on Vegetable Production Systems and Consumption Patterns in South and Southeast Asia on 11-13 October in Bangkok, Thailand. The main objectives of the workshop were to explore the state of the art of agricultural economics research in vegetable production, marketing, distribution, and consumption; find out the types and sources of available secondary data sets in vegetables; and identify the gaps in research to set future research priorities for national and international agricultural economists working with vegetable systems.

The workshop identified future research agenda and planned for impact assessment of ongoing AVNET and SAVERNET activities.

South Asian Vegetable Research Network

Summary

The South Asian Vegetable Research Network (SAVERNET) Midterm Workshop was held on 12-15 September 1994 at the Atria Hotel, Bangalore, India, and attended by 50 vegetable scientists from Bangladesh, Bhutan, India, Nepal, Pakistan, and Sri Lanka. Specifically, the midterm workshop had the following objectives: to discuss and exchange notes on the progress made so far in each country in each subnetwork activity with a view to strengthen ongoing research activities; to understand issues that may arise and ascertain ways and means to manage the issues; to agree on minimum midcourse fine-tuning, if necessary, and arrange for minor modifications in the work plan based on current needs; to discuss strategies for planning of the next phase (1995-98) based on the progress and expected impact of the first phase; and to agree on necessary adjustments in the financial plan within each subnetwork and within each country.

Subnetwork I

Exchange and Evaluation of Elite Varieties

A total of 69 varieties out of 120 varieties of 13 kinds of vegetables were evaluated for yield and quality parameters by the member countries. In onion, 8 out of 13 varieties were tested. Agrifound Dark Red and Arka Niketan performed well in Bangladesh and Sri Lanka; Red Creole in Nepal; Pusa Madhavi did well in India and Nepal. In tomato, 13 out of 19 varieties were tested. The varieties T 245, Pusa Early Dwarf, and Arka Vikas performed well. In Nepal and Pakistan, superior performance was observed in tomato varieties Pusa Early Dwarf and Punjab Chhuhara. In Sri Lanka, the varieties Ratan, K.W.R., and Pant Bahar performed well. Tomato varieties T 245 and T 89 were found resistant to bacterial wilt in India. In chili, 13 out of 16 varieties were evaluated. Performance of Sri Lankan chili varieties ML 2, KA 2, and Pusa Jawala of India was better in India. Farmers in North India preferred the color of KA 2 which had equally good yield. In eggplant, 11 out of 19 varieties were evaluated. The variety Pusa Purple Long performed well in Bangladesh, Pakistan, and Sri Lanka. Superior performance was also observed in brinjal varieties Nurki, Pant Samrat, and Thinnevelly Purple.

The six SAVERNET member countries agreed to exchange 117 varieties of 12 vegetable crops.

Subnetwork II

Bacterial Wilt Studies

In Bangladesh, out of 29 tomato hybrids tested, the following six showed 100% resistance: IND X CHN, 8 X 6, 6 X IND, 6 X CHN, IND X 6, and IND X 10. The data are for 1 year only and are not from replicated trials. Out of 56 lines tested for 2 years under field conditions and 1 year under artificial screening, six showed complete resistance, namely, TM 72, TM 91, TM 81, TM 80, TM 85, and TM 89. In eggplant, 12 varieties were tested and only two, Pant Rituraj and Thinnevelly White, showed moderate resistance

In Bhutan, bacterial wilt in brinjal, chili, and tomato is not a problem and, hence, research on this topic shall be dropped.

In India, a survey of four provinces where the disease is more prevalent was conducted. It showed that high humidity and high temperature generally favor the wilt in these areas. Crop rotation pattern showed considerable variation in wilt incidence. Paddy and maize were found to be good intercrops, while potato, beans, cabbage, ginger, and wheat rotations did not reduce wilt. Out of 21 advanced breeding lines from AVRDC evaluated at the Indian Institute of Horticultural Research (IIHR), Bangalore, 14 lines proved resistant even after 105 days after planting. Two lines, CLN 675-206 D4-2-2-0 with yield potential of 1.35 kg/plant and CLN 675-BC$_1$FC-285-0-21-0 with yield of 1.55 kg/plant, are promising. The average fruit weight in both these lines was about 73 g.

In Nepal, Race 2 was responsible for bacterial wilt in brinjal and Race 3 in tomato. This needs confirmation for another year.

In Pakistan, survey work in 42 locations in four provinces was done and infected soil and plant samples of chili and tomato were collected. Laboratory testing showed 3 to 10% incidence of wilt. Biovar III is mostly responsible for tomato wilt, although Biovar II is also present in potato.

In Sri Lanka, survey and sampling were conducted in 21 locations in the up-country area. The total wilt incidence (at flowering and at harvesting) during 1993 and 1994 ranged from 0 to 33% and 0 to 30%, respectively. Biovar III was the most common type, while Biovars II and IV were also prevalent in the highlands. Tomato lines BL 162, BL 311, and CLN 65-349-2-0 expressed resistance. Soil amendments like calcium oxide, 2 t/ha, urea, 200 kg N/ha and sunhemp (*Crotalaria diversifolia* L.), and 10 t/ha walsurinkankantha (*Titonia diversifolia*) suppressed the expression of bacterial wilt in tomato. Survey and sampling were done in 21 farmers' fields in the midcountry during flowering stage in 1992. Bacterial wilt incidence varied from 0 to 35%. Biovar III is responsible in this region. Tomato variety PBC 204, PBC 375, and PBC 473 were found to be resistant to bacterial wilt. None of the brinjal varieties were found resistant.

Leaf Curl Virus Studies

In Bangladesh, only three out of 29 F_1 screened in 1992 showed some field tolerance, with less than 7% infected plants. The disease incidence in the other lines ranged from 13 to 73%. Another 56 lines/cultivars were screened in 1993. These included germplasm received from AVRDC and other sources with reported resistance/tolerance to tomato yellow leaf curl virus.

In India, two institutes are involved in leaf curl virus research, the ICAR, Project Directorate of Vegetable Research Sunderpure, Varanasi in North India, and IIHR, Bangalore, South India. In North India, a total of 178 tomato accessions comprised of varieties, hybrids/germplasm accessions, and two advanced breeding lines, H 24 and H 36, resulting from crosses of *L. esculentum* x *L. hirsutum* and

L. esculentum x *L. pimpinellifolium*, respectively, were screened for leaf curl virus resistance under natural field conditions. Eight accessions (EC 1113, EC 89248, EC 428838, EC 121210, EC 92951, EC 6033, EC 3176, and Lerica) were resistant, i.e., none of the plants showed any disease symptoms. A total of 168 lines showed varying degrees (4-81%) of leaf curl infection, whereas two lines (EC 163611 and EC 8822) showed 100% infection. Of the two advanced breeding lines tested in two consecutive years, H 24 showed an average disease incidence of 9%; H 36 showed 14% compared to 64% of the susceptible checks. None of the 135 hot pepper accessions screened were resistant, although four lines (PDAC 26, PDAC 24, PDAC 14, and HOE 808) showed disease incidences below 20%. Six lines (PDAC 44, PDAC 42, PDAC 20, IC 119604, RHRC 16-5, and Katrain Local 1) had a disease incidence of 100%.

None of the 11 sweet pepper lines screened were resistant. In South India, a survey of the major pepper growing areas in Karnataka and Andhra Pradesh was conducted. A total of 150 samples were collected and tested by indirect ELISA. Cucumber mosaic virus (CMV), potato virus Y (PVY), pepper mosaic virus (PMV), and tobacco mosaic virus (TMV) were the most prevalent and were detected in more than 90% of the samples. In addition, 97 samples, showing symptoms typical of geminivirus infection (such as yellowing, curling, and blistering of the leaves, stunting, etc.) were tested at AVRDC by DNA hybridization using a mixture of three digoxygenin-labeled DNA probes (of TLCV-India, TLCV-Taiwan, and TYLCV-Egypt). Only six samples gave a positive reaction.

In Nepal, only about 35% of the surveyed plants showed typical symptoms. Only five out of 35 samples tested positive with the TLCV-India probe for the presence of geminivirus. Other viruses such as CMV and TMV appear more serious than tomato leaf curl virus. In Dhading and Para, more than 80% of the surveyed plants showed leaf curl and yellowing symptoms. Farmers attributed 90% of the yield losses to this.

Of 351 and 88 samples collected in Pakistan in 1993 and 1994, respectively, the most important virus on tomato was tomato bushy stunt virus (TBSV). Tomato aspermy virus (TAV) was second in importance with 32 and 79% infected samples in 1993 and 1994, respectively. A high percentage (46%) of the tomato crop was infected with potato leaf roll virus (PLRV) in 1993. The virus was, however, not found at all in the 1994 surveys. CMV was found in 26% of the samples collected in 1993 and in 67% of the samples collected in 1994. Potato virus X was found in 12 and 31% of the samples in 1993 and 1994, respectively. PVX, TMV, tomato yellow top virus (TYTV), and PVY were also encountered. Twenty samples with typical leaf curl symptoms were collected and tested by DNA hybridization with a mixture of P32-labeled probes (TLCV-Taiwan, TLCV-India, TYLCV-Egypt, ToMoV-Fla, and MYMV). Sixteen samples tested positive, indicating the presence of a geminivirus. The virus could be transmitted by grafting to *Nicotiana tabacum* Samsun and Xanthi.

The tomato geminivirus has been partially purified. Initial comparative serological tests using monoclonal antibodies to the Indian cassava mosaic virus (ICMV) and polyclonal antibodies to the African cassava mosaic virus (ACMV) indicated that the geminivirus which causes leaf curl virus symptoms in Pakistan may be different from the Indian tomato leaf curl virus. Of 504 chili samples collected in 1993, CVMV and CMV were the most important, with 20% of the samples infected followed by TMV (9%), PVY (7%), PVX (3%), potato virus E (PVE) (2%), pepper mild mottle virus (PMMV) (1%), and PVMV (1%). TSWV was not detected. Mixed infection with several viruses was a common feature. From preliminary screening tests, some cultivars/lines found with resistance to TMV (Anaheim TMR 23, Chi 3) and CMV (VC 16a) were identified.

In Sri Lanka, a survey for leaf curl virus was conducted in the major chili-growing areas of the dry zone. Twenty-four samples were sent to AVRDC and the University of Wisconsin for testing by DNA hybridization, using a mixture of either radio or digoxygenin-labeled DNA probes of TYLCV-Egypt, TLCV-Taiwan, TLCV-India. Eight of the samples gave a positive reaction, clearly indicating the presence of a geminivirus in chili in Sri Lanka.

Integrated Pest Management of Diamondback Moth

In Bangladesh, among the winter vegetables, crucifers are planted to a maximum area of about 92,000 ha. In recent times, DBM has emerged as a regular and major pest of crucifers. Its host range extends to eight cruciferous vegetables. Some important natural enemies like *Diadromus collaris*, *Cotesia plutellae*, and *Oomyzus sokolowskii* have been identified. An existing structure was modified to create a parasitoid-rearing facility.

In Bhutan, DBM was one of the several pests found to occur (*Pieris brassicas*, *Mamestra brassicas*, and *Agrotis* spp. *Brevicoryne brassicas*) on crucifers. Parasitoid-rearing facilities were completed. Three species of parasitoids, viz., one larval and two larval/pupal, were collected and await identification. Rate of parasitism by these parasitoids ranged from 0 to 50%.

In India, DBM is the most important pest of crucifers. An existing structure was renovated to rear parasitoids. A simple technique was developed to collect DBM eggs on tissue paper and is being used routinely. Parasitoid-rearing techniques were refined to get an optimum sex ratio. Two field trials were conducted to evaluate releases of *Diadegma semiclausum* under different pest-management practices. The use of mustard as trap crop and spraying 4% neem seed kernel extract (NSKE) gave good control of DBM and other pests. A pilot project on IPM was conducted at Ooty in Nilgiris (2000 m asl), Tamil Nadu. About 18,000 *D. semiclausum* and 6000 *Cotesia plutellae* were released.

In Nepal, a survey on DBM and its natural enemies was initiated. An existing rearing room was renovated and an air-conditioning unit was installed. A continuous supply of DBM was maintained. An unidentified parasite was recorded.

In Pakistan, DBM occurs in all cruciferous vegetables at different times of the year. The percentage infestation by *Cotesia plutellae* on field collected larvae from plains in Lahore, Sahiwal, and Hyderabad ranged from 2 to 22%. Taxilla, near Islamabad, was identified as a pilot project site for the implementation of IPM by combining trap cropping, biological, and chemical control methods. An existing laboratory was modified for mass-rearing of selected parasitoids.

In Sri Lanka, DBM and looper, *Plusia eriosoma*, are the most important pests. A high incidence of DBM and looper was observed in the dry and wet seasons, respectively. *C. plutellae* was the common parasitoid with 0-90% parasitism on DBM. Cabbage looper and cut worm, *Agrotis* spp., were controlled with sprays of *Bacillus thuringiensis* and insect growth regulators.

Management of Heat, Moisture, and Other Physical Factors of Tomato and Chili

In Bangladesh, an experiment was designed using different mulching materials to minimize the irrigation requirement. It was concluded that tomato can be produced in the winter with initial single irrigation and by covering the plots with black polythene/rice straw. Another experiment was designed to produce summer tomato under protection in combination with raised bed techniques and application of hormone. The bed was covered with a transparent polythene sheet with bamboo structure like a tunnel. One percent tomatotone was equally effective as 2% tomatotone. By using 1% tomatotone (4 chlorophenoxy acetic acid 0.15%), commercial adoption is economical. This experiment clearly demonstrated that tomato can be produced in hot, humid, high rainfall summer under polythene tunnel with tomatotone application (1%). Bhutan did not participate in this activity. However, some work needs to be initiated with emphasis on screening varieties of chili, *Capsicum*, and tomato for cold tolerance.

In India, a technique for screening the germplasm for high temperature was developed based on the cell membrane stability (CMS). A good correlation between CMS and percent injury due to high temperature stress and osmotic adjustment was observed (r^2 =0.87). Studies on the response of tomato to moisture stress indicated that moisture stress during fruit development accelerated the process of

fruit drop before ripening. Tomato cv. Arka Saurabh, KT 1, Sioux, Pusa Early Dwarf, Red Rock, RFS 1, and RFS 2 maintained better osmotic adjustment and yielded better under water stress conditions. These cultivars had good recovery capacity. Tomatotone was effective in improving the fruit-set of tomato under high temperature (35°C day and 25°C night temperature) conditions. Fruit-set varied from 75 to 100% in the sprayed tomato plants under high temperature conditions. Proline content in anthers was high under high temperature conditions in high temperature-tolerant tomato cultivars, which might be required to confer high temperature tolerance to pollen germination. Variety IIHR 1224 can be used for summer cultivation. Mepiquat chloride, a water soluble growth regulator at a concentration of 125 ppm, can be used for improving the yield under moisture-stress conditions. Chili cv. CA 960 can be successfully grown under rainfed conditions.

No program was alloted to Nepal. However, it was felt that some work should be initiated under this network

In Pakistan, four tomato varieties were grown under four moisture depletion levels under glasshouse conditions. Maximum yield was recorded under 40% moisture depletion of available water. Cv. Lyp 1 gave maximum yield. Glutamine synthetase activity was more at 70% moisture depletion level. Another experiment was conducted to study the effect of different colored plastic mulches and use of hormone at flowering stage of tomato grown under high temperature. Maximum yield was observed with green plastic mulch followed by red and black. PCPA (15 ppm) had a positive effect on fruit-set of tomato under high temperature conditions.

In Sri Lanka, the effect of four mulching materials on the performance of chili grown under rainfed conditions was tested. All mulching materials maintained higher soil moisture content and low temperature than the control. Black polythene maintained the lowest soil moisture content. Rice straw and guinea grass recorded the lowest temperature. Pods per plant and dry chili yields were not significantly affected by the different mulching materials.

Philippines–AVRDC Outreach Program

International Hot Pepper Trial

Summary

Eighteen and 16 hot pepper cultivars were evaluated in the International Hot Pepper Trial (INTHOPE) at the BPI-Los Baños National Crop Research and Development Center (LBNCRDC) and at the Bohol Agricultural Promotion Center in Tagbilaran City, respectively, during the 1994 dry season. In both locations, significant differences in actual yield and other horticultural characters were observed among entries.

At BPI-LBNCRDC, entries PSP 11 (5.7 t/ha), PBC 473 (4.9 t/ha), and Rotan (4.4 t/ha) significantly outperformed the lowest yielding check, Kawit (2.2 t/ha) in total yield.

Of the 16 entries evaluated at the Bohol Agricultural Promotion Center, KA 6-5, MI 1, IR, Yuak, and Szechuan 8 produced statistically similar marketable yields which ranged from 9.0 to 15.6 t/ha.

Introduction

INTHOPE is a cooperative trial coordinated by AVRDC since 1991 to facilitate the exchange and evaluation of hot pepper landraces and elite germplasm among its network collaborators worldwide. Through INTHOPE, adapted germplasm with good horticultural characters could be used as introduced cultivars in appropriate production regions.

Materials and Methods

The dry season evaluation of INTHOPE 3 was conducted in two locations: BPI-LBNCRDC and Bohol Agricultural Promotion Center in Tagbilaran City.

The trial at BPI-LBNCRDC consisted of 16 entries from AVRDC and two local check cultivars (Hotshot and Kawit). The varieties were sown on 12 December 1993 and transplanted on 24 January 1994. Each entry was set in the field in double row 4.8 m^2 subplots (1.2 x 4.0 m) spaced 60 cm between rows and 40 cm between hills. The trial was laid out in a randomized complete block design (RCBD) with three replications.

Only the 16 varieties from AVRDC were evaluated at Bohol Agricultural Promotion Center. Sowing and transplanting of the different entries were done on 6 January and 17 February 1994, respectively. The field was laid out in RCBD with two replications. Each entry was transplanted in 5-m^2 subplots (1.0 x 5.0 m) and 10 plants were maintained per subplot.

In both locations, the cultural management and data gathering procedure were based on the suggested INTHOPE procedure provided by AVRDC.

Results and Discussion

BPI-LBNCRDC trial

The total yield ranged from 2.2 t/ha for Kawit to 5.7t/ha for PSP 11 with a grand mean of 3.6 t/ha (table 1). None of the high-yielding entries significantly outperformed Hotshot with 3.8 t/ha. However, the yields of PSP 11, PBC 473 (4.9 t/ha) and Rotan (4.4 t/ha) differed significantly from Kawit.

The yield components and other horticultural characters measured differed significantly among varieties (table 1). C 01664, PBC 370, Pant C-1, Perennial HDV, and Rotan had the highest number of fruit both on per plant and per hectare basis.

IR had the longest fruits (13.9 cm), while Yuak had the widest (3.0 cm) which differed significantly from the rest of the entries.

Days to flower and to first harvest varied from 37 to 50 days and 78 to 95 days, respectively, with Rotan exhibiting the latest in both characters.

Table 1. Field performance of 18 hot pepper cultivars evaluated under the INTHOPE trial during the 1994 dry season at BPI-LBNCRDC[a]

Entry	Yield (kg/ha)	No. of fruit/ ha	No. of fruit/ plant (million)	Fruit size (cm) length	Fruit size (cm) width	Plant (cm) height	Plant (cm) width	Days to flower	Days to 1st harvest
PSP 11	5.7 a	2.8 c-e	86 cd	5.8 gh	0.9 f-h	51.1 gh	15.9 c	41.0 b-e	78 d
PBC 473	4.9 ab	1.4 f	54 d-f	10.9 c	1.0 d-f	68.3 cd	19.5 b	47.0 ab	87 b
Rotan	4.4 a-c	3.0 cd	116 bc	11.2 bc	0.5 k	88.7 a	29.4 a	50.0 a	95 a
Pant C-1	4.0 a-d	4.7 ab	158 ab	5.6 h	0.7 hi	60.1 d-g	23.1 ab	42.0 b-e	87 b
Mun	4.0 a-d	0.7 f	38 e-g	9.3 d	1.5 b	65.4 de	21.6 bc	42.0 b-e	87 b
MI 1	4.0 a-d	1.6 d-f	70 de	8.2 e	0.9 f-h	65.8 de	23.0 ab	45.0 a-c	87 b
C 01664	3.9 a-d	5.7 a	191 a	3.5 i	0.6 jk	83.4 ab	28.5 a	47.0 ab	87 b
IR	3.8 a-d	0.6 f	27 e-g	13.9 a	1.2 c	48.5 h	18.2 bc	47.0 ab	78 d
PBC 370	3.8 a-d	5.4 ab	198 a	3.5 i	0.7 i	77.4 bc	19.3 bc	43.0 b-e	87 b
Hotshot	3.8 a-d	0.8 f	33 e-g	11.7 b	1.0 de	56.9 e-h	18.4 bc	39.0 de	78 d
KA 6-5	3.6 a-d	1.5 ef	61 de	6.4 fg	1.0 d-f	53.7 f-h	20.6 bc	43.0 b-e	87 b
Yuak	3.1 b-d	0.4 f	10 g	7.1 f	3.0 a	50.0 gh	16.9 bc	45.0 a-c	87 b
PBC 371	3.0 b-d	1.717 d-f	83 cd	3.8 i	0.8 hi	63.3 def	16.8 bc	39.0 de	81 cd
Luang	3.0 b-d	0.5 f	18 fg	8.0 e	1.38 b	85.9 ab	21.0 bc	49.0 a	92 ab
Perennial ADV	2.8 b-d	4.1 bc	146 b	3.3 i	0.7 i-k	66.7 de	17.4 bc	44.0 a-d	87 b
Szechuan 8	2.3 cd	0.6 f	29 e-g	10.9 c	0.9 e-g	53.6 f-h	17.4 bc	41.0 b-e	87 b
Tiwari II	2.2 cd	1.4 f	66 de	6.5 fg	0.8 g-i	53.4 f-h	17.2 bc	37.0 e	84 cd
Kawit	2.2 d	0.6 f	27 e-g	8.8 de	1.1 d	47.3 h	17.1 bc	39.0 d-e	78 d
Mean	3.6	2.1	78	7.7	1.0	63.3	20.1	42.7	85
CV (%)	31.84	33.38	27.4	4.88	6.96	8.46	16.43	7.39	4.2

[a] Transplanted 24 January 1994 and harvested from 12 April to 30 June 1994 with seven harvests. Data are means of double-row 4.8-m² subplots distributed in RCBD and replicated three times
Means followed by a common letter are not statistically significant at 5% level (DMRT)

Bohol Agricultural Promotion Center trial

Marketable yield, yield components, and other horticultural characters of the different cultivars showed highly significant differences among entries.

Marketable yield varied from 3.3 to 15.6 t/ha for C 07664 and Szechuan 8, respectively (table 2). The difference in yield of Szechuan 8 was not significant compared to those of Yuak, IR, MI 1 and KA 6-5, but it differed significantly from the rest of the entries.

The highest number of marketable fruits harvested per hectare was recorded in KA 6-5 (5.5 M), PBC-370 (5.0 M) and MI 1 (4.2 M).

Fruit length ranged from 2.9 cm for Perennial HDV to 12.3 cm for IR and fruit width from 0.6 cm for Rotan to 3.7 cm for Yuak.

Variety IR was the earliest to flower at 14 days from transplanting and, consequently, one of the earliest to be harvested. The latest to flower (33 days) and to first harvest (73 days) were C 07664 and Luang, respectively.

Results of the INTHOPE trials show that higher yield levels were observed in Bohol Agricultural Promotion Center than at BPI-LBNCRDC. Variation in the performance of the entries may be attributed to differences in the growing environments as well as the management practices employed.

Table 2. Field performance of the INTHOPE hot pepper entries evaluated during the 1994 dry season at Bohol Agricultural Promotion Center[a]

Entry	Marketable yield (t/ha)	No. of marketable fruit/ha (million)	Fruit size (cm) Length	Fruit size (cm) Width	Days to Flower	Days to 1st harvest
Mun	6.4 cd	0.8 ef	8.5 e	1.6 c	18 c-e	59 b
Szechuan 8	16.4 ab	2.3 c-f	11.3 b	1.12 f-h	15 d-e	57 b
Yuak	13.9 ab	0.6 f	9.4 d	3.7 a	17 c-e	59 b
IR	12.6 a-c	0.7 f	12.3 a	1.4 d	14 e	59 b
PBC 473	8.0 b-d	1.4 d-f	11.4 b	1.30 de	17 c-e	59 b
MI 1	13.7 ab	4.2 a-c	8.0 f	1.05 gh	22 b-d	61 b
Pant C-1	8.9 b-d	5.479 a	5.5 i	0.89 i	19 c-e	59 b
KA 6-5	9.0 a-d	2.9 b-f	6.5 h	1.25 d-f	22 b-d	59 b
PBC 371	7.1 cd	3.2 a-e	4.2 k	1.13 eg	23 bc	59 b
Tiwari II	8.0 de	3.170 a-e	5.1 j	0.84 i	16 c-e	57 b
PSP 11	6.8 cd	2.8 b-f	5.6 i	0.98 h	17 c-e	57 b
Luang	4.2 d	0.5 f	7.4 g	1.80 b	28 ab	73 a
PBC 370	4.6 d	5.0 ab	3.3 l	0.78 i	23 bc	61 b
Rotan	7.8 b-d	3.5 a-d	11.0 c	0.64 j	22 b-d	61 b
Perennial HDV	4.7 e	3.850 a-d	2.9 m	0.79 i	22 b-d	59 b
C 07664	3.3 d	3.6 a-d	3.4 l	0.76 ij	33 a	61 b
Mean	8.26	2.8	7.2	1.25	15.23	60
CV (%)	32.95	35.32	0.85	4.7	20	3.23

[a] Transplanted 17 February 1994 in a 5.0-m² subplot and harvested from 15 April to 7 June 1994 with 14 harvests. Data are means of 5.0-m² subplots distributed in RCBD with two replications

Means followed by a common letter are not statistically significant at 5% level (DMRT)

Chinese Cabbage Preliminary Yield Trial

Summary

Twelve and 16 AVRDC lines and commercial varieties of Chinese cabbage were evaluated during the 1993-94 dry and wet seasons, respectively to determine their field performance in the lowland tropics.

Significant differences among entries in both seasons were noted in head yield, mean head weight, and harvest rate. The highest yielder during the dry season was Hybrid No. 83-20 (13.0 t/ha) and Hybrid No. 83-16 (13.7 t/ha) during the wet season. Both significantly outyielded the check Reyna Elena. Six entries, mostly hybrids, gave a higher mean head weight (414 to 484 g) compared to the check Reyna Elena (378 g) during the wet season, but the difference was not significant. Var. Tropical Quick was rated highly resistant to soft rot in both seasons.

Introduction

Imported varieties of crucifers from temperate countries are commonly grown by local vegetable farmers. In most cases, these varieties have poor heat tolerance, and some perform poorly when grown in the lowland hot tropics. After two seasons of initial screening in a nonreplicated trial, the promising commercial varieties, together with the AVRDC lines were entered in the PYT for further evaluation.

Materials and Methods

Twelve Chinese cabbage entries (5 AVRDC lines and 7 commercial varieties) were evaluated during the dry season (transplanted 10-11 March 1994) and 16 (10 AVRDC lines and 6 commercial varieties) during the wet season (transplanted 4 August 1994). Each entry was planted in 1 x 4.8 m raised subplots distributed in RCBD and replicated three times. Each subplot had two furrows set 50 cm apart. One seedling was transplanted with 40 cm between hills within rows. Two side-dressings, each with 30.0-7.5-15.0 kg/ha N, P_2O_5, and K_2O were applied 10 to 20 days after transplanting. Plots were mulched with 5 t/ha rice straw at transplanting. The plants were sprayed with contact and systemic insecticides and irrigated as needed. Data were collected as prescribed in the AVRDC procedures for Chinese cabbage evaluation trial.

Results and Discussion

Dry season trial

Marketable yield ranged from 4.9 t/ha for commercial variety Summer Harvest 45 Days to 13.0 t/ha for Hybrid No. 83-20 and with significant differences among entries (table 3). The grand mean for the 12 entries was 8.9 t/ha. The second highest yielder was Hybrid No. 85-202 (11.5 t/ha) closely followed by the check Reyna Elena (11.1 t/ha).

There were no significant differences in other yield components measured. The heaviest heads (avg. 418.5 g) were produced by Hybrid No. 85-202 closely followed by Hybrid No. 83-20 (410 g). The highest head solidity was obtained in Hybrid No. 83-20 (0.72 g/cc). The harvest rate among entries ranged from 41.7 to 95% with a mean of 74%. Soft rot infection ranged from 1.7% (Exp. 86-27, Tropical Shot, and Tropical Pride) to 17.7% (Summer Harvest 45 Days). Insect damage/pest infestation ranged from 1.7% (Tropical Pride and Hybrid No. 82-156) to 9.0% (Summer Harvest 45 Days).

Table 3. Yield performance of AVRDC Chinese cabbage lines and promising commercial varieties under the PYT during the wet and dry seasons 1994[a]

Entry	Unadjusted yield (t/ha)		Mean head wt (g)		Harvest rate (%)		Solidity (g/cc)[b]		Soft rot rating (%)		Insect rating (%)	
	DS	WS	DS	WS	DS	WS	DS	WS	DS	WS	DS	WS
Summer Harvest 45 Days	4.9 f	7.0 e-f	349.5	426.7 a	41.7 d	49.7 d	0.61	0.39	17.7	5.0	9.0	0.0
Exp. 86-27	9.8 b-d	10.4 a-f	388.4	483.8 a	76.3 a-d	68.0 a-d	0.58	0.47	1.7	15.0	3.3	1.7
Tropical Shot	9.9 b-d	7.9 d-f	310.4	297.2 b	95.0 a	80.3 a-d	0.58	0.37	1.7	7.3	8.3	0.0
Tropical Quick	7.1 d-f	9.6 b-f	282.8	328.3 b	73.3 b-d	88.0 ab	0.53	0.52	12.3	7.0	3.0	0.0
Tropical Delight	8.2 c-f	12.7 a-c	379.7	414.3 a	65.0 de	91.7 a	0.47	0.40	0.0	0.0	3.3	0.0
Tropical Pride	9.0 c-e	-	376.4	-	71.7 cd	-	0.44	-	1.7	-	1.7	-
Tropical Rapid	8.8 c-e	11.1 a-e	355.7	390.5 ab	74.7 a-d	85.3 a-c	0.47	0.39	7.0	0.0	3.0	0.0
Reyna Elena (ck)	11.1 bc	8.6 c-f	378.4	378.0 ab	88.3 a-c	68.0 a-d	0.63	0.35	5.0	13.7	0.0	6.7
Hybrid No. 83-20	13.0 a	13.5 ab	410.0	449.1 a	93.3 ab	90.0 a	0.72	0.35	3.3	0.0	0.0	0.0
Hybrid No. 82-156	8.8 c-e	11.2 a-e	349.0	370.5 ab	76.0 a-d	91.0 a	0.59	0.33	8.3	2.5	1.7	0.0
Hybrid No 85-202	11.5 b	-	418.5	-	83.3 a-d	-	0.50	-	16.7	-	-	-
Corazon (BSU-IPB)	5.3 ef	6.0 f	314.7	333.3 ab	49.0 ef	51.3 d	0.65	0.41	3.0	22.3	0.5	1.7
77M (3)-40	-	6.4 f	-	348.7 ab	-	55.3 b-d	-	-	-	9.0	0.0	9.3
77M (3)-27	-	12.0 a-d	-	391.3 ab	-	92.3 a	-	0.40	-	0.0	0.0	0.0
Hybrid No. 83-16	-	13.7 a	-	434.7 a	-	94.0 a	-	0.43	-	0.0	0.0	0.0
Hybrid No 82-157	-	11.0 a-e	-	367.8 ab	-	90.7 a	-	0.38	-	0.0	0.0	1.7
B-40	-	7.24 ef	-	384.3 ab	-	56.3 b-d	-	0.43	-	0.0	0.0	0.0
77M (2/3)-46	-	9.9 b-f	-	419.1 a	-	69.3 a-d	-	0.48	-	5.0	0.0	0.0
Grand mean	8.9	9.9	359.5	388.6	74.0	76.5	0.56	0.41	6.7	5.3	3.9	1.3
CV (%)	22.4	22.9	15.8	14.2	14.6	21.1	21.5	18.3	-	-	-	-

[a] Dry season varieties were transplanted on 10-11 March 1994 and harvested 21 April to 6 May 1994; wet season varieties were transplanted on 4 August 1994 and harvested 13 September to 4 October 1994

[b] An appropriate measure of head firmness expressed in g/cc

Note: Means in each vertical column followed by the same letter are not statistically different at 5% level (DMRT)

The poor performance of Chinese cabbage in terms of marketable yield during the trial was due to high temperature, excessive precipitation, and delayed planting during the season. The temperature ranged from 23.9°C (min.) to 32.1°C (max.), while total rainfall was 207.52 mm.

Wet season trial

There were highly significant differences among entries in marketable yield which ranged from 6.0 t/ha for Corazon to 13.7 t/ha for Hybrid No. 83-16 (table 3). The grand mean was 9.9 t/ha for the 16 entries. Hybrid No. 83-20 had the second highest yield of 13.5 t/ha.

Exp. 86-27 had the heaviest head with a mean of 483.8 g which was statistically comparable to Hybrid No. 83-20 (449.1 g), Hybrid No. 83-16 (434.7 g), 77M (2/3)46 (419.1 g), Summer Harvest 45 Days (420.7 g), and Tropical Delight (414.3 g).

Hybrid No. 82-156 had the highest head solidity of 0.52 g/cc, but it was not significantly different from the check Reyna Elena with 0.35 g/cc. Harvest rate among entries ranged from 49.7 to 94%, with a grand mean of 76.5%. There was high incidence of soft rot infection on Corazon (22.3%) and pest infestation on 77M (3)-40 (9.3%).

In general, the performance of Chinese cabbage during the off-season (wet season) was poor in terms of marketable head yield due to high temperature and excessive precipitation. The mean temperature ranged from 23.9°C (min.) and 31.5°C (max.). Total precipitation during the crop cycle was 358.14 mm.

Tomato Preliminary Yield Trial

Summary

Six F_1 fresh market indeterminate tomatoes and 10 cherry tomatoes were compared to the check Pope to determine their yield potentials and agronomic characters during the wet season. FMTT 105 an F_1 indeterminate, significantly outyielded the check, while CHT 33-7C-14-5-2, a cherry type, produced yields comparable to Pope. The performance of the entries was greatly affected by a series of typhoons at vegetative and reproductive stages.

Introduction

Preliminary yield trials on fresh market determinate and indeterminate, cherry, and, processing tomato were conducted during the wet and dry seasons. The screening for heat tolerance was given emphasis during the off-season (wet season) trial.

Materials and Methods

Six F_1 indeterminate fresh market and 10 cherry tomatoes were evaluated during the wet season (transplanted 30 May 1994). A 5-m^2 single row subplot with 12 plants per row was used for each entry. The subplots were distributed in RCBD and replicated three times. All the subplots were mulched with rice straw at about 5 t/ha. The AVRDC cultural requirements were followed in raising the crop. The indeterminate F_1s and cherry tomatoes were provided with trellis.

The indeterminate fresh market tomatoes were harvested at green-ripe stage, and the cherry tomatoes when almost all fruits in the bunch were red-ripe. Data on total and marketable yields, important horticultural characters, and reactions to pests and diseases were analyzed statistically.

Results and Discussion

FMTT 105, an F_1 indeterminate hybrid, significantly outyielded the check Pope. Total yield ranged from 2.9 to 25.2 t/ha. The rest of the indeterminate hybrids produced yields comparable to Pope. Of the 10 cherry tomatoes, three lines (CHT 111, CHT 160, and CHT 33-7C-14-5-2) gave total yields comparable to the check. The low yields of the other cherry tomato entries were due to low heat tolerance.

The small-fruited cherry tomatoes flowered early at 23 days, except for CHT 14-174C-30-2-5 which flowered the latest at 26.7 days from transplanting. First harvest was done from 61 to 67 days from field setting. The highest fruit setting score of 4.0 was recorded on CHT 33-7C-14-5-2, a cherry type. The F_1 indeterminate with higher yields had lower fruit setting than the heat-tolerant cherry tomatoes because of their bigger fruit size.

Line CHT 14-171C-20-6-0 was rated most susceptible to TMV, while Bonanza, an East/West variety was most resistant. The degree of severity of fruitworm infestation among entries varied. The least infested was CHT 14-171C-16-1-0-0 (score: 1.34) which was comparable to Pope but significantly different from the five other entries. Fruit cracking was observed on all test lines although its effect was not significant.

The general performance of the entries, particularly yield, was low because of heavy rainfall (total of 452.5 mm) and strong winds due to four typhoons and high mean temperature (31.05°C min.) in June and July at the vegetative and reproductive stages of the crop.

Tomato General Yield Trial

Summary

Eighteen determinate fresh market tomatoes were compared to the checks Pope and Maigaya during the dry season. Five entries gave yields comparable to Pope (30.9 t/ha). The most promising entries were CL 5915-223 D4-2-1-0 (30.2 t/ha), CL 5915-153 D4-3-4-0 (30.4 t/ha), and CL 143-0-10-3-1-0-1-10 (30.2 t/ha).

During the wet season, two sets of trials were conducted on determinate and indeterminate tomatoes. In set 1 with seven indeterminate fresh market test lines, only FMTT-77 (21.0 t/ha) significantly outyielded the check Pope (14.7 t/ha). In set 2, 17 determinate entries were evaluated against Pope. Only two harvests were obtained from the general yield trial (GYT) because the plants were destroyed by typhoon. However, CL 5915-93 D4-1-0-3 significantly outyielded the check and the other entries.

Introduction

Tomato lines/varieties selected from the preliminary trial were further evaluated in the general yield trial. For the determinate and indeterminate fresh market tomatoes, screening for off-season production during the wet season was given emphasis to identify entries with high yield potentials, heat tolerance, and resistance to tropical pests and diseases.

Materials and Methods

Eighteen determinate tomatoes were evaluated against the checks Pope and Maigaya during the dry season. The entries were transplanted on 23 February 1994 and harvested four times from 2 to 18 April 1994.

Two sets of trials were conducted during the wet season. In set 1, seven AVRDC F_1 indeterminate tomatoes and Bonanza from East/West Seed Co. were evaluated against Pope. The entries were transplanted on 4 May and harvested six times from 29 July to 23 August 1994. In set 2, 17 determinates were compared to Pope. Transplanting was done on 28 June and harvesting on 26 and 31 August 1994.

In both seasons, the entries were transplanted in single-row 5-m^2 subplots distributed in RCBD with four replications. Twelve plants were maintained per entry per subplot. Triple-14 fertilizer was applied basally at 1 tablespoon per hill, equivalent to 600 kg/ha. The plants were sidedressed 10 and 20 days after transplanting, with equal proportions of urea, solophos, and muriate of potash at 1 tablespoon per hill. At this rate, total NPK applied was equivalent to 168-164-324 kg/ha. The plants were sprayed at weekly intervals against pest and diseases.

Total and marketable yields, important horticultural characters, and reaction of the test entries to pests and diseases were recorded and analyzed statistically.

Results and Discussion

Dry season trial

The check Pope gave the highest total yield of 30.9 t/ha. However, five test entries had yields comparable to Pope but were significantly different from Maigaya. The promising entries were: CL

5915-223 D4-2-1-0, CL 5915-153 D4-3-4-0, CL 143-0-10-3-1-0-1-10, CLN 466 BC_1-44-7-5, and CLN 657 BC_1 F_5-206-0-0. Line CL 143-0-10-3-1-0-1-10 produced the highest marketable yield of 28.2 t/ha. Fruit size ranged from 22 to 56 g/fruit. Five entries produced fruits which were significantly bigger than the checks Pope and Maigaya. The highest fruit setting score of 5.0 was observed on CL 143-0-10-3-1-0-1-10. The earliest to flower was Pope at 18 days, and latest was Alton and CLN 466 BC1-44-7-5 at 26 days from transplanting. The peak of harvest was from 73 to 79 days from transplanting. The differences among entries were not statistically significant. Reactions to fruitworms and fruit cracking significantly differed among entries. Line CLN 452 BC_1 F_5-143-7-10 with a rating of 2.25 was the most susceptible to fruitworm, and CLN 95-280 D5-1-7-0 and CLN 475 BC_1 F_5-265-9-0 to fruit cracking.

Wet season trials

In set 1 line FMTT 77 significantly outperformed the checks and the rest of the entries in total (21.4 t/ha) and marketable (21.0 t/ha) yields. The entries varied in dates of flowering and maturity, but the differences were insignificant. The difference in fruit setting rate which varied from 2.5 to 3.0 were likewise not significant among entries. The fruit of the test entries which ranged from 42 to 82 g were significantly bigger than that of the check Pope. Four of the seven FMTT lines were significantly susceptible to TMV. Fruitworm infestation varied, but the differences were not significant.

Data on marketable yield of set 2 on determinate tomato were based on two harvests as the plants were destroyed by typhoon in September. From partial results, CL 5915-93 D4-1-0-3 with marketable yield of 3.7 t/ha significantly outyielded the check and the rest of entries. This line flowered (22 days) and matured (61.0 days) early compared to other entries. All entries had generally small fruits, ranging from 12.0 to 25.0 g/fruit. TMV infection varied significantly among entries; it was highest (3.5) on Pope and CL 5915-223 D4-2-1-0.

Tomato Regional Yield Trial

Summary

Maigaya gave the highest total yield of 32.5 t/ha. Significant differences on various parameters were observed.

Introduction

RYT on vegetables is conducted simultaneously in 12 stations strategically located in various parts of the country. Its primary objective is to identify lines/hybrids adapted to the different regions. Results from four season tests are used as basis for regional/national recommendations of new varieties.

The report covers only the results of the RYT on tomato conducted at the POP in Los Baños, Laguna.

Materials and Methods

Nine test entries were evaluated against the check Maigaya during the dry and wet seasons. The test entries were developed/selected by the following institutions: four open-pollinated lines from the Department of Agriculture (DA)-Dalwangan Seed Farm; two F_1 hybrids from the Institute of Plant

Breeding (IPB, UPLB); and three open-pollinated lines from AVRDC-POP. During the dry season, the entries were transplanted on 16 February and harvested six times from 11 April to 16 May 1994.

For the wet season, two trials were conducted. The first trial was transplanted 14 June and the second on 16 August 1994. Both trials were destroyed by continuous rains due to a series of typhoons.

RYT was conducted following standard national procedure for regional trial. The entries were distributed in RCBD with four replications, using four-row plots. The rows were 5 m long and set 1 m apart. Twelve plants were transplanted per row. Experimental data were taken from the two inner rows of each subplot with an area of 10 m^2. Data on yield were based on total and marketable yields. Other horticultural traits of the entries were also recorded.

Results and Discussion

There were significant differences among entries on all parameters measured (table 4).

Consistent patterns on fruit yield among entries were noted. The check Maigaya produced the highest marketable yield of 28.9 t/ha. Six other test entries with yields ranging from 20.8 to 26.2 t/ha were comparable to Maigaya. The best was CL 5915-93 D4-1-0-C-1.

CL 5915 93 D4-1-0-C-1 had the biggest fruit (54.5 g) and Maigaya the smallest (17.0 g). A high fruit setting rate of of 5.0 was recorded on Maigaya and AVR 035 which was not significantly different from three other test entries, including CL 5915 93 D4-1-0-C. Maturity ranged from 66 to 86 days from transplanting, with Maigaya maturing the earliest. The most susceptible entries to fruit cracking were CL 5915-204 (G) D4-0 and DSF-82352; AVR-035 and CL 5915 93 D4-1-0-C-1 were susceptible to fruitworm.

Of the nine test entries, CL 5915 93-D4-1-0-C-1 was the most promising. Its marketable yield and fruit setting rate are similar to the check Maigaya. Its fruit is much bigger and more resistant to cracking than that of Maigaya. Its only limitation is its susceptibility to fruitworm.

Table 4. Potential and actual yields and horticultural traits of tomato entries under the regional yield trial, DS 1993-94[a]

Entry	Yield (t/ha) Total	Yield (t/ha) Marketable	Fruit size (g)	Fruit-set rating	Days to Flowering	Days to Maturity[b]	Rating[c] Cracking	Rating[c] FW
DSF-8234	24.3 a-d	22.5 a-c	32.0 bc	3.25 c	21.0 f	76 bc	3.00 bc	2.25 c
DSF-8505	22.9 b-d	20.8 a-c	33.5 b	3.00 c	22.5 e	76 bc	3.00 bc	2.50 c
DSF-82352	19.1 d	16.7 c	39.0 b	2.75 c	23.0 de	86 a	4.00 a	2.25 c
DSF-82353	19.3 cd	18.0 bc	30.7 bc	3.25 c	23.0 de	81 ab	2.25 c	2.25 c
AVR-035	25.3 a-d	22.6 a-c	38.2 b	5.00 a	27.0 a	76 bc	2.25 c	3.50 a
AVR-039	24.8 a-d	22.1 a-c	39.5 b	4.00 b	24.0 cd	72 c	2.50 c	3.25 ab
CL 5915-93 D4-1-0-C-1	28.8 ab	26.2 ab	54.5 a	4.25 ab	24.5 bc	74 c	2.25 c	3.50 a
CL5915-204 (G) D4-0	28.5 a-c	24.4 a-c	24.0 cd	4.75 ab	22.0 ef	71 cd	4.00 a	2.75 bc
EG Alton	26.4 a-d	24.8 a-c	30.5 bc	4.50 a	25.0 b	83 a	3.00 c	2.75 bc
Maigaya (ck)	32.5 a	28.9 a	17.0 d	5.00 a	19.0 g	66 d	3.50 ab	2.25 c
Grand mean	25.19	22.69	33.9	3.97	23.05	76.23	2.89	2.72
CV (%)	22.32	22.39	15.68	12.13	2.95	5.10	21.35	16.68

[a] Transplanted 16 February and harvested six times from 11 April to 16 May 1994; data are means of 10-m^2 subplots distributed in RCBD and replicated four times

[b] Peak of harvest

[c] Rating scale of 1 = none to 5 = severe; Cr = fruit cracking and Fw = fruitworm

Note: Means followed by the same letter in each columm are not statistically different at 5% level (DMRT)

Mungbean Preliminary Yield Trial

Summary

The study was conducted to identify mungbean lines with high bean yield potentials, desirable agronomic characters, and disease resistance, particularly to Cercospora leaf spot (CLS) and powdery mildew (PM).

A total of 44 entries were evaluated in two sets of trials during the crop year 1993-94. For the dry season planting, the grand mean bean yield for both sets of trials was generally low due to adverse soil allelopathy and climatic conditions. Severe soil moisture stress prevailed during the entire crop season. During the wet season, VC 4180-B-5, VC 4169-B-5, and VC 4211-2 outyielded the check Pag-asa 7, but the difference was not statistically significant. Negligible infections of CLS and virus diseases were noted during the season.

Introduction

PYT is the first series of field trials conducted on lines and hybrids that have attained homozygosity. It aims to identify lines adapted to local agroclimatic conditions, with high bean yield potentials, early and uniform maturity, resistance to common pests and diseases, tolerance to lodging, and with acceptable eating and processing qualities. After two to four seasons of PYTs, the most promising lines are elevated to GYT.

Materials and Methods

For 1993-94, two sets of trials were evaluated. Set 1, with 20 entries was planted during the dry (10 November 1993) and wet (4 July 1994) seasons. Set 2 with 24 entries was planted during the dry season (10 November 1993). In all trials, the entries were sown in two-row, 5-m-long subplots distributed in RCBD and replicated three times. The rows were spaced 0.6 m apart.

Cultural management and data collection were based on the standard procedure suggested in the AVRDC International Cooperator's Guide for Mungbean Evaluation Trial.

Results and Discussion

In set 1, the dry season planting produced very low mean bean yield of 167.8 kg/ha. The poor performance of the entries may be attributed to soil allelopathy and moisture stress problems.

For the wet season, no significant differences in bean yield were observed among the entries. The yield ranged from 686.3 kg/ha for VC 4169-B-2 to 1047 kg/ha for VC 4180-B-5 with a grand mean of 890.15 kg/ha. Three entries, VC 4180-B-5 (1047 kg/ha), VC 4169-B-5 (1043.3 kg/ha), and VC 4211-2 (1041.7 kg/ha) outyielded the check Pag-asa 7 (981.7 kg/ha).

VC 4297-1-B-5 and VC 4180-B-9 produced the biggest seeds of 6.9 g/100 seeds which was statistically significant compared to the checks Pag-asa 7 (6.3 g) and BPI-Mg9 (6.5 g). All the entries flowered between 36 to 39 days and matured in 57 to 59 days from planting. Negligible symptoms of CLS and virus diseases were observed during the cropping season.

In set 2, the 24 mungbean entries evaluated during the dry season performed poorly due to adverse soil and climatic conditions. The data from the trial were not validated. The test entries were not evaluated during the wet season.

Mungbean General Yield Trial

Summary

The study was conducted to evaluate selected promising lines from the PYT for yield potentials, adaptability to local agroclimatic conditions, and resistance to common pests and diseases.

Twenty-eight entries were evaluated in two sets of trials during the crop year 1993-94.

In set 1, the mean bean yield was 410.7 kg/ha. The highest yielding entry, VC 3995-B-5, with 641.9 kg/ha had a yield comparable to the check BPI-Mg9 (522.5 kg/ha). No incidence of CLS and virus diseases was recorded.

For set 2, three test entries significantly outyielded the check BPI-Mg9. The mean bean yield was 856 kg/ha. Significant differences were also observed among the entries on other yield parameters evaluated. The entries were resistant to moderately resistant to CLS and virus infections.

Introduction

Selected materials from PYT were further evaluated under GYT for yield, adaptability to local agroclimatic conditions, and reactions to common pests and diseases. Results obtained from this trial served as bases for further evaluation of promising entries in RYT or the National Cooperative Trial before possible varietal release for commercial production.

Materials and Methods

Twenty-eight entries were evaluated in two sets of trials during the crop year 1993-94. Set 1 with 14 entries was planted during the dry season (20 January 1994).

Set 2 with 14 entries was planted during the dry (10 November 1993) and wet seasons (4 July 1994). A 4-row plot, 5 m long with three replications was used in both sets of trials.

AVRDC-suggested cultural management practices and data collection procedure were used.

Results and Discussion

For set 1, a mean bean yield of 410.7 kg/ha was recorded. The yield ranged from 280 kg/ha for the check Pag-asa 7 to 641.9 kg/ha for VC 3995-B-5. Only VC 3995-B-5 outyielded the check BPI-Mg9 but the difference was not significant. Both VC 3995-B-5 and VC 4509 A had the heaviest 100-seed weights of 6.0 g. All the entries flowered in 34 to 35 days and matured in 52 to 53 days from planting. No incidence of CLS and virus infections was recorded.

During the wet season, the same set of entries was planted in a nonreplicated plot for seed increase. Since the entries already completed the four-season trial, the promising lines/accessions will be selected for inclusion in the RYT.

For set 2, there were significant differences in bean yield, 100-seed weight, days to maturity, plant height, pod per plant, seeds per pod, and CLS and virus ratings among the 14 entries during the wet season.

Bean yields ranged from 484.8 kg/ha for VC 4140-B-3 to 1178.8 kg/ha for VC 4195-B-5, with a grand mean of 856 kg/ha. VC 4195-B-5, VC 4195-B-2, and VC 4140-B-1, significantly outyielded the check BPI-Mg9.

Entries VC 4150-B-2, VC 4195-B-6, and Pag-asa 7 had the biggest seeds (6.3 g/100 seeds). The entries flowered in 37 to 39 days and matured in 58 to 60 days from planting. The entries were resistant to moderately resistant to CLS and virus infections.

Mungbean Regional Yield Trial

Summary

RYT was conducted to evaluate the performance of promising lines/varieties in eight to nine locations in bean yield potentials and reactions to major pests and diseases.

During the dry season, the crop performed poorly due to soil allelopathy and moisture stress problems. The grand mean bean yield was only 132 kg/ha, hence, the trial was invalidated.

For the wet season, a grand mean yield of 934.59 kg/ha was recorded. Four test entries (EGM 3876, EGM 2764 (Y), IPBM 85-4-11, and IPBM 83-33-20) outyielded the check MG50-10A. Significant differences were observed among the entries on almost all the yield parameters measured.

Introduction

RYT for mungbean was undertaken in cooperation with IPB, BPI, and other institutions involved in the varietal improvement of field legumes. Eight to nine cooperators participated in the evaluation of the performance of the promising lines/accessions. Results obtained from the RYT served as the basis for recommendation/ approval by the National Seed Industry Council (formerly Philippine Seedboard) of new varieties for commercial production.

Materials and Methods

Selected promising lines from either PYT or GYT were submitted to the National Legume Technical Committee of the National Seed Industry Council for inclusion in RYT. The entries were evaluated for four to six seasons in different cooperating stations. Entries that consistently equaled or outperformed the checks are recommended for release either regionally or nationally.

For 1993-94, 10 entries were evaluated during the dry season and eight during the wet season. In both season trials, each entry was planted in 5-m-long four-row subplots spaced 0.6 m apart and distributed in RCBD replicated four times.

The standard cultural management and procedure in evaluating the entries were based on the Guidelines of the Upland Crop National Cooperative Trial (UCNCT) of the Philippine Seedboard.

Results and Discussion

The results of the wet season trial are presented in table 5. There were significant differences among entries in bean yield, seed weight, flowering, maturity, plant height, and number of pods per plant.

The highest yielding entries, EGM 3876 (1181 kg/ha) and EGM 2764 (Y) (1117.5 kg/ha), were comparable to the checks MG50-10A (873 kg/ha) and BPI-Mg9 (869 kg/ha). Line EGM 3902 produced the biggest seeds with 7.5 g/100 seeds which was significantly different from the checks MG50-10A and BPI-Mg9 with 6.3 and 6.4 g/100 seeds, respectively. Among entries, maturity ranged

from 54 to 60 days from planting, plant height from 47 to 59 cm, and pods per plant from 7 to 9. Majority of the entries produced 10 seeds per pod and were resistant to moderately resistant to CLS and virus diseases.

Table 5. Field performance of the eight mungbean entries under RYT during the wet season 1994 conducted at BPI-LBNCRDC[a]

Entry	Yield (kg/ha)	100-seed wt (g)	Days to Flowering	Days to Maturity	Plant ht (cm)	Pod/ plant	Seeds/ pod	Plants harvested 6 m²	Disease rating[b] CLS	Disease rating[b] Virus
EGM 3876	1181.3 a	6.4 b	37 ab	58 b	58.9 a	9 a	10	146	1.3	1.0
EGM 2764 (Y)	1117.5 ab	5.4 d	35 c	54 e	57.8 ab	7 b	10	180	1.8	1.0
IPBM 85-4-11	897.0 bc	5.9 b-d	38 a	58 b	57.4 ab	8 ab	10	140	1.0	1.0
IPBM 83-33-20	885.0 bc	5.8 cd	38 a	60 a	54.2 b	7 b	10	165	1.3	1.0
MG50-10A (R. ck)	873.0 c	6.3 bc	36 bc	55 de	57.8 ab	9 a	10	132	1.	1.0
BPI-Mg9 (N ck)	868.8 c	6.4 b	36bc	56 cd	50.1c	9a	10	120	1.8	1.0
EGM 3902	837.0 c	7.5 a	37 ab	57 bc	49.0 c	8 ab	9	123	1.5	1.0
IPBM83-80-26	817.3 c	5.4 d	36 bc	55 de	47.2 c	8 ab	10	133	1.8	1.0
Grand mean	934.59	6.12	36.5	56.56	54.06	7.93	9.65	142.19	1.47	1.0
CV (%)	10.76	3.7	2.3	1.89	4.58	13.72	4.56	12.27	30.26	-

[a] Planted 4 July 1994. Data are means of 6-m² subplots, distributed in RCBD and replicated four times
[b] Rated on a scale of 1 to 5, where 1 = highly resistant and 5 = highly susceptible
Note: Means followed by the same letter are not significantly different at 5% level (DMRT)

Soybean Preliminary Yield Trial

Summary

The study aimed to evaluate and identify lines with high bean yield potentials, less sensitivity to photoperiodism, resistance to common pests and diseases, and other desirable characters.

Twenty promising accessions/lines from AVRDC and other sources were evaluated in the PYT during the dry and wet seasons of 1993-94.

During the dry season, only lines TGX 239-250-13 and G-90086 with bean yields of 1.9 and 1.7 t/ha, respectively, outperformed the check UPL-Sy4 with 1.7 t/ha, but the difference was not statistically significant. Seed weight, plant height, and maturity were significantly different among entries.

The wet season trial was planted late due to continuous heavy rains and typhoons.

Introduction

Promising selections from the AVRDC advanced soybean lines were evaluated in the PYT during the dry and wet seasons to identify lines/accessions with high bean yield potentials, resistance to common pests and diseases and other desirable agronomic characters.

Materials and Methods

Twenty test entries were evaluated against the standard checks BPI-Sy4 and UPL-Sy4 during the dry and wet seasons. Plantings were made on 17 January 1994 for the dry season and on 16 August 1994 for the wet season. In both trials, the entries were drilled in two-row, 5-m² subplots distributed

in RCBD and replicated three times. Complete fertilizer at 30-30-30 kg/ha was applied at planting. The AVRDC procedure for evaluating soybean was followed.

Results and Discussion

During the dry season, lines TGX 239-250-13 (1.9 t/ha) and G 90086 (1.7 t/ha) outyielded the check UPL-Sy4 (1.7 t/ha), but the differences were not statistically significant. The bean yields of the 20 entries ranged from 1.1 to 1.9 t/ha, with a grand mean of 1.4 t/ha. Significant differences among entries on 100-seed weight, days to flowering and maturity, and plant height at maturity were noted. The entries matured in 74 to 82 days from planting. All entries were resistant to soybean rust and moderately susceptible to mite infestation.

The relatively small seed size of the entries, their early maturity, and moderately high infestation of mites were attributed to the drought condition from pod-filling stage to harvest.

Soybean General Yield Trial

Summary

Selected lines from PYT were evaluated in GYT to further verify their yield potentials, resistance to common pests and diseases, and to identify their desirable agronomic characters.

Of the 16 entries evaluated during the dry season, only Jupiter R, SJ 4, and GC 60017-2-2 gave comparable yields as the check UPL-Sy4 with a bean yield of 2.5 t/ha.

Introduction

Selected promising lines from PYT were further tested for bean yield potentials and reactions to major pests and diseases, and to identify other desirable agronomic characters. The selections from the GYT were finally evaluated in RYT for regional and/or national recommendation.

Materials and Methods

Sixteen test entries were evaluated against standard checks BPI-Sy4 and IPB-Sy4 during the dry and wet seasons. The dry season trial was planted on 17 January 1994 and wet season trial on 16 August 1994. In both trials, the entries were sown in four-row, 10-m^2 subplots distributed in RCBD and replicated three times. Complete fertilizer of 30-30-30 kg/ha of N, P_2O_5, and K_2O was applied at planting. Cultural management and data collection were based on the AVRDC International Cooperator's Guide for Soybean Evaluation Trial.

Results and Discussion

Of the 16 test entries evaluated during the dry season, only Jupiter R, SJ 4, and GC 60017-2-2 had comparable yields as the highest yielding check UPL-Sy4, with a bean yield of 2.5 t/ha and a grand mean yield of 1.6 t/ha. All entries were resistant to moderately resistant to mites and susceptible to soybean rust.

Soybean Regional Yield Trial

Summary

Eleven and eight entries (including the national and regional checks) were evaluated in RYT during the dry and wet seasons, respectively. The study aimed to verify the performance of promising strains/varieties in the different regions of the Philippines in bean yield potentials, reactions to major pests and diseases, and other desirable agronomic characters.

Results from the BPI-LBNCRDC during the dry season showed that five of the test entries outyielded the check BPI-Sy4, but only IPB-Sy 201-013 and EGSy 93-62 showed significant differences.

Introduction

RYT for soybean was conducted in cooperation with IPB and other institutions involved in the legume varietal improvement program. Coordinated by the Field Legume Technical Working Group of the National Seed Industry Council, promising entries from various national breeding programs were assembled and evaluated for four seasons in 8 to 12 cooperating stations using common standard procedures. The study was designed to identify high-yielding varieties with desirable agronomic attributes adapted to the different regions of the country. Recently, to regionalize varietal recommendation, promising entries in RYT in a given region were further evaluated for two seasons in the technology adaptation trial before a variety was released in the regions.

Materials and Methods

Eleven and eight entries, including the national and regional checks were evaluated in eight to nine cooperating stations during the dry and wet seasons of 1993-94. In both seasons, each entry was planted in four-row, 10-m^2 subplots, distributed in RCBD and replicated four times. The standard procedures for evaluating soybean prescribed in UCNCT of the Philippine Seedboard was followed by all cooperating stations.

Results and Discussion

Out of 11 entries evaluated during the dry season, five outyielded the check BPI-Sy4 with a bean yield of 2.1 t/ha (table 6). However, only the yields of lines IPB-Sy 201-013 and EGSy 93-62 were significant over that of the check. Significant differences in 100-seed weight, days to flowering and maturity, plant height, and number of pods per plant among entries were noted. All entries were resistant to moderately resistant to soybean rust and mites.

Table 6. Mean bean yield and other agronomical characters of soybean lines under RYT during the dry season, 1993-94[a]

Entry	Bean yield (t/ha)	100-seed weight (g)	Days to Flowering	Days to Maturity	Plant height at maturity (cm)	Pods/ plant	Disease/pest rating[b] rust	Disease/pest rating[b] mites
IPB Sy 201-013	3.9 a	13.2 a	33	82 a	42.7 de	39	2.0	1.9
EGSy 93-62	3.5 a	13.6 a	32	80 b	50.5 cd	31	2.2	1.6
LGSy 12-12	2.4 b	12.0 b	30	75 d	37.0 e	39	2.8	2.0
EGSy 93-147	2.3 bc	11.3 bc	29	75 d	35.4 e	35	2.2	2.0
LGSy 5-8	2.2 bc	10.9 c	20	75 d	41.6 de	32	2.5	2.1
BPI-Sy4 (R ck)	2.1 b-d	11.4 bc	31	76 d	39.0 e	40	2.8	2.5
EGSy 93-18-7	2.1 b-d	14.0 a	30	73 e	38.5 e	38	3.2	2.0
IPB Sy 85-16-8	1.9 b-d	9.4 d	31	80 b	55.8 bc	28	2.2	1.6
PSB Sy1 (N ck)	1.8 cd	11.0 bc	29	80 b	39.4 e	28	2.5	1.9
IPB Sy 85-17-19	1.8 cd	9.1 d	36	78 c	65.1 ab	32	2.2	1.9
IPB Sy 85-3-11	1.7 d	9.7 d	40	82 a	70.6 a	26	2.2	1.8
Grand mean	2.3	11.4	32	78	46.9	33	2.5	1.9
CV (%)	13.47	5.45	0.70	0.80	13.89	-	14.93	10.8

[a] Dry season planted 17 January 1994; data are means of 5-m² subplots, distributed in RCBD with four replications

[b] Rated on a scale of 1 to 5, with 1 = highly resistant and 5 = highly susceptible

Note: Means followed by the same letter are not significantly different at 5% level (DMRT)

Pilot Testing of Vegetable Soybean

Summary

Six selected vegetable soybean lines were pilot tested in Los Baños, Laguna and Lipa, Batangas during the dry season and in seven test sites during the wet season of 1993-94. The study was designed to evaluate the performance of the lines in the different regions of the country; to encourage the widespread use of vegetable soybean as snack and as vegetable; and to promote its cultivation, especially by small farmers.

Of the six lines pilot tested, AGS 190 and AGS 191 were most promising in fresh pod yield, seed size, and eating qualities. The two lines produced mean number of graded pods per 500 g within the international standard during the dry season. This showed the importance of irrigation in the production of vegetable soybean. The mean pod length and pod width of AGS 190 and AGS 191 were within acceptable international standard.

Inspite of the promising qualities of AGS 191 as a vegetable soybean, its limitation was its short plant stature and short seed storage life.

Introduction

Vegetable soybean is a rich and cheap source of vitamins, minerals, protein, energy, and fiber. It is a very versatile crop that fits well into cropping systems, has soil-enriching properties, and high income value for small farmers. However, in spite of these advantages, the crop is still relatively unknown to most farmers in the country.

Because of its fine taste, high nutritional value, and export potential, vegetable soybean has been included as a priority crop in the nutrition and production programs of most Department of Agriculture regional offices. In support of this program, POP evaluated AVRDC vegetable soybean accessions and selected the six most promising lines adapted to local agroclimatic conditions. To promote this new crop to the farmers, a two-year pilot testing on the promising lines has been conducted with the station in cooperation with the DA regional offices.

Materials and Methods

The six vegetable soybean lines selected from PYT and found adapted to local conditions were: AGS 186, AGS 190, AGS 191, G 10478, G 10500, and G 10503.

The lines were evaluated during the wet and dry seasons by the DA cooperators from different regions. In each trial, the entries were sown in four-row 6-m-long subplots distributed in RCBD and replicated four times. The rows were spaced 50 cm apart during the dry season and 60 cm during the wet season. The plants were thinned to 5 cm within the row in both seasons. The seeds were inoculated with rhizobium bacteria at planting. Triple-14 complete fertilizer was applied basally at 200 kg/ha and side-dressed twice with 100 kg/ha each of Triple-14 at 25 and 40 days from planting. Standard cultural practices were observed in raising the crop.

Data were recorded for days to emergence, flowering and maturity (fresh pods), plant height, number of nodes and branches per plant, plant stand at harvest, fresh pod yield, fresh 100-seed weight, fresh pod width and length, and number of graded two- and three-seeded pods per 500 g. The entries were also rated for reaction to common pests and diseases.

Results

Dry season

The dry season trial was conducted in Los Baños, Laguna and in Lipa, Batangas.

Fresh pod yield. The data obtained on fresh pod yield were significantly different between entries in Lipa but not in Los Baños. However, in both sites, AGS 191 showed the highest mean yield (7.2 t/ha in Los Baños and 9.9 t/ha in Lipa) or an average of 8.6 t/ha. This was followed by AGS 190 with 7.4 t/ha and AGS 186 with 7.1 t/ha from the two sites.

Fresh 100-seed weight. There were significant differences among entries in 100-seed weight in both test sites. AGS 191 had the largest seed size of 57.70 g/100 seeds, closely followed by AGS 190 with 56.25 g and AGS 187 with 49.05 g.

Number of graded pods per 500 g. The entries varied significantly in the number of graded pods per 500 g in both locations. Entries AGS 191 (210) and AGS 190 (216) had the least number of graded pods per 500 g. They were the biggest podded entries evaluated for vegetable soybean.

Length and width of two-seeded pods. The length of two-seeded pods also varied significantly among entries in the two test sites. The entries with the longest pod were AGS 191 (5.0 cm) and AGS 190 (4.9 cm). Pod width ranged from 1.2 to 1.3 cm.

Maturity. Mean maturity of the AGS lines was about 70 days and G lines about 61 days from planting. Those planted in Los Baños matured 4 to 6 days earlier than those planted in Lipa during the dry season.

Pest and disease ratings. All entries were affected by soybean rust. Leaffolder and mite infestations were light.

Wet season

During the wet season, the pilot trial was conducted in seven locations. However, only the reports from Los Baños, Lipa, and Tiaong have been completed and are reported.

There were significant differences among entries on fresh pod yield, fresh 100-seed weight, number of graded pods per 500 g, length and width of two-seeded pods, plant height, and bacterial pustule rating from the three test sites.

Fresh pod yield. AGS 191 significantly outperformed all entries in the three test sites with an overall mean of 18.8 t/ha. This was followed by AGS 190 with a grand mean of 11.0 t/ha, but AGS-190 was comparable to other entries in the two test sites.

Fresh 100-seed weight. Entries AGS 191 with an over-all mean of 69.71 and AGS-190, with 68.66 g/100 seeds were comparable , but significantly outperformed the other entries in the three test sites.

Number of graded pods per 500 g. The best entries were AGS 191 and AGS 190 with the least number of graded pods per 500 g of 169 and 174, respectively.

Length and width of two-seeded pods. AGS 191 had the longest pod length from the three test sites with a mean of 5.25 cm, followed by AGS 190 with 5.2 cm. AGS 191 and AGS 190 had the widest pod (1.4 cm).

Maturity. For fresh pod production, all the AGS lines were ready for harvest in 77 days and the G lines in 66 days from planting under local conditions.

Plant height at maturity. The tallest among the entries was AGS 187 with a mean plant height of 57.8 cm followed by AGS 190 with 42.6 cm. Plant stature of the rest of the entries ranged from 34 to 35 cm.

Disease rating. All entries were moderately susceptible to soybean rust infection, except for G 10503 which was rated moderately resistant.

On bacterial pustule, the least infected entries (moderately resistant) were AGS 190 and AGS 191, while those with high infection ratings (moderately susceptible) were G 10478 and G 10503.

Discussion

Results of the two season trial in three test sites showed that AGS 191 and AGS 190 were the most promising vegetable soybean lines in fresh pod yield, seed size, and eating qualities. The two lines consistently outyielded the other entries in all test sites in both seasons.

As in other vegetable crops, however, the availability of soil water, especially at flowering and pod development stages is most important in the production of vegetable soybean to meet international standards. During the wet season, only AGS 190 and AGS 191, with means of 174 and 169 graded pods per 500 g, respectively, met the international standard maximum requirement of 175 graded pods per 500 g. The mean pod length and width of AGS 190 and AGS 191 were within acceptable international standards. However, during the dry season, the pod size produced by all test entries were below standard requirements for vegetable soybean. This was due to insufficient soil moisture, especially at pod formation and development stages.

Inspite of the promising qualities of AGS 191, its plant height averaged only 28 and 34 cm during the dry and wet season, respectively. Because of its bigger seed, AGS 191 had shorter seed storage life than other entries, thus producing poor plant stand in the field. Solutions to these problems may be formulated through proper irrigation and seed processing and storage studies.

Further piloting trials on vegetable soybean in more test sites will be conducted during the next two crop seasons for more conclusive results.

Sweet Potato Germplasm Collection and Maintenance

Summary

Seventy-two sweet potato accessions from AVRDC and from local sources and 18 from the International Potato Center (CIP) were maintained and propagated in nursery plots. These accessions were evaluated at POP and most were made available to different institutions for research purposes.

Introduction

In breeding, the source of germplasm is an important criterion to select for the best varieties for commercial production. With the increasing demand for sweet potato as food and feed, it is imperative that a germplasm collection of sweet potato varieties and accessions from AVRDC and local sources be maintained to continuously propagate sufficient planting materials for research purposes.

This study was therefore conducted to: (1) collect and maintain sweet potato varieties and accessions; (2) propagate sufficient planting materials for research purposes; and (3) make available planting materials to other researchers and growers.

Materials and Methods

Several entries from AVRDC and from local sources were maintained in 12-inch-diam clay pots and kept in the greenhouse. When enough planting materials were propagated, each accession was further multiplied in 6-m^2 plots under field conditions.

Results and Discussion

A total of 72 accessions are now being maintained in the station. Most of the propagated materials from these accessions are given free to different institutions for research purposes. Moreover, new entries have been received from CIP for multiplication.

Sweet Potato Preliminary Yield Trial

Summary

Eleven entries of AVRDC sweet potato accessions/lines were evaluated against BPI-Sp2 during the wet and dry seasons to identify high-yielding lines and other desirable agronomic characters.

No yields were taken during the wet season, but scab rating revealed that CN 1517-142 was moderately susceptible to the disease. During the dry season trial, no entries significantly surpassed the yield of check variety BPI-Sp2. CN 942-47 produced the longest root of 15.4 cm.

Introduction

Sweet potato [*Ipomoea batatas* L. (Lam)] is an important crop with various uses. Thus, a continuous supply of the storage roots to meet the demand for processing and utilization must be maintained. In sweet potato, evaluation is necessary to minimize genetic variability and attain stable yields in promising clones. This study, therefore, aims to identify the most promising sweet potato clones in root yield potential and reaction to diseases.

Materials and Methods

Eleven sweet potato accessions were evaluated against BPI-Sp2 (check variety) on 4 August 1993 and 17 November 1993, for wet and dry season trials, respectively. Each entry was planted in double-row 6-m^2 subplots distributed in RCBD in three replications. Planting distance was set at 50 x 25 cm between rows and hills, respectively. The cultural management practices (hilling-up, weeding, fertilizer rate application, pest control) and the data collection procedure employed were according to the standard AVRDC procedure on sweet potato trials.

Results and Discussion

The wet season trial was a failure and no roots were harvested. Harvesting of the roots coincided with intermittent heavy rains which resulted in rotting of the fleshy roots.

Scab rating during this trial was also taken. Seven entries, CN 1423-170, CN 941-32, CN 1489-89, Miracle, CN 942-47, Bicol variety, and BPI-Sp2 (check variety), showed some degree of resistance to sweet potato scab with a rating of 1 (no symptom of disease on foliage and stem). CN 1517-142 was moderately susceptible to scab.

During the dry season trial, 11 lines were evaluated. No accession outyielded the check variety (BPI-Sp2). CN 942-47, which has a low yield had the longest root (15.4 cm).

Sweet Potato General Yield Trial

Summary

The marketable yields during the dry season were generally low due to drought. CN 1309-18 produced the highest marketable yield of 10.0 t/ha and the widest root of 5.0 cm, but the difference from those of the check BPI-Sp2 was not significant.

During the 1994 wet season trial, CN 1229-2 produced the highest marketable root yield of 17.5 t/ha, which did not significantly differ from that of BPI-Sp2, with marketable yield of 14.7 t/ha. Moreover, all entries, except CN 1229-14, showed some degree of resistance to scab. CN 1309-18 was infected with virus.

Introduction

Sweet potato can be grown throughout the year even in marginal areas. Due to the growing demand for the crop in the domestic market, traditional varieties need to be improved. Therefore, promising AVRDC entries were evaluated to reduce wide genetic variation and to identify clones which are high yielding, stable, and resistant to pests and diseases.

Materials and Methods

Eleven sweet potato accessions which are promising from the previous PYT were evaluated for two wet seasons (planted on 4 August 1993 and 11 May 1994) and one dry season (planted on 25 January 1994 and harvested on 17 May 1994). In these three trials, all entries were planted in double-row 6-m^2 subplots, distributed in RCBD in four replications. Planting distance was 50 x 25 cm between rows and hills, respectively. All cultural management and yield parameters were based on AVRDC recommendations.

Results and Discussion

The mean marketable yield for all entries was low at 4.2 t/ha during the dry season trial. Only CN 1309-18, with a marketable yield of 10.0 t/ha, exceeded the yield of the check BPI-Sp2 (7.3 t/ha) but the difference was not significant. CN 1229-14 had the lowest yield of 1.2 t/ha. The low yield was attributed to unexpected drought during the cropping season.

CN 1346-9 (11.9 cm), CN 1229-16 (11.6 cm), and CN 1216-10 (10.7 cm) significantly exceeded the root length of the check variety (9.0 cm). On the other hand, only CN 1309-18 produced the widest root (5.0 cm), which is a contributing factor to its high-yielding ability.

During the wet season trial on 4 August 1993, roots were not harvested due to continuous rain. This condition resulted in rotting of roots in the field.

The marketable root yield of sweet potato during the wet season trial ranged from 5.2 to 17.5 t/ha. CN 1229-2, the highest yielder, surpassed the check variety BPI-Sp2, but no significant difference was observed. Two entries, CN 1346-9 and CN 1216-10, significantly produced the longest roots of 14.0 and 14.2 cm, respectively, compared to BPI-Sp2 (check variety) with 12.1 cm. However, no entries exceeded the root diameter of the check variety (6.1 cm).

The scab disease rating was also taken. All entries, except for CN 1229-14, showed some degree of resistance to the disease. CN 1309-18, a moderate yielder, was infected with an unidentified virus disease.

ROC – AVRDC Cooperative Program

Regional Yield Trials

Summary

In 1993-94, 53 regional yield trials (RYTs) were conducted in cooperation with the Republic of China (ROC) national program. Promising AVRDC lines of soybean, vegetable soybean, mungbean, and cherry tomato were evaluated.

Among the 10 soybean entries evaluated at AVRDC, four lines, i.e., IRRI 12, HL77-79, TN 4, and KS 2164 outyielded the check entries and were stable and promising. Yields ranged from 2.6 to 4.2 t/ha. TN 4 also had larger grain weight of 26.8 g/100 seeds. Results of vegetable soybean trials showed that GC 84126-13-1-2 and GC 84126-13-4-4 both produced 7.1 t/ha which was the highest yield in spring 1994. In mungbean RYTs, VC 3738B produced the highest yield of 1.8 t/ha in autumn 1993. Yields of VC 3737A, VC 3907A, VC 4152B, VC 4386A, and VC 4442A were comparable to that of the check Tainan No. 5.

Cherry tomato CHT 261 and CHT 264 performed well in terms of yield and soluble solids (°Brix). The entries of cherry tomato RYTs were changed in 1994 because of the popularity of Santa, a small and long-fruit variety in Taiwan. CHT 151, CHT 154, and CHT 155 were superior compared to Santa in yield. However, Santa still had the highest soluble solids content.

Introduction

RYTs were conducted to evaluate promising AVRDC lines/varieties in the field under different seasons and locations. At AVRDC headquarters, RYTs were organized in cooperation with the national program of ROC. They were supported by the ROC Council of Agriculture and conducted by AVRDC in cooperation with various District Agricultural Improvement Stations (DAIS) and the Taiwan Agricultural Research Institute.

The purpose of RYTs is to identify promising vegetable varieties for release to farmers. In past years, 12 AVRDC vegetable varieties have been named and released by national programs in Taiwan. They have significantly contributed to farmers' incomes. The performance of some AVRDC-released vegetables in Taiwan in 1993 is given in table 1. Among them, vegetable soybean Kaohsiung No. 1 comprised 91% of total vegetable soybean growing areas. Soybean Kaohsiung No. 10 and mungbean Tainan No. 5 also occupied more than 75% of the total growing areas for these crops.

In 1993-94, four crops, i.e., soybean, vegetable soybean, mungbean, and cherry tomato, were included in AVRDC RYTs. A total of 53 RYTs were carried out under different crop seasons and locations.

Table 1. Performance of released AVRDC vegetable varieties in Taiwan, 1993

Crop	Released name	Planted area (ha)	Planted area (%)
Soybean	Tainan No. 1	955.9	17.6
	Kaohsiung No. 10	4,078.7	75.1
Vegetable soybean	Kaohsiung No. 1	6,208.6	91.0
Mungbean	Tainan No. 5	275.6	76.7
	Tainan Sel. No. 3	58.6	16.3
Fresh market	Taichung ASVEG No. 4	259.2	24.0
Tomato	Hualien ASVEG No. 5	77.4	7.2

Materials and Methods

In 1993-94 crop seasons, 53 RYTs were conducted for four crops: soybean, vegetable soybean, mungbean, and cherry tomato. Details of planting seasons, number of locations, number of lines, dates of sowing, and dates of transplanting for each crop are given in table 2. The experimental design for each RYT was a randomized complete block design (RCBD) with four replications. Plant spacing for different crops in different seasons are listed in table 3. The cultural management and data recording procedure were based on the standards for RYTs. Fruit and bean qualities were analyzed by AVRDC's Analytical Laboratory.

Table 2. Number of locations (NL), number of entries (NE), and sowing/transplanting dates (SD/TD) of RYTs, including AVRDC materials, in Taiwan

Crop	Coordinated by	Year	Season	NE	NL	SD/TD[a]
Soybean	Tainan DAIS	1993	Sp	12	10	1/24-3/26
		1993	Su	12	8	6/19-8/5
		1993	Au	12	1	9/10
Vegetable soybean	Kaohsiung DAIS	1993	Su	14	5	6/22-7/29
		1993	Au	14	4	9/22-10/06
		1994	Sp	14	7	1/28-2/26
		1994	Su	14	4	6/21-8/16
Mungbean	Tainan DAIS	1993	Su	10	3	6/27-7/09
		1994	Sp	10	3	2/25-3/14
Tomato (cherry)	Tainan DAIS	1993	Au	8	4	7/21
		1994	Sp	7	4	3/24-7/17

[a] Data represent the sowing duration of RYTs in all locations for soybean, vegetable soybean, and mungbean; for cherry tomato data represent the first sowing and the latest transplanting dates of all locations

Table 3. Plant spacings in RYTs

Crop	Space between rows (cm)		Space between plants (cm)		Plants per hill
	Spring & summer	Autumn	Spring & summer	Autumn	
Soybean	50	30	10	15	2
Vegetable soybean	50	50	10	10	2
Mungbean	50	–	10	–	2
Cherry tomato	100	100	50	50	1

Results and Discussion

Soybean. In 1993, soybean RYTs were done in 10 locations in spring, 8 locations in summer, and 1 location in autumn (table 2). Twelve entries, including check varieties KS 8 and KS 10 were evaluated.

The highest yielders were KS 2164 (4.2 t/ha), HL 77-79 (3.4 t/ha), and TN 6 (3.2 t/ha) for spring, summer, and autumn, respectively (table 4). HL 77-79 outyielded the two check varieties in all three seasons. In addition, IRRI 12, HL 77-79, and KS 2164 were more stable and produced yields up to 10 t/ha per year from three crops. GC 84036 was the earliest in terms of days to maturity (DTM) in spring. The average DTM for summer and autumn were both 88 days. GC 84036 produced the most number of pods per plant in summer; however, its grain size was too small (16.4 g/100 seeds) to get a significant yield. HL 77-79 and IRRI 13 had more number of pods/plant across the three seasons. KS 2095 had the heaviest 100-seed weight of 30.1 g in spring, while TN 4 had the heaviest 100-seed weight in both summer and winter crops.

Table 4. Yields and agronomic characteristics of soybean RYTs, AVRDC, 1993[a]

Entry	Yield (t/ha)			Days to maturity			Pods/plant			100-seed wt (g)		
	SP	SU	AU	SP	SU	AU	SP	SU	AU	SP	SU	AU
IRR I12	3.5	3.2	3.1	114	91	90	32	44	26	25.6	18.8	21.8
IRR I13	3.7	2.7	2.8	114	97	95	30	48	32	22.2	17.5	18.8
GC 81027	3.1	2.8	2.7	111	87	84	24	38	25	18.1	14.9	16.8
GC 84036	2.7	3.0	2.6	98	84	84	27	54	27	18.9	16.4	19.6
HL 77-33	3.2	3.0	2.7	107	85	82	23	47	25	23.5	17.6	21.2
HL 77-79	3.9	3.4	3.1	114	91	95	27	44	34	23.2	17.7	20.9
TN 4	3.7	3.2	2.6	108	87	90	17	34	27	20.5	23.9	26.8
TN 6	2.9	3.2	3.2	105	91	95	15	41	26	22.9	17.8	24.7
KS 2095	3.1	3.3	3.0	107	91	90	21	41	22	30.1	22.5	24.5
KS 2164	4.2	2.9	2.7	113	85	84	26	34	24	24.2	15.7	17.4
KS 8	3.5	2.8	2.5	113	81	87	24	31	23	27.6	19.2	19.1
KS 10	3.4	2.7	1.6	113	81	90	18	30	30	18.9	18.2	19.5
Mean	3.4	3.0	2.7	109.8	88	88	23.7	40.4	26.8	22.9	18.4	20.9
CV (%)	8.1	7.2	5.7	0.8	–	–	16.7	14.5	10.2	6.7	5.0	4.7
LSD (5%)	0.4	0.3	0.2	1.2	–	–	5.0	7.5	3.5	2.0	1.2	1.3

[a] Sowing dates: SP: 28 January 1993; SU: 15 July 1993; AU: 10 September 1993

Protein and oil contents of soybeans are shown in table 5. The average protein content of TN 6 was 43.9% dry matter (DM) which was higher than the mean for the check varieties. TN 4 had the highest oil content across the three growing seasons with a mean of 23.2%.

Vegetable soybean. A total of 20 RYTs were conducted in 1993-94 crop seasons. Eleven entries, along with three check varieties, were included. KVS 256 was the earliest with an average of 71.5 days (table 6). The number of pods in 500 g marketable pods ranged from 183.0 (GC 84137-8-2-1) to 235.5 (KVS 351). The mean seed weight accounted for 54.4% of the pod weight. GC 84126-13-1-2 had a comparable shelling percentage to the control variety KS No. 2.

Yields of vegetable soybean fluctuated widely in various crop seasons (tables 7 and 8). The check variety KS No. 3 had stable total plant yield in summer in AVRDC or in other locations. In 1993 AVRDC trials, none of the 11 entries outyielded KS No. 3 in summer and KS No. 2 in autumn (table 7). However, KVS 363 in other locations performed very well. In summer, it yielded 7.8 t/ha of marketable pods. It had an average yield of 7.3 t/ha in two crop seasons, the highest among the entries. GC 84128-17-2-1 also produced comparable yield as the check varieties.

Table 5. Protein and oil contents of soybean RYTs, AVRDC, 1993[a]

Entry	Protein (% DM)				Oil (% DM)			
	SP	SU	AU	Mean	SP	SU	AU	Mean
IRR 112	42.1	41.3	41.4	41.6	20.9	21.3	19.9	20.7
TARI 13	42.2	42.9	41.8	42.3	22.4	21.3	21.1	21.6
GC 81027	43.9	42.4	43.0	43.1	21.6	21.5	20.5	21.2
GC 84036	43.1	41.8	42.6	42.5	20.0	20.9	19.5	20.1
HL 77-33	42.4	44.0	42.6	43.0	21.3	20.1	20.0	20.5
HL 77-79	41.4	42.7	42.4	42.2	22.3	21.5	20.4	21.4
TN 4	39.8	40.1	40.6	40.2	23.6	23.7	22.2	23.2
TN 6	44.8	42.6	44.2	43.9	20.0	21.4	19.4	20.3
KS 2095	41.3	41.6	41.4	41.4	21.3	21.3	20.4	21.0
KS 2164	39.9	41.8	42.2	41.3	24.3	22.9	21.8	23.0
KS 8	42.1	44.5	43.1	43.2	20.6	19.9	19.5	20.0
KS 10	40.3	40.3	39.9	40.2	23.2	23.7	22.3	23.1
Mean	41.9	42.2	42.1	42.1	21.8	21.6	20.6	21.3
CV (%)	0.8	0.9	1.3		0.9	1.1	1.3	
LSD (5%)	0.5	0.5	0.7		0.3	0.3	0.4	

[a] Sowing dates: SP: 28 January 1993; SU: 15 July 1993; AU: 10 September 1993

Table 6. Agronomic characteristics and bean quality of vegetable soybean RYTs, AVRDC, 1993–1994[a]

Entry	Days to maturity	Pods/ 500 g	Shelled bean (%)	Dry matter (%)	Protein (% DM)	Oil (% DM)	Sugar (% DM)	Fiber (% DM)	Color[b]
KVS 133	76.0	192.8	54.6	30.4	42.6	19.1	11.0	4.8	3.1
KVS 256	71.5	191.8	53.2	28.4	42.2	19.5	11.2	4.9	2.4
KVS 363	82.5	206.8	52.5	33.6	43.7	20.7	10.6	4.7	3.5
KVS 351	82.0	235.5	54.9	32.9	43.0	20.3	11.1	4.8	2.8
Ryokkoh 74	74.5	169.8	53.8	31.1	43.3	19.5	11.3	4.7	2.5
GC 84126-13-1-1	82.5	190.5	56.3	31.6	41.9	21.1	11.1	5.0	2.4
GC 84126-13-1-2	84.3	192.8	56.8	32.3	42.1	21.7	10.6	5.1	2.6
GC 84126-13-4-4	84.5	186.8	55.5	32.4	42.1	21.4	10.4	5.1	2.8
GC 84128-9-2-1	82.0	198.0	55.8	33.1	43.0	21.6	10.2	5.1	2.9
GC 84128-17-2-1	85.0	188.0	54.3	33.2	44.7	20.6	10.0	4.6	3.9
GC 84137-8-2-1	84.8	183.0	50.4	32.6	43.1	21.0	10.7	4.9	2.8
KS No. 2 (ck)	84.0	208.3	56.9	33.2	44.3	19.4	10.8	4.6	2.7
KS No. 3 (ck)	86.8	184.8	54.9	33.1	45.4	20.5	10.2	4.7	3.3
Ryokkoh (ck)	74.0	190.0	51.6	31.0	42.9	19.4	11.4	4.8	2.4
Mean	81.0	194.2	54.4	32.1	43.2	20.4	10.8	4.8	2.9

[a] Data represent the means of the four crop seasons

[b] Color value: lower value means darker green

Table 7. Yield of vegetable soybean RYTs, AVRDC, 1993[a]

Entry	Total plant yield (t/ha)						Marketable pod yield (t/ha)					
	AVRDC			Others[b]			AVRDC			Others[b]		
	SU	AU	Mean	SU	AU	Mean	SU	AU	Mean	SU	AU	Mean
KVS 133	23.3	18.7	21.0	20.0	16.0	18.3	5.2	3.4	4.3	5.2	5.6	5.4
KVS 256	17.1	17.7	17.4	13.0	17.2	15.1	3.3	4.0	3.7	3.6	5.9	4.8
KVS 363	25.9	22.0	24.0	25.7	17.9	21.8	6.2	3.2	4.7	7.8	6.7	7.3
KVS 351	27.7	20.2	24.0	22.2	18.4	20.3	7.1	1.5	4.3	5.6	6.5	6.1
Ryokkoh 74	21.6	19.8	20.7	18.2	17.7	18.0	4.8	3.5	4.2	5.4	7.2	6.3
GC84126-13-1-1	24.8	22.3	23.6	21.9	18.0	20.0	6.8	3.7	5.3	5.4	6.1	5.8
GC 84126-13-1-2	26.2	21.9	24.1	20.2	18.4	19.3	5.2	3.7	4.5	5.4	6.1	5.8
GC 84126-13-4-4	27.9	22.1	25.0	21.1	18.6	19.9	5.4	2.9	4.2	5.3	6.8	6.1
GC 84128-9-2-1	22.7	23.2	23.0	20.9	18.1	19.5	6.0	4.9	5.5	5.5	5.7	5.6
GC 84128-17-2-1	24.1	22.3	23.2	26.6	18.2	22.4	7.3	4.4	5.9	7.1	7.0	7.1
GC 84137-8-2-1	22.0	23.7	22.9	23.5	17.5	20.5	6.1	4.9	5.5	5.2	5.9	5.6
KS No. 2 (ck)	24.2	22.0	23.1	21.0	17.8	19.4	4.7	5.6	5.2	6.3	6.7	6.5
KS No. 3 (ck)	27.0	23.4	25.2	25.0	20.5	22.8	8.5	5.1	6.8	5.8	7.9	6.9
Ryokkoh (ck)	22.4	21.5	22.0	17.4	17.4	17.4	5.0	2.9	4.0	4.3	5.9	5.1
Mean	24.1	21.5	22.8	21.2	18.0	19.6	5.8	3.8	4.9	5.6	6.4	6.0
LSD (5%)	3.2	2					1.9	2.1				

[a] Sowing dates: SU : 15 July 1993; AU : 9 September 1993

[b] Data represent means of other four locations in summer and other three locations in autumn

Table 8. Yields of vegetable soybean RYTs, AVRDC, 1994[a]

Entry	Total plant yield (t/ha)						Marketable pod yield (t/ha)					
	AVRDC			Others[b]			AVRDC			Others[b]		
	SP	SU	Mean	SP	SU	Mean	SP	SU	Mean	SP	SU	Mean
KVS 133	22.1	14.0	18.1	20.2	16.5	18.4	5.9	2.5	4.2	5.6	3.6	4.6
KVS 256	21.1	5.4	13.3	17.6	11.9	14.8	5.2	1.2	3.2	4.7	2.6	3.7
KVS 363	22.4	14.4	18.4	32.6	20.9	26.8	6.4	2.8	4.6	6.8	5.7	6.3
KVS 351	23.6	14.5	19.1	20.1	18.8	19.5	4.8	2.8	3.8	5.6	4.5	5.1
Ryokkoh 74	21.0	9.7	15.4	18.1	17.5	17.8	5.9	2.0	4.0	5.6	5.0	5.3
GC 84126-13-1-1	24.3	15.1	19.7	18.9	19.7	19.3	6.4	3.2	4.8	5.5	6.5	6.0
GC 84126-13-1-2	21.3	15.1	18.2	20.6	20.0	20.3	7.1	3.0	5.1	5.9	5.7	5.8
GC84126-13-4-4	19.7	13.3	16.5	19.9	18.7	19.3	7.1	2.8	5.0	6.2	5.3	5.8
GC84128-9-2-1	15.3	13.0	14.2	22.2	17.7	20.0	4.3	3.0	3.7	6.3	3.7	5.0
GC84128-17-2-1	23.2	14.3	18.8	20.2	21.4	20.8	6.1	4.0	5.1	6.3	5.9	6.1
GC84137 3-2-1	18.0	11.7	14.9	22.5	17.6	20.1	5.8	2.4	4.1	6.2	4.4	5.3
KS No. 2 (ck)	23.1	16.2	19.7	20.4	20.5	20.5	5.7	3.7	4.7	6.4	4.9	5.7
KS No. 3 (ck)	18.3	17.0	17.7	19.7	20.7	20.2	4.5	4.3	4.4	6.7	5.5	6.1
Ryokkoh (ck)	22.7	10.0	16.4	17.9	16.0	17.0	5.1	2.0	3.6	4.9	3.6	4.3
Mean	21.1	13.1	17.1	20.8	18.4	19.6	5.7	2.8	4.3	5.9	4.8	5.4
LSD (5%)	3.1	1.9					1.2	0.7				

[a] Sowing dates: SP: 25 February 1994; SU : 19 June 1994

[b] Data represent means of other six locations in spring and other three locations in summer

In the AVRDC 1994 spring trial, the highest yield of 7.1 t/ha was given by both GC 84126-13-1-2 and GC 84126-13-4-4 (table 8). Seven other entries outyielded the check varieties. However, none of the entries were superior to KS No. 2 and KS No. 3 in marketable pod yield in autumn. Results from other locations differed from those at AVRDC. KVS 363 and GC 84128-17-2 were promising and had comparable yield to KS No. 3. The 1994 summer trial was influenced by typhoons, so all entries produced much lower yields compared with those in 1993.

Mungbean. Mungbean RYTs were conducted in three locations in summer 1993 and spring 1994. Ten entries, including the check variety TN No. 5, were included in both seasons. However, four lines VC 3907A, VC 4250A, VC 4394A, and VC 4541A in the 1993 summer trial, were replaced by VC 4678B, VC 4828B, VC 6040B, and VC 6066B in 1994 (tables 9 and 10). In 1993, only VC 3738B outyielded TN No. 5; however, the difference was not significant (table 9). Plant heights ranged from 47.2 to 54.5 cm. TN No. 5 had the lightest 100-seed weight of 6.0 g; while VC 4386A had the heaviest of 6.7 g. In spring 1994, none of the 10 entries outyielded the check variety TN No. 5 (1.4 t/ha). However, VC 3737A, VC 4152B, VC 4442A, and VC 4678B produced comparable yields with TN No. 5 (table 10). The crop was influenced by rain, therefore, yields were generally low compared to those in the previous spring trials. VC 4828B had the heaviest 100-seed weight of 7.0 g. Number of pods per m² ranged from 111 to 138 pods. In other locations, none of the entries were superior to the check TN No. 5 in summer 1993 trials. However, three entries, VC 4152B, VC 4541A, and VC 4442A, outyielded TN No. 5 in spring 1994. The highest average yield of 2.6 t/ha was given by VC 4541A compared to 2.4 t/ha of TN No. 5.

Table 9. Yields and agronomic characteristics of mungbean RYT, AVRDC, summer 1993[a]

Entry	Yield (t/ha)	Plant ht (cm)	100-seed wt (g)	Pods/m²	Seeds/pod
VC 3737A	1.8	49.3	6.3	164	11.0
VC 3738B	1.8	48.3	6.2	189	11.3
VC 3907A	1.6	49.6	6.4	163	11.2
VC 4152B	1.5	47.8	6.3	156	11.6
VC 4250A	1.5	48.2	6.4	156	11.7
VC 4386A	1.7	54.3	6.7	170	11.3
VC 4394A	1.5	52.8	6.1	147	11.0
VC 4442A	1.7	48.8	5.5	170	11.6
VC 4541A	1.5	51.7	5.5	148	11.5
TN No. 5 (ck)	1.6	47.2	6.0	158	11.9
Mean	1.6	49.8	6.1	162.1	11.4
CV (%)	13.0	4.4	3.0	20.1	4.6
LSD (5%)	0.31	3.2	0.3	47.3	0.8

[a] Sowing date: 15 July 1993

Table 10. Yields and agronomic characteristics of mungbean RYT, AVRDC, spring 1994[a]

Entry	Yield (t/ha)	Plant ht (cm)	100-seed wt (g)	Pods/m²	Seeds/pod
VC 3737A	1.4	46.9	6.2	138	11.0
VC 3738B	1.2	40.7	6.2	119	11.1
VC 4152B	1.3	44.1	6.3	121	11.6
VC 4386A	1.2	44.1	6.7	124	11.1
VC 4442A	1.4	44.0	6.3	125	11.0
VC 4678B	1.3	47.2	6.5	129	11.7
VC 4828B	1.1	40.0	7.0	111	11.1
VC 6040B	1.2	42.3	6.1	123	11.7
VC 6066B	1.1	46.2	6.6	135	11.2
TN No. 5	1.4	44.4	6.4	125	11.3
Mean	1.2	44.0	6.4	124.9	11.3
CV (%)	8.4	5.0	5.1	14.2	4.8
LSD (5%)	0.15	3.2	0.5	25.8	0.8

[a] Sowing date: 1 March 1994

Cherry tomato. In autumn 1993, eight cherry tomato entries were evaluated in four locations. Only the check variety Santa had a small cylindrical fruit. Another check, Sandy, and the rest of the entries had round fruit. CHT 261 and CHT 264 produced the highest yields of 46.1 and 41.9 t/ha, respectively (table 11). Santa produced a yield of only 19.2 t/ha; however, it had the highest soluble solids content of 5.4 °Brix. The high soluble solids content makes Santa very popular in Taiwan. It also had the smallest fruit size (7.1 g). Sandy had the highest fruit weight of 17.6 g.

In the 1994 spring trial, entries were changed; there were seven entries tested in four locations (table 12). All entries, except CHT 264, had small cylindrical fruit similar to Santa. CHT 264 outyielded the other entries significantly (35.3 t/ha compared to the mean yield of 15.0 t/ha). CHT 151 had the highest yield of 14.7 t/ha when CHT 264 was excluded. Santa had the lowest yield of 9.3 t/ha, but had the highest soluble solids content. CHT154 with 6.0 °Brix was also outstanding. Fruit size ranged from 3.9 to 6.1 g.

Table 11. Yields and horticultural characteristics of cherry tomato RYT, AVRDC, autumn 1993[a]

Entry	Yield (t/ha)	Days to maturity	Fruit size (g)	Soluble solids (°Brix)	Acidity (%)	pH value	Color[b]
CHT 217	32.7	97	10.1	5.4	0.47	4.2	2.06
CHT 261	46.1	101	9.0	4.9	0.41	4.2	2.09
CHT 264	41.9	100	8.7	5.1	0.37	4.3	1.95
CHT 266	17.3	97	9.4	5.3	0.42	4.2	2.11
CHT 267	14.1	97	8.1	4.7	0.37	4.3	2.15
CHT 268	14.9	97	8.8	5.2	0.43	4.3	2.01
CHT 224	9.3	95	9.8	4.9	0.39	4.3	2.07
Santa	19.2	98	7.1	5.4	0.46	4.2	2.05
Sandy	35.1	93	17.6	4.9	0.43	4.3	2.29
Mean	25.6	97	9.8	5.1	0.42	4.3	2.09
CV (5%)	14.1	3.5	6.6	11.1	14.41	2.1	8.19
LSD (5%)	5.5	5.0	1.0	0.8	0.09	0.1	0.25

[a] Planting dates: sowing, 26 August 1993; transplanting, 21 September 1993
[b] Low value=light red color; high value=dark red color

Table 12. Yields and horticultural characteristics of cherry tomato RYT, AVRDC, spring 1994[a]

Entry	Yield (t/ha)	Days to maturity	Fruit size (g)	Soluble solids (°Brix)	Acidity (%)	pH value	Color[b]
CHT 151	14.7	74	4.4	5.2	0.40	4.1	0.93
CHT 152	10.9	75	4.4	5.7	0.48	4.1	0.63
CHT 154	12.1	79	5.0	6.0	0.49	4.0	0.91
CHT 155	12.8	77	4.1	5.5	0.46	4.1	0.76
CHT 157	9.8	77	3.9	5.1	0.44	4.1	1.14
CHT 264	35.3	75	6.1	4.8	0.48	4.0	1.00
Santa	9.3	83	5.6	6.1	0.59	4.1	0.98
Mean	15.0	77	4.8	5.5	0.48	4.1	0.91
CV (5%)	16.2	1.5	10.8	5.0	6.99	0.8	19.44
LSD (5%)	3.6	1.7	0.8	0.4	0.05	0.1	0.26

[a] Planting dates: sowing, 28 February 1994; transplanting, 1 April 1994
[b] Low value=light red color; high value=dark red color

CHT 261 and CHT 264 also performed very well in other locations. However, market acceptability was low. In the 1994 spring trials, the new entries with Santa fruit shape exhibited high-yielding potential, more heat tolerance, and less cracking compared to check variety Santa. CHT 151 and CHT 152 had very good fruit-set, while CHT 154 and CHT 155 had larger fruit comparable to Santa.

Seed Production and Distribution

Seeds of AVRDC-released varieties or promising lines were multiplied to supply the demonstration trials or RYTs nationwide. In 1993-94, 480 kg of mungbean, 550 kg of soybean, and 190 kg of vegetable soybean were produced (table 13). A small amount of 630 g of fresh market tomato FMTT 33 was also produced. These seeds will be distributed to the national programs.

Table 13. Seed production and distribution of AVRDC-released varieties

Crop	Variety	Seed amount (kg)
Tomato	FMTT 33	0.63
Mungbean	Tainan No. 5	480
Soybean	Tainan No. 1	200
	KS No. 10	350
Vegetable soybean	KS No. 1	190

Varietal Evaluation of Nonprincipal Vegetables

Summary

Varietial observation trials of lettuce, snap bean, and yard-long bean were conducted in 1993-94. There were four lettuce observation trials in summer and autumn 1993, spring and summer 1994. In summer, larger space (0.75 x 0.2 m) and higher beds were applied to improve drainage in the rainy and hot seasons. For leaf lettuce, Sierra was the top yielder in these four crop seasons. It produced the lowest yield of 15 t/ha in summer 1994 and the highest of 47 t/ha in autumn 1993. Summergold, Calmar, and Lagomor VML were promising with stable yields among the 11 crispyhead lettuce varieties. However, Brogan had the best heads during summer. Among 10 butterhead varieties, Regina 71 and Carmona had stable high yields. Yields of Regina 71 were 17, 32, 24, and 15 t/ha in these four planting seasons. For romaine lettuce, Maravimor Clara VML, and Augustus PVP were stable and had high yields.

Snap bean observation trials were conducted in autumn 1993 and spring 1994. Among 54 bush-type varieties, NY 91-2504, Paradise, Greenerop, and Gator Green Improved performed well with more than 17 t/ha in autumn and more than 12 t/ha in spring. For pole-type snap bean, Jemmy, Witsa, and Taichung No. 16-N were promising. They yielded more than 20 and 12 t/ha in autumn 1993 and spring 1994, respectively. For the 19 yard-long bean varieties tested in autumn 1993 and spring 1994, the average yield in 1994 was higher than that in 1993. Tun 205 produced 13.6 t/ha in 1993 and Tun 209 produced 16.3 t/ha in 1994. White Pod had the heaviest pod weight of 28.9 g in 1994.

Introduction

The purpose of varietal evaluation of nonprincipal vegetables at AVRDC is to look for improved varieties with potential as principal crops at AVRDC or for national programs through collection, evaluation, and selection. Currently, lettuce, snap bean, yard-long bean, broccoli, and cauliflower are included in the evaluation of nonprincipal vegetables in the country program.

In 1993-94, four trials for lettuce (including leaf lettuce, crispyhead, butterhead, and cos) were conducted to evaluate the collected varieties. Snap bean and yard-long bean were also included in the nonprincipal crop evaluation trials. Fourteen pole-type and 54 bush-type varieties of snap bean and 19 yard-long bean varieties were grown to observe their horticultural characteristics and yields.

Materials and Methods

Lettuce. Leaf, crispyhead, butterhead, and romaine lettuce were evaluated in four trials in 1993-94. In summer 1993, seeds were sown on 27 July and seedlings transplanted on 25 August. In autumn 1993, the sowing and transplanting dates were 21 October and 19 November. For spring and summer 1994 trials, the sowing/transplanting dates were 9 March/8 April and 1 August/6 September, respectively. Spacing for spring and autumn trials was 50 cm between rows and 20 cm between plants. The width of the bed was 100 cm and there were two rows in one bed. The height of the bed was 15 cm above the furrow. Spacing for summer trials was larger with 75 cm between rows and 20 cm between plants. Beds of 150 cm were used. There were two rows in one bed with a height of 25 cm. The bed was mulched with rice straw in all trials. The experimental design was RCBD with three replications. Because of limited seeds and poor germination, some varieties were missing or were replaced by other varieties.

Snap bean. Two observation trials were conducted to evaluate 14 pole-type snap bean varieties in autumn 1993 and spring 1994. Seeds were directly sown in the bed. There were two rows in one bed. Spacing was 75 cm between rows and 40 cm between plants. Each plot was 4.8 m long with 24 plants. The design was RCBD with two replications. Two other observation trials were carried out to observe 54 bush-type varieties. The sowing seasons and dates were the same as those for the pole type. Spacing was 75 x 10 cm, and the design was single row in one bed. Each plot was 6 m long. The experimental design was RCBD with two replications in 1993 and three replications in 1994.

Yard-long bean. Nineteen yard-long bean varieties were included in two trials in 1993-94. The sowing dates and the spacing were the same as those for the pole-type snap bean. However, no replication was employed in the yard-long bean trials.

Cultural practices and management were according to the procedures adopted by farmers for each crop. Yields and other horticultural characteristics, especially their performance under high temperatures were recorded.

Results and Discussion

Leaf lettuce. Twelve leaf lettuce varieties were included in the trials (table 14). The average yield (22.6 t/ha) of autumn 1993 was the highest across the four trials. Mean yields in the two summer trials were only 9.2 and 11.1 t/ha, which were only half of those of the spring and autumn trials. High temperatures enhanced early bolting which caused the low yields in summer. Sierra was the most promising, outyielding all other varieties in the four trials. Its yield in autumn 1993 was as high as 46.8 t/ha. In spite of late maturity, it was the best variety for summer. Although the yields in autumn were high, it took a longer time for lettuce to mature at 77.8 days. Local Improved was the earliest (51 days) in summer 1993 while Prizemor Oscura VML was the latest (98 days) in autumn 1993.

Table 14. Results of leaf lettuce variety trials, 1993-94[a]

Entry	Days to harvest (DAS)				Yield (t/ha)			
	SU 93	AU 93	SP 94	SU 94	SU 93	AU 93	SP 94	SU 94
Celtus	–	77	58	65	–	17.3	24.8	9.4
New Red Fire	87	77	68	74	12.0	21.6	14.3	13.9
Local Improve	51	72	58	68	5.0	18.7	19.8	8.6
Red Salad Bowl	71	77	66	77	14.3	16.7	19.8	10.0
Rapidmor Oscura VML	58	71	62	71	9.2	17.7	22.0	14.9
Local	61	72	58	64	8.1	22.1	17.4	8.4
Simpson's	66	70	59	65	7.7	16.7	15.2	9.2
Prizehead	–	88	65	–	–	20.1	17.9	–
Loose Leaf	54	66	58	61	6.2	14.7	15.7	4.6
Rapidmor Clara VML	54	72	58	68	5.3	17.3	16.8	9.1
Sierra	71	93	69	76	15.1	46.8	26.5	21.1
Prizemor Oscura VML	–	98	63	71	–	42.1	21.3	13.2
Mean	63.6	77.8	61.8	69.3	9.2	22.6	19.3	11.1
CV (5%)	–	8.6	2.8	1.8	–	13.5	14.0	20.7
LSD (5%)	–	11.3	3.0	2.1	–	5.2	4.6	9.8

[a] Planting dates: sowing/transplanting: SU 93: 27 July 1993/25 August 1993; AU 93: 21 October 1993/19 November 1993; SP 94: 9 March 1994/8 April 1994; SU 94: 1 August 1994/6 September 1994

Crispyhead lettuce. Most crispyhead lettuce performed very well in autumn 1993. Yields of Calmar, Summergold, and Lagomor VML even exceeded 40.0 t/ha (table 15). Summergold, Lagomor VML, and Georgia were more promising in autumn and spring. In general, yields and heading performance were poor in summer. Most varieties produced only very loose heads with low market values. Brogon and Sun had better heads in summer 1993 and Brogon and Greenfield in 1994. Heading was poor in spring 1994 since it was warm. Georgia and Brogan produced more compact and good heads than other varieties in spring 1994. It took an average of 93 days (after sowing) to harvest crispyhead lettuce in autumn. The heads were large and compact. In contrast, it took less than 80 days to harvest them in the other three crops, but the heads were small and loose.

Table 15. Results of crispyhead lettuce variety trials, 1993-94[a]

Entry	Days to harvest (DAS)				Yield (t/ha)			
	SU 93	AU 93	SP 94	SU 94	SU 93	AU 93	SP 94	SU 94
Georgia	73	95	69	77	7.2	39.7	35.1	17.8
Great Lakes	67	96	72	75	13.0	19.0	26.0	21.1
Calmar	74	96	72	74	16.7	41.7	17.5	16.7
Summergold	79	96	72	80	16.1	42.2	28.3	13.9
Great Lakes 659	72	92	72	84	8.0	33.5	23.2	12.6
Lagomor VML	87	100	72	82	7.1	41.8	27.3	10.6
Brogan	74	89	72	80	12.0	29.8	23.9	14.4
Sun	72	95	72	80	8.1	35.0	20.9	16.3
Radiant	66	92	72	76	12.5	39.4	19.5	17.4
Greenfield	87	86	72	76	7.1	32.9	15.2	18.2
Vanguard 75	–	–	–	67	–	-	–	9.5
Mean	75	92.9	71.7	77.3	10.5	35.4	23.7	15.3
CV (5%)	–	4.4	2.0	1.6	–	17.5	28.9	22.0
LSD (5%)	–	6.9	2.5	2.1	–	10.6	11.7	5.7

[a] Planting dates: sowing/transplanting: SU 93: 27 July 1993/25 August 1993; AU 93: 21 October 1993/19 November 1993; SP 94: 9 March 1994/8 April 1994; SU 94: 1 August 1994/6 September 1994

Butterhead. The performance of Carmona in 1993 was stable and promising; however, it was excluded in the subsequent trials because of lack of seeds (table 16). The top yielders in the four trials were Carmona (17.4 t/ha), Regina 71 (31 t/ha), Felicia (25.4 t/ha), and Buttercrunch (17.0 t/ha) from summer 1993 to summer 1994. These four varieties also had stable yields compared with other varieties. For the two summer trials, Buttercrunch had the highest yield followed by Kagranager Summer and Regina 71.

Romaine lettuce. Yields of romaine lettuce were not stable in 1993-94. Because of seed deficiency, only two varieties were evaluated in all four trials. Augustus had high and stable yield in autumn 1993 (45.6 t/ha) and spring 1994 (31.6 t/ha). The highest yielder in summer 1993 was Kalura (19.7 t/ha) and Carolangueu (17.2 t/ha) in summer 1994. The average yields of romaine lettuce were the highest among the four types of lettuce evaluated regardless of season. However, its fluctuation was also the largest. The mean yield was only 12 t/ha in summer, but in autumn it reached 41.5 t/ha (table 17).

Table 16. Yields and days to harvest of butterhead lettuce variety trials, 1993-94[a]

Entry	Days to harvest (DAS)				Yield (t/ha)			
	SU 93	AU 93	SP 94	SU 94	SU 93	AU 93	SP 94	SU 94
Anuenue	59	–	–	–	8.0	–	–	–
Felicia	68	97	67	–		16.9	28.0	25.4
Brado RS	57	97	62	67	6.9	20.9	17.3	6.9
Regina 71	79	86	65	80	12.6	31.7	23.0	14.2
Semira	71	88	65	78	5.6	29.8	12.8	6.3
Carmona	69	86	–	–	17.4	27.2	–	–
Kagranager Summer	66	86	65	76	10.1	24.9	17.1	14.9
Okayama Salad	61	81	–	–	9.3	20.7	–	–
Buttercrunch	60	79	64	74	11.0	26.0	23.0	17.0
Mantilia	69	87	65	77	7.8	24.2	14.9	13.0
Wild Boston	–	–	64	78	–	–	22.8	11.2
Solista ez 84	–	–	64	78	–	–	13.2	11.2
Wonder of the 4 Seasons	–	–	62	67	–	–	14.7	5.5
Mean	65.9	86.6	64	74.9	10.6	25.9	18.4	11.1
CV (5%)	–	7.5	3.1	3.1	–	12.4	19.4	14.9
LSD (5%)	–	11.3	3.4	4.0	–	5.6	6.1	2.9

[a] Planting dates: sowing/transplanting: SU 93: 27 July 1993/25 August 1993; AU 93: 21 October 1993/19 November 1993; SP 94: 9 March 1994/8 April 1994; SU 94: 1 August 1994/6 September 1994

Table 17. Yields and days to harvest of romaine lettuce variety trials, 1993-94[a]

Entry	Days to harvest (DAS)				Yield (t/ha)			
	SU 93	AU 93	SP 94	SU 94	SU 93	AU 93	SP 94	SU 94
Floricos LG	69	–	–	–	9.2		–	–
Kalura	71	–	–	–	19.7		–	–
Prizemor Clara VML	66	87	–	–	14.3	34.2	–	–
Augustus PVP	64	79	64	70	9.5	45.6	31.6	13.1
Parris Island	71	82	63	65	5.6	37.0	24.6	9.1
Maravimor Clara VML	–	99	64	–	–	45.7	20.2	–
Valmaine	–	–	65	65	–	–	26.1	9.7
Carolangueu	–	–	62	73	–	–	22.1	17.2
Tall Guzmaine H1	–	–	67	–	–	–	6.6	–
Mean	48.2	86.6	64.0	68.2	11.7	41.5	26.0	12.1
CV (5%)		6.7	2.8	3.1		13.0	13.9	19.1
LSD (5%)		10.9	3.3	3.9		10.2	6.6	4.4

[a] Planting dates: sowing/transplanting: SU 93: 27 July 1993/25 August 1993; AU 93: 21 October 1993/19 November 1993; SP 94: 9 March 1994/8 April 1994; SU 94: 1 August 1994/6 September 1994

Results of lettuce trials revealed that autumn is the most favorable season for growing any type of lettuce. In contrast, the majority of lettuce varieties are sensitive to high temperature which causes immature bolting and low yield in summer.

Snap bean. Results of pole snap bean variety trials are shown in table 18. The highest yielder in autumn 1993 was Witsa with 23.8 t/ha. The other varieties with yields of more than 20 t/ha were Taichung No. 16-N (21.4 t/ha), Taichung No. 11-N (20.4 t/ha), and Jemmy (20.2 t/ha). Pole-type varieties matured in 62-74 days after sowing. Poroto (Green) had the heaviest pod weight of 18.2 g. The top four yielders in spring 1994 were also the top four in 1993, with a different ranking: Jemmy (12.4 t/ha), Witsa (12.3 t/ha), Taichung No. 16-N (12.3 t/ha), and Taichung No. 11-N (11.8 t/ha). All varieties matured in 65-71 days in spring 1994. Poroto (Green) still had the heaviest pod weight of 14.3 g. Poroto (Green) and (Waxy) had flatter and wider pods than the other 12 varieties.

The top 20 yielders of bush snap bean in 1993 and 1994 trials are listed in tables 19 and 20. In the autumn 1993 trial, NY 70 produced 19.3 t/ha, followed by Green (19.0 t/ha), and Fesca (17.9 t/ha). Greenerop also had the heaviest pod weight of 11.6 g (table 19). The average yield of the top 20 varieties in spring 1994 was lower than the top 20 in autumn 1993 (tables 19 and 20). The highest yielder in 1994 was NY 91-2504 with 14.3 t/ha. This was followed by Paradise (14.0 t/ha), Greenerop (12.8 t/ha), and BBI GV-2 (12.8 t/ha). Greenerop seemed to be more stable, and it also had the heaviest pod weight.

Yard-long bean. Yields and other horticultural characteristics of the 19 yard-long bean varieties varied with planting seasons and varieties (table 21). Tun 205 (13.6 t/ha) had the highest yield in autumn 1993, followed by Tun 209 (12.5 t/ha), and Tun 210 (11.8 t/ha). Tun 232 had the the heaviest pod weight of 24.0 g. There were seven other varieties with pod weights higher than 20 g. Tun 233 produced pods with an average length of 61 cm and an average weight of 22.8 g, while the shortest pods were produced by Tun 204 with a length of 22 cm and a weight of 9.0 g. All varieties matured in 66-79 days after sowing.

Table 18. Yields and horticultural characteristics of pole snap bean variety trials, 1993-94[a]

Variety	Days to maturity		Pod length (cm)		Pod width (cm)		Pod weight (g)		Yield (t/ha)	
	AU 93	SP 94	AU 93	SP 94	AU 93	SP 94	AU 93	SP 94	AU 93	SP 94
Kentucky Blue	67	71	17	14	0.84	1.03	11.0	6.9	18.2	4.3
Emerite	64	69	17	15	0.80	0.82	7.9	6.1	15.8	3.6
Jemmy	59	66	17	15	0.89	1.26	10.8	8.7	20.2	12.4
M.F. AG-480	67	69	15	14	0.85	1.17	9.8	9.2	18.2	10.8
Poroto (Green)	64	69	17	16	0.79	2.35	18.2	14.3	16.3	7.6
Poroto (Waxy)	63	69	17	16	0.71	2.28	17.0	11.6	19.0	5.2
Kentucky Wonder	66	66	18	17	0.88	1.27	11.2	10.9	17.6	11.7
Paichia	69	68	15	15	0.83	1.05	8.3	7.3	16.8	9.7
Witsa	66	67	17	18	0.88	1.11	10.3	9.4	23.8	12.3
Unknown	62	65	15	16	0.85	0.96	8.3	6.8	19.7	10.9
Black Creasback	69	69	15	14	0.80	1.01	7.9	6.8	19.0	4.1
Taichung No. 11-N	66	66	17	16	0.85	1.05	8.7	6.6	20.4	11.8
Taichung No. 12-N	74	68	15	17	0.81	0.96	9.4	8.7	11.4	6.1
Taichung No. 16-N	70	68	16	18	0.84	1.12	9.3	8.6	21.4	12.3
Mean	66	68	16	16	0.83	1.25	10.6	8.7	18.4	8.8

[a] Sowing dates: AU 93: 16 September 1993; SP 94: 21 March 1994

Table 19. Yields and horticultural characteristics of top 20 bush snap bean varieties in autumn 1993[a]

Variety	Days to maturity	Pod Length (cm)	Pod Width (cm)	Pod weight (g)	Yield (t/ha)
NY70	62	12.2	1.10	8.1	19.3
Greencrop	66	13.0	0.86	11.6	19.0
Fesca	66	13.7	0.65	7.7	17.9
Gator Green Improved	64	15.5	0.87	8.4	17.7
Bush Kentucky Wonder No. 125	66	12.3	0.96	9.4	17.5
NY 91-2504	62	13.8	0.95	7.6	17.4
Paradise	62	13.4	0.92	7.8	17.2
Nerina RS	65	13.6	0.77	7.6	16.8
Greenpak	66	16.3	0.87	8.1	16.8
Kentucky Wonder CWS	66	14.2	0.88	9.3	16.5
W-290	63	15.9	0.86	9.0	16.4
Jemmy	64	16.1	1.05	8.7	16.3
Sentry	65	12.9	0.81	6.3	16.2
Derby (Stokes seeds)	65	14.5	0.93	8.4	16.0
Derby (Agway seeds)	62	14.0	0.97	8.0	15.9
NY 2458	58	13.7	0.97	7.9	15.5
Slenderette	63	14.3	0.85	6.3	15.1
Venture	61	15.7	0.88	8.4	15.1
Vilbel	66	16.2	0.73	7.1	14.7
Superstar	62	13.0	0.78	6.9	14.6
Mean	63.7	14.2	0.88	8.1	16.6

[a] Total varieties were 54; sowing date: 16 September 1993

Table 20. Yields and horticultural characteristics of top 20 bush snap bean varieties in spring 1994[a]

Variety	Days to maturity	Pod Length (cm)	Pod Width (cm)	Pod weight (g)	Yield (t/ha)
NY 91-2504	61	11.5	0.87	6.1	14.3
Paradise	65	11.7	0.92	6.0	14.0
Greencrop	63	15.9	1.17	7.7	12.8
BBI GV-2	70	12.1	0.89	5.6	12.8
NY 2458	62	11.4	0.84	5.4	12.7
NY 2915	67	12.8	0.92	5.2	12.6
Gator Green Improve	65	14.4	0.77	5.8	12.3
Selenderette	66	11.2	0.85	5.3	12.1
NY 91-2503	64	10.9	0.88	5.3	12.0
Pulobaeda	65	11.9	0.95	6.2	11.8
Mimoso Rasteiro (AG-461)	69	11.0	0.93	6.0	11.7
Superstar	67	12.2	0.95	6.2	11.6
Meal	62	13.0	0.86	4.9	11.3
Greenpak	69	12.6	0.95	5.9	11.2
Bush Kentucky Wonder No. 125	71	13.4	1.16	6.1	10.9
NY70	65	10.1	0.90	6.4	10.4
Astrel	68	11.1	0.78	3.2	10.1
Nerina RS	70	10.4	0.81	4.5	9.9
Derby (Stokes seeds)	65	12.5	0.83	5.0	9.9
Sentry	71	11.0	0.91	4.2	9.8
Mean	66.3	12.1	0.91	5.5	11.7

[a] Total varieties were 54; sowing date: 21 March 1994

Table 21. Yields and horticultural characteristics of yard-long bean variety trials, 1993-94[a]

Variety	Days to maturity		Pod length (cm)		Pod width (cm)		Pod weight (g)		Yield (t/ha)	
	AU 93	SP 94	AU 93	SP 94	AU 93	SP 94	AU 93	SP 94	AU 93	SP 94
Green Pod	75	87	41	41	0.92	1.08	23.2	20.4	7.5	8.8
Tun 204	72	83	22	22	0.69	0.73	9.0	5.8	6.2	5.6
Tun 205	69	77	38	45	0.68	0.73	14.9	9.2	13.6	11.0
Tun 206	71	83	38	47	0.69	0.78	14.8	9.0	9.1	8.3
Tun 207	71	80	29	50	0.75	0.77	16.3	12.2	10.1	14.3
Tun 208	72	81	26	28	0.62	0.82	11.5	7.2	7.0	9.0
White Pod	69	85	54	54	0.87	1.18	20.7	28.0	11.1	14.7
Tun 209	70	80	42	49	0.75	0.81	16.8	14.9	12.5	16.3
Tun 210	70	78	37	44	0.73	0.77	18.0	12.7	11.8	16.0
Tun 211	69	77	32	32	0.80	0.67	14.3	12.7	9.4	14.8
Tun 214	66	83	30	28	0.67	0.86	11.0	9.0	6.1	8.2
Tun 232	78	85	56	52	0.79	0.92	24.0	19.1	4.0	15.2
Tun 233	77	86	61	51	0.86	0.88	22.8	19.0	3.7	12.8
Local Black	72	81	43	55	0.90	1.12	21.3	25.7	6.6	8.3
Tun 238	70	86	52	56	0.87	1.21	19.7	22.5	10.9	6.9
Tun 243	79	86	38	43	0.85	1.17	–	21.9	0.2	12.7
Tun 244	73	84	41	43	0.81	0.85	20.0	16.5	9.0	13.8
Tun 252	77	87	45	–	0.74	–	21.5	22.5	1.4	2.8
Tun 255	79	84	49	44	0.65	0.86	22.0	26.3	1.5	9.4
Mean	73	83	41	44	0.77	0.90	17.9	16.6	7.5	11.0

[a] Sowing dates: AU 93: 16 September 1993; SP 94: 21 March 1994

In spring 1994, all varieties matured in 77-87 days. Tun 209 had the highest yield of 16.3 t/ha, followed by Tun 210 (16.0 t/ha), and Tun 232 (15.2 t/ha). Yields in spring were higher than those in autumn indicating that yard-long bean grows better in a warmer climate. Five varieties, i.e., Tun 205, Tun 207, White Pod, Tun 209, and Tun 210, produced more than 10 t/ha in the two planting seasons. White Pod had the heaviest pod weight of 28.0 g in 1994. Green Pod, White Pod, Local Black, Tun 252, and Tun 255 had pod weights of more than 20 g in both crops. Tun 238 had the longest average pod length of 56 cm.

Other Activities

The reception of domestic visitors is one of the major activities of the ROC Cooperative Program. In 1994, a total of 62 groups, consisting of 4096 persons from ROC visited AVRDC. The visitors included members of farmers' associations, officials and researchers of agricultural research institutes, and students. A total of 301 international visitors were received by the Program.

Bangladesh-AVRDC Agricultural Research Project

Summary

The AVRDC-USAID-Bangladesh project continues to facilitate the introduction and development of adaptive technologies, as well as germplasm introduction for varietal improvement. This will help boost the year-around vegetable production in Bangladesh. More germplasm accessions from AVRDC, the Asian Regional Center (ARC) AVRDC, the Natural Resources Institute (NRI), and the South Asian Vegetable Research Network (SAVERNET) countries have been received. A total of 164 lines/varieties were introduced in 1994. The AVRDC project assists the Bangladesh Agricultural Research Institute (BARI) identify promising lines. Promising lines of tomato, okra, kangkong, edible-podded pea, mungbean, Malabar spinach, chili, summer cauliflower, garden pea, vegetable soybean, and Chinese cabbage were identified and are under preliminary yield trial (PYT). To ensure a year-round tomato crop, protected cultivation with polythene cover in June, July, and August has been included, along with other techniques such as raised bed, grafting, and use of tomatotone spray and heat-tolerant lines. This year, cropping in a large area under polythene protection was demonstrated. Lines TM 0111 and TM 0367, developed from AVRDC germplasm, were promising for summer and summer-rainy season planting.

The AVRDC project organized a symposium on Recent Advances in Vegetable Development of Bangladesh and three in-country training programs, namely, Vegetable Growing and its Utilization, Vegetable Breeding and Genetic Analysis, and Off-season and Protected Vegetable Production. A total of 133 scientists, teachers, and extension workers were trained. Project members participated in the internal and central research reviews for 1994-95 on vegetable crops at BARI and assisted in identifying research priorities. A farmers' field day on Chinese cabbage was organized by the On-Farm Research Division (OFRD), BARI, Tangail, in collaboration with the Vegetable Section on 9 December 1993. One hundred farmers participated. Also, a farmers' field day on tomato cultivation was organized by the Regional Agricultural Research Station (RARS), Ishurdi, on 1 February 1994. A total of 150 farmers and extension workers attended. A seminar-cum-farmers' training on mungbean and 1-day field demonstration-cum-training on summer tomato production for farmers and NGOs were also organized in collaboration with BARI and extension agencies.

Nineteen technology transfer training programs on winter and summer vegetables for 570 farmers and eight for 200 extension workers were completed in 1994. The project organized 927 farmers' field demonstrations on new vegetable varieties and technologies throughout the country. This project also supported the participation of three BARI and Bangladesh Agricultural Research Council (BARC) scientists and planners in international symposia and study tours. Through the AVRDC project, two books and one booklet for researchers, extension workers, and farmers were produced and released.

Introduction

Emphasis is being given on developing or introducing new varieties and technologies that can extend vegetable production year-round and ensure production by breeding for resistance to diseases and insects and tolerance to environmental stresses. Many new varieties and technologies were generated by BARI in collaboration with AVRDC. The Bangladesh-AVRDC project is working to overcome the problems by introducing germplasm based on need, testing, and identification of promising lines. Two heat-tolerant tomato lines, TM 0111 (CL 11d-0-1) and TM 0367 (CL 143-0-10-3), selected from AVRDC germplasm are at the advanced stage of release for summer cultivation. In winter 1994, five tomato lines were identified for secondary yield trials (SYTs). Okra line OK 0285, edible-podded pea line GP 007, mungbean line NM 92, and kangkong line KG 002 were selected for regional yield trials (RYTs). Promising lines in chili/pepper, Chinese cabbage, vegetable soybean, brinjal, summer cauliflower, Malabar spinach, garden pea were identified for PYT. Three in-country trainers' training programs on important aspects of vegetable crops for scientists, research managers, extension workers, including NGOs, were organized. Efforts have been undertaken for the transfer of identified varieties and technologies to the farmers through training, demonstrations, and field days by involving on-farm research scientists, extension agencies, including NGOs, throughout the country.

Introduction of Germplasm

In 1994, 164 lines of different vegetable crops were introduced from AVRDC, ARC-AVRDC, SAVERNET member countries, and NRI (table 1) and tested at BARI.

Table 1. Germplasm collection introduced during 1994

Crops	No. of lines
Tomato	32
Vegetable soybean	12
Hot pepper	58
Sweet pepper	3
Mungbean	7
Chinese cabbage	10
Onion	8
Bittergourd	1
Brinjal	1
Asparagus	2
Muskmelon	3
Garden beans	4
Zucchini	1
Miscellaneous	22

Research

Regular visits to the experimental areas were made to check the performance of ongoing vegetable crop experiments at BARI headquarters and its research stations, Farming Systems Research (FSR) sites, and farmers' fields. These helped scientists in planning and implementing vegetable research programs.

Tomato Germplasm Evaluation

Out of 37 tomato genotypes tested in winter 1993-94, lines TM 0763, TM 0736, TM 0748, TM 0729, TM 0734, TM 0739, and TM 0740 were quite promising in yield and other characters. The yield of these lines ranged from 50.1 to 62.9 t/ha.

AVRDC Lines for Secondary Yield Trial (SYT)

Harvesting for SYT of six lines (table 2) which were transplanted on 8 November 1993 was completed on 26 February 1994. Lines CHT 280, FMTT 260, FMTT 301, and FMTT 304 were recommended for further testing.

Table 2. Yield and yield-contributing characters of selected tomato lines under SYT

Lines	Days to flowering	Days to 1st harvest	Number of fruit/ plant	Fruit weight/ plant (g)	Yield/ plant (kg)	Yield (t/ha)
CL 5915-206 D4-2-2-0-4	31	64	37	63.0	1.3	63.0
FMTT 260	40	60	28	85.3	1.7	85.3
CHT 280	36	57	25	85.9	1.7	85.9
FMTT 301	24	57	29	82.4	1.6	82.4
FMTT 304	36	60	28	81.2	1.6	81.2
Ratan	31	67	30	65.4	1.3	65.4

Summer Tomato

To demonstrate and reconfirm the usefulness of the technology, two crops were grown at BARI on a large scale during summer 1994. The first crop, with AVRDC lines TM 0111 and TM 0367, was sown on 5 May and transplanted on 5 June. The first tomatotone spraying was done on 30 June; the second on 7 July. Harvest was from 28 July onwards.

For the second crop, three lines, namely, TM 0111, TM 0367 and TM 0054, were sown on 10 June and transplanted on 11 July. First tomatotone spraying was done on 7 August; the second on 13 August. Harvest started from 28 August onwards. The performance of both crops was quite good. Average yields of 20 and 18 t/ha for the first crop, and 21, 19, and 16 t/ha for the second crop were obtained from these lines, respectively.

Large-scale summer tomato cropping using seeds of two lines, TM 0111 and TM 0367, was also demonstrated at Thakurgaon, Pahartali, Hathazari, Jessore, and Jamalpur Research Stations of BARI.

In 1994, about 200 demonstrations of these lines were conducted by the Department of Agricultural Extension (DAE), NGOs, and Bangladesh Agriculture Development Corporation (BADC) in farmers' fields.

Trial on AVRDC F_1 Cherry Tomato and Tropical Lines

Eight hybrids and two tropical lines collected from AVRDC were sown in an observational trial on 7 April, transplanted on 11 May, and harvested from 1 July onwards. Fruiting continued to 15 September.

Three hybrids, namely, CHT 499, CHT 500, and CHT 501, were quite promising (table 3). These lines gave sufficient number of fruit under high temperature without the use of hormone (tomatotone).

Table 3. Yield and yield contributing characters of eight heat-tolerant tomato germplasm accessions

Genotype	Plant height (cm)	Days to flowering	Fruit color	Fruit shape	Fruit size (cm) Length	Fruit size (cm) Diameter	No. of locules /fruit	TSS (%)	Avg. fruit wt (g)	No. of fruit/ plant	Avg. yield plant (g)
CHT 264	161	32	Red	Round	2.8	2.7	2.0	4.9	2.0	49.9	561
CHT 437	135	38	Red	High round	2.6	2.4	2.0	4.0	8.6	60.2	492
CHT 491	110	35	Red	Heart-shaped	4.1	3.4	2.6	5.8	8.6	67.8	521
CHT 492	133	30	Red	Lengthened cylindrical	3.3	2.9	2.0	4.9	12.0	40.2	450
CHT 500	139	30	Deep red	Round	3.1	3.1	2.2	5.4	15.0	89.3	1,280
CHT 499	167	29	Red	Heart-shaped	2.5	2.4	2.0	4.9	13.4	82.6	960
CHT 501	121	27	Red	Lengthened cylindrical	3.6	2.9	2.0	4.9	14.4	78.5	1,030
CL-143-0-1 -3-0-1-10	135	37	Light red	Round	2.5	2.1	2.0	4.1	6.1	21.1	115

Chinese Cabbage

A Chinese cabbage evaluation trial of 11 AVRDC lines and a local line was conducted in winter 1993-94 to select promising lines under Bangladesh conditions. Six lines which gave more than 38 t/ha (table 4) were selected for further testing.

On-farm trials on new Chinese cabbage line 1-2-70 were quite successful. Farmers preferred this new vegetable variety and they were able to sell this new crop even in the local markets.

Table 4. Yield and yield-contributing characters of different AVRDC Chinese cabbage lines tested in winter 1993-94

Lines	No. leaves/ plant	Total plant wt (g)	Head weight (g)	Head length (cm)	Head breadth (cm)	Yield (t/ha)
77M(3)-35	11.5	1,507	944	19.5	11.9	38.9
62 (ASVEG No. 1)	10.9	1,468	1,052	16.8	13.7	39.0
B 40	15.5	1,639	1,060	22.2	13.4	39.2
85-216 (F_1)	9.2	1,722	1,124	17.4	11.1	41.7
No. 82-157 (F_1)	12.7	1,438	985	16.5	11.8	36.5
85-202 (F_1)	9.2	1,399	912	16.9	11.9	33.8
77M(3)-27	13.0	1,672	1,081	19.9	11.4	40.6
82-156 (F_1)	12.7	1,318	795	14.9	11.4	29.4
77M(2/3)-46	12.7	1,566	1,077	16.4	13.5	39.9
77M(3)-40	15.9	1,153	619	17.7	9.4	22.9
83-20 (F_1)	8.0	1,328	945	15.9	11.6	35.0
CVSRC line	14.2	1,632	936	19.9	13.1	34.7

AVRDC Mungbean

At present, mungbean contributes 8% to the total pulse production in Bangladesh. Mungbean is regarded as one of the most popular and precious pulse crops. It is more expensive than other pulses. There is a good scope to increase area and production of this crop provided there are short-duration, high-yielding, synchronous, yellow mosaic virus (YMV)-, cercospora leaf spot (CLS)-resistant/tolerant varieties, and lines suitable for specific cropping patterns. Among the AVRDC germplasm accessions tested, line NM 92 has been identified as quite promising; it possesses most of the desirable characters.

In 1994, in a trial at the Institute of Postgraduate Studies in Agriculture (IPSA), the agronomic performance of 26 genotypes of mungbean, including one local check (Kanti), was evaluated during early kharif. Among the genotypes, NM 92 produced the highest yield (1.2 t/ha), closely followed by VC 2773, NM 94, NM 51, Pusa 9173, and VC 1137A. Genotypes NM 92 and NM 94 showed less than one primary branch per plant on the average compared to 1.7 branches per plant in Kanti. The genotypes NM 92 and NM 94 matured within 49-58 days after emergence (DAE) compared to 58-70 DAE in all other genotypes. Severe mungbean yellow mosaic virus (MYMV) infection (25-90%) was observed in most of the genotypes, but the MYMV infection was very low in NM 51 (1-5%), Pusa 9173 (1-5%), NM 92 (0-1%), NM 94 (0-1%), and VC 1137A (1-2%).

At BARI, RARS, Ishurdi, a germplasm evaluation trial was conducted during kharif II, August-October 1993, on 300 AVRDC lines. Forty-eight lines were isolated, and 11 lines were identified as highly promising for multiple characters. These lines were C 31-24, A 47-20, E 50-3, E 50-22, VC 6144-b-18-2B, VC 6148-B-17-2B, VC 6163-B-20-2B, B 48-7, B 48-13, VC 6146-B-20-2B, and VC 6146-B-15-2B.

Six new AVRDC mungbean lines, namely, VC 6144 D, VC 6144 C, VC 6144 B, VC 6144 A, VC 6173 A, and VC 6173 B, were tested at BARI. The experiment was conducted late on 9 April, and was completed on the first week of July. Lines VC 6144 A, VC 6173 B, and NM 92 showed better performance in yield, uniform maturity, and tolerance to virus (table 5).

Table 5. Performance of AVRDC mungbean lines at BARI

Entries	Days to flowering	Days to maturity	Plant height (cm)	Pods/ plant	Seeds/ pod	100-seed wt (g)	Yield/ 2 m² (g)	Yield (kg/ha)	Disease[a] CLS	YMV
VC 6144 A	37	68	68.0	21.7	6.6	4.9	196	980	-	-
VC 6144 B	40	68	62.7	20.9	6.8	4.7	180	900	*	-
VC 6144 C	38	68	71.2	12.5	6.8	4.5	165	825	-	-
VC 6144 D	40	68	73.4	12.6	6.6	4.5	160	800	-	-
VC 6173 A	37	64	59.5	12.2	7.2	5.3	168	840	**	-
VC 6173 B	36	65	62.5	11.7	6.2	5.3	158	790	*	-
NM 92	29	59	44.2	24.4	5.8	4.2	190	950	-	-
Kanti	39	60	51.5	18.5	6.2	2.4	180	900	-	-

[a] No disease infestation; * -low infestation; ** -severe infestation

Summer Cauliflower

Four crops of summer cauliflower using two lines, namely, CL 026 (an introduction from Taiwan) and CL 136 local, were grown in summer 1994. The first crop was sown on 10 April, transplanted on 10 May, and harvested from 25 June 1994 and for two weeks thereafter. The second crop was sown on 14 May, transplanted on 17 June, and harvested from 8 August onwards. The third crop was sown on 10 July, transplanted on 10 August, and harvested on the last week of September. The fourth crop was sown on 30 July, transplanted on 2 September, and harvested from 20 October to 5 November 1994. Only CL 026 (from Taiwan) formed very good curds in the first three sowings, whereas in the fourth sowing, line CL 136 also formed curds of 400-600 g. The average curd weight of line CL 026 ranged from 300 to 500 g.

Vegetable Soybean

Two vegetable soybean lines, namely, GC 84134-P-9-3-15-1 and GC 84136-P-4-1-8, were identified as promising. Seeds of 10 vegetable soybean lines, namely, AGS 292, AGS 334, AGS 190, AGS 332, AGS 333, AGS 335, AGS 331, GC 83005-9, GC 83011-35, and GC 83094-7, were sown on 1 October for evaluation.

Pepper/Chili

Seven chili genotypes received from SAVERNET countries were tested at BARI from September 1993 to June 1994 (table 6). Varieties MI 2 and KA 2 from Sri Lanka were promising in yield, earliness, and pungency.

The International Hot Pepper Trial Network (INTHOPE 4) was started this year, and a nursery of 16 lines was established on 29 September. These lines were transplanted on 1 November at BARI.

Table 6. Comparative performance of chili varieties at BARI

Variety	Country	Plant height (cm)	No. of primary branches/ plant	Days to first harvest	Days to last harvest	Fruit color (green stage)	Fruit color at ripe stage	Fruitness	Fruit length (cm)	Fruit girth (cm)	Yield (t/ha)
Yatsufusa	Nepal	28.2	5.7	124.1	250.1	Green	Red	Very hot	5.5	0.8	1.4
MI 2	Sri Lanka	45.6	6.0	121.3	264.1	Green	Red	Medium hot	5.8	1.0	3.1
CA 8	Sri Lanka	41.3	6.7	122.5	272.3	Green	Deep red	Medium hot	5.7	0.8	2.2
Pusa Jawala	India	61.2	4.0	127.3	222.1	Light green	Red	Medium hot	7.2	0.7	1.5
Sha-Ema	Bhutan	61.2	3.3	130.1	253.3	Light green	Red	Very hot	5.7	1.5	1.3
Suryamukhi	Nepal	64.4	9.0	124.4	263.4	Deep green	Red	Very hot	2.4	0.7	1.2
KA 2	Sri Lanka	44.2	6.3	123.3	270.8	Deep green	Deep red	Very hot	6.6	1.2	2.4

Garden Pea

Seeds of six early garden pea lines GP 003, GP 004, GP 006, GP 018, GP 023, and GP 028 were sown on 28 October and 15 November. First harvest was completed from 15 to 27 December 1993 and second harvest from 15 January to 1 February 1994 (table 7). Lines GP 003, GP 006, and GP 023 matured very early. Meanwhile, lines GP 006 and GP 028 gave better yield.

Out of 11 garden pea lines tested during the main season, GP 015 and GP 011 were quite promising. Harvesting from the main season crop which was sown on 16 November 1993 was from 25 January to 7 March 1994.

Table 7. Performance of early garden pea lines at BARI

Line	Days to flowering	Plant height (cm)	No. of branches/ plant	Length of pod (cm)	Breadth of pod (cm)	No of pods/ plant	Wt of pods/ plant	Yield (t/ha)	No. of seed/ pod	100-seed wt (g)
GP 003	30	24.5	1.3	7.6	1.2	5.1	23.9	2.3	5.3	48.7
GP 004	39	20.3	1.4	7.4	1.2	6.9	22.9	2.3	5.3	39.0
GP 006	32	33.7	2.0	7.8	1.3	6.5	29.4	3.0	5.0	49.8
GP 018	30	29.5	1.7	7.5	1.4	5.6	24.7	2.5	5.5	53.0
GP 023	29	30.0	1.7	7.3	1.4	5.3	26.1	2.6	5.6	53.3
GP 028	35	31.2	2.3	8.1	1.3	6.4	30.5	3.1	5.3	50.3

RYT of Edible-Podded Garden Pea

Line GP 007 was tested under RYT at different locations. At BARI central station in Gazipur, three sowings of the new line were done on 1 and 15 November and 1 December 1993 with five replications. First harvest was done from 20 January to 13 February, second from 7 to 17 February, and third 27 February to 25 March 1994 (table 8).

Table 8. Yield and yield-contributing characters of edible-podded pea line GP 007 introduced from AVRDC

Sowing time	Days to 50% flowering	Plant height (cm)	No. of branch/ plant	No. of pods/ plot	No. of pods/ plant	Weight of pods/ plot	Pod size Length (cm)	Pod size Breadth (cm)	No. of seeds (g)	Yield (t/ha)
1st sowing (11/1/93)	58	73	3.3	664	8.3	4,250	8.6	2.3	5.9	5.3
2nd sowing (11/16/93)	55	102	4.5	1,904	23.8	8,567	9.1	2.2	6.0	10.7
3rd sowing (12/1/93)	61	105	4.6	1,375	17.2	7,741	8.9	2.1	6.2	9.7

The experiment was conducted during winter 1993-94 at Joydebpur, Jamalpur, Jessore, Ishurdi, Pahartali, and Rahmatpur. In all locations, the advanced line GP 007 produced an average of 10 t/ha of edible pod when sown on 15 November (table 9). RYT in all locations gave good performance.

Table 9. Performance of edible-podded garden pea line GP 007 in different regions

Location	Days to flowering[a]			No. of pods/plant[a]			Yield (t/ha)[a]		
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Joydebpur	58	55	61	8.3	23.8	17.2	5.3	10.7	9.7
Jessore	61	57	58	12.0	24.3	19.4	6.4	10.0	7.2
Jamalpur	57	54	55	28.0	23.0	14.0	11.0	10.5	8.3
Pahartali	56	54	51	13.0	14.0	13.0	7.3	7.8	7.4
Rahmatpur	51	50	50	25.0	22.0	13.0	9.5	8.3	6.2

[a] 1st sowing = 1 November; 2nd sowing = 15 November; 3rd sowing = 1 December

Brinjal

A trial on 12 SAVERNET brinjal varieties was conducted to compare the yield potential and other characters under Bangladesh conditions during winter 1993-94. Pusa Purple Long (India) gave the highest number of fruit per plant (56) and produced the highest yield (51 t/ha), followed by Pusa Kranti (India) and Pant Rituraj. The lowest yield was recorded in variety Islampuri from Bangladesh (17 t/ha) with four fruit per plant.

New Cabbage Line Development

AVRDC cabbage line 1-1-52 has a wide range of genetic variabilities. Therefore, it is being improved through mass selection. In 1993-94, a large population was grown to select superior and desirable plants. This line produces seeds under local climatic conditions. Composited seeds of the cabbage line 1-1-52 produced from the selected plants in 1992-93 were sown in the seedbed on 1 September 1993. One thousand seedlings were planted in October 1993. In the eighth generation, an average of 15% of the plants produced loose head or flower buds during harvest. To get 90-95% marketable compact heads, further selection is needed. A total of 150 plants were selected and incised to facilitate flower stalk development. Seeds were collected from individual plants and composited for next year's selection and evaluation.

Radish

A total of 15 lines, including four lines provided by the AVRDC project, were sown on 2 November 1993 and harvested from 20 December 1993 to 2 January 1994. Lines RH 033 (71.1 t/ha), RH 042 (70.1 t/ha), and RH 031 (70.1 t/ha) were selected for further testing.

Okra

The performance of okra advanced line OK 0285, with a local variety as check, was tested under RYT at RARS, Ishurdi, Jessore, Jamalpur, and Hathazari and at OFRD, BARI at Bogra, Rangpur, and Ishurdi. Three NGOs, namely, Rangpur Dinajpur Rural Service (RDRS), Gono Kallyan Trust (GKT), and Bangladesh Rural Advancement Committee (BRAC), also tested this line in their project areas. All the varieties were sown in the last week of March and were completed in early August 1994. The new line has very good yields. It was also found to be highly tolerant to yellow vein mosaic virus (YVMV) at all the test locations. The yield of the new okra line OK 0285 at different locations in 1993-1994 is presented in table 10.

Table 10. Yield performance of okra line OK 0285 at different locations

Parameter	Joydebpur	Jessore	Jamalpur	Ishurdi	Hathazari
Days to flowering	43	26	47	48	52
No. of fruit/plant	28	27	22	21	24
Weight of fruit/plant (g)	550	525	420	380	399
Yield (t/ha)	10.8	10.3	8.3	7.5	7.8

Onion

A trial to evaluate seven onion genotypes collected from India, Nepal, and Bangladesh under SAVERNET was completed. Agri Found Dark Red gave the highest yield (39.7 t/ha), followed by Arka Niketan (38.0 t/ha), and Pusa Madhavi (30.1 t/ha) from India. The lowest yield was recorded in variety Taherpuri from Bangladesh.

Technology Identification

Bangladesh has already released 14 vegetable varieties through BARI and other national institutes. It is important to replace the old varieties with the new to get production and quality benefits. A number of lines which were identified with the help of the AVRDC project are in the pipeline and some are at the final stage for release like summer tomato (TM 0111, TM 0367), okra (OK 0285), kangkong (KG 002), mungbean (NM 92), and edible-podded pea (GP 007). Promising lines of Chinese cabbage, chili, vegetable soybean, early- and main-season garden pea, radish, Malabar spinach, summer cauliflower, and other vegetables were also identified.

Similarly, a number of production technologies like raised bed, fertilizer doses, dates of sowing, grafting technique, hormone application for fruit-setting, protected cultivation, nursery raising, use of polythene bags to raise seedlings, etc. have been standardized. These varieties and technologies need to be transferred to the farmers through different extension agencies, including NGOs, so that the farmers can benefit from these innovations.

Homestead Vegetable Production

Homestead vegetable production has good potential to increase vegetable production in Bangladesh. At present, both the production and consumption of vegetable crops are less than the requirement. This situation can be improved by year-round growing of vegetables in the homestead. However, it is constrained by lack of appropriate varieties and production package. About 13 million homesteads are located in the rural areas of Bangladesh. A small increase in vegetable production in each homestead can change the production level. Different models are being tested to develop a home garden model for year-round vegetable production and better nutrition involving proper varieties, use of intercropping combinations, and soil and pest management. At BARI headquarters, two new intensive vegetable homestead models with seven main beds and a corner bed planted to different vegetables, spices, and quick-growing fruit are being tested, along with the already released homestead vegetable production Kalikapur model as check.

In the new models, emphasis is given to intensive cultivation and development of a cropping system for vegetables for higher yield (per unit area and time), better nutrition, and more profit.

The homestead vegetable production technology is being disseminated, especially to landless and marginal households where malnutrition is a serious problem.

Internal and Central Research Review for 1994-95

On 3-5 July 1994, an internal review of research progress for 1993-94 was done. The proposed research programs for 1994-95 on vegetable crops were also discussed.

The central program and review committee reviewed research programs on horticulture on 13-16 August 1994. The vegetable research programs for 1994-95 were discussed extensively. Experiments were proposed on germplasm collection and evaluation (20), varietal improvement and cultural management of solanaceous vegetables (7), crucifers (9), vegetable legumes (10), cucurbits (11), leafy vegetables and okra (4), intensive vegetable production (2), vegetable seed production and technology (3), and breeders' seed production and maintenance (7). In the proposed research programs, more emphasis will be given to summer and year-round vegetables, as well as to new crops like asparagus, vegetable soybean, edible-podded and garden peas, and others.

Manpower Development

In-country Trainers' Training

The AVRDC project arranged three short-term, in-country trainers' training programs on vegetable growing and utilization, vegetable breeding and genetic analysis, and off-season and protected vegetable production (table 11) involving 133 participants from BARI, DAE, BADC, Bangladesh Agricultural University (BAU), Bangladesh Institute of Nuclear Agriculture (BINA), IPSA, agriculture colleges, private vegetable seed growers, Grameen Krishi Foundation, and NGOs.

A total of 30 lectures for the first training, 36 lectures and practical classes for the second, and 23 lectures for the third were arranged. Resource persons for these training programs were from AVRDC and BARI.

International Symposium and Study Tour

The project extended support to two BARI vegetable scientists, namely, Dr. S.M. Monowar Hossain, chief scientific officer (olericulture) and Mr. M. Mozammel Haque, principal scientific officer (vegetables) to attend and present papers in the 29th International Horticulture Congress held at Kyoto from 21 to 27 August 1994. They also had a short study tour related to vegetable crops and seed production in Japan after the congress.

The project facilitated support for Dr. M.A. Hamid Miah, member-director (Planning and Evaluation), BARC to participate in the 3rd Asian Farming Systems Symposium and visit ARC-AVRDC from 6 to 11 November 1994. Dr. Hamid Miah acted as theme commentator and was elected to the Board of Governors of the Asian Farming Systems Association (AFSA) for 2 years (1994-96) during the 3rd Symposium of AFSA held in Manila on 10 November. On 11 November, he visited ARC-AVRDC in Thailand and observed various field, training, and laboratory activities.

Table 11. In-country trainers' training programs

Training program	Date/duration	No. of participants
1. Vegetable Growing and Utilization	11/22-25/1993	47
2. Vegetable Breeding and Genetic Analysis	5/28/1993-2/2/1994	45
3. Off-season and Protected Vegetable Production	6/18-23/1994	41

Technology Transfer

Vegetable Technology Transfer Training Programs

Extension workers' training. Eight training programs on transfer of vegetable technology for extension workers were completed in 1994 at RARS Jamalpur, Hathazari, Ishurdi, Jessore, Khagrachari, Rahmatpur, and ARS Thakurgaon. In each training, there were 25 participants from different extension agencies, including NGOs. A total of 200 extension workers were trained. Twenty lectures on different vegetable technologies were delivered by the experts in each training course. Also, field visits and practical classes were arranged. Demonstration plots on the new varieties and new technologies were prepared in advance at each station for the participants.

Farmers' training. Nineteen training programs were arranged for the farmers under PL-480 funding at RARS, Jamalpur, Hathazari, Khagrachari, Rahmatpur, Ishurdi, Jessore, ARS, Thakurgaon, Raikhali, Ramgarh, and Pahartali. A total of 570 progressive farmers were trained from January to June 1994. In each training, 10 topics were covered to showcase new technologies on vegetable production. Demonstration plots on new varieties were prepared at each station and technologies were shown to the farmers. Field visits were also arranged. Seeds of the new varieties were given to farmers to test in their farms.

Farmers' Field Days

Farmers' field day on Chinese cabbage at Tangail. OFRD, Tangail, in collaboration with the Vegetable Section of BARI organized a farmers' field day on Chinese cabbage at FSR site, Palima on 9 December 1993. One hundred progressive farmers attended. Chinese cabbage cultivation and its problems were discussed. Since farmers were satisfied with the performance of this new crop in their fields and with its potential to capture the early winter market, many are interested to grow Chinese cabbage in the coming season.

Farmers' field day on tomato at Ishurdi. A field day on tomato cultivation was organized at BARI-RARS, Ishurdi, on 1 February 1994. A total of 150 farmers and extension workers, including NGO staff, attended. Tomato production techniques and problems were discussed in detail. Farmers were taken to the tomato-growing area in Pakshi, Ishurdi, where the farmers grow winter tomato in an area of about 300 acres.

Practical training on summer tomato production technology. A practical training program to transfer summer tomato production technology to farmers and extension workers, including a number of NGO staff, was held on 8 March 1994 at BARI. More than 100 farmers and extension workers attended. After the training, all the farmers were given summer tomato seed varieties. A booklet in Bangla on summer tomato cultivation was also released. Ten thousand copies of this booklet were prepared for distribution to farmers and extension workers.

Farmers' training-cum-seminar on mungbean at Rahmatpur, Barisal. Development of high-yielding, disease-resistant varieties is a prerequisite for sustaining mungbean production in Bangladesh. The lone variety (Barisal Local) extensively grown in the southern districts was seriously susceptible to YMV and other diseases. In collaboration with AVRDC, IPSA, and BARI, a mungbean varietal improvement program was launched. Two AVRDC lines, namely, NM 92 and NM 94, which were found quite promising, are being tested at the experiment station and in the farmers' plots. To enhance varietal development activities and to develop future programs effectively, a 1-day training-cum-seminar on mungbean involving scientists, extension workers, and farmers was organized at RARS, Rahmatpur, Barisal, on 11 June 1994. Farmers explained their problems in mungbean

cultivation. Discussions of the problems and prospects for mungbean cultivation in the Barisal area were held. Eighty participants, including farmers and NGO staff, attended this training.

Field demonstration-cum-training on summer-rainy season tomato production. This was held on 7 September 1994 at BARI. Fifty-two progressive farmers, extension officials of DAE, and NGO staff attended the training program. Problems and prospects of summer and summer-rainy season tomato cultivation were discussed. A demonstration on summer tomato, summer cauliflower, virus-free okra, and other summer vegetables in the field was prepared for the participants.

Demonstration-cum-field day on mungbean at Saturia. A mungbean and demonstration field day was organized at Saturia, Manikgonj, on 31 October 1994. A total of 109 women mungbean growers participated. Scientists from IPSA and BARI explained about the new mungbean variety and production techniques. Visits to several farmers' demonstration plots growing the new AVRDC mungbean line NM 92 were organized.

Interaction with the NGOs. The AVRDC Bangladesh project links with NGOs for the transfer of technologies through training and field demonstrations. This year, it arranged 927 demonstrations on new varieties and technologies of summer and winter vegetable crops in farmers' fields with the help of BRAC, GKT, the Mennonite Central Committee (MCC), RDRS, DAE, and BADC at different locations in the country.

New Vegetable Varieties and Technology Demonstrations

A total of 184 on-station, on-farm, and 927 farmers' field demonstrations were arranged through extension agencies, including NGOs, in different regions in 1993-94 (table 12).

Table 12. Demonstrations on new varieties and technologies

Crops	On-station HQ/RARS	NGOs/Farmers' field demonstrations
Winter tomato	27	115
Edible-podded pea	16	-
Radish	27	150
Chinese cabbage	17	30
China sak (summer)	10	121
Bati sak (summer)	20	90
Kangkong (summer)	10	22
Okra	15	35
Amaranth (summer)	7	19
Red amaranth (winter)	15	37
Summer tomato	7	136
Country bean (IPSA var.)	-	35
Brinjal	6	31
Mungbean	6	101
Homestead	1	15

National and International Linkages

Close linkages were maintained with national entities such as BARI, IPSA, BINA, BAU, BADC, DAE, and NGOs by inviting them to most training programs, the national symposium organized at BARI, and through the exchange of germplasm, new varieties, and technologies. The AVRDC project facilitated the participation of national planners, scientists, and extension workers involved in vegetable research and development programs in international symposia, study tours, and exchange of ideas with AVRDC and other international scientists.

National Symposium on Vegetable Development

A 2-day national symposium on Recent Advances in Vegetable Development of Bangladesh was held on 24-25 April 1994 at BARI. Horticulturists and Bangladesh Society for Horticultural Science (BSHS) members working all over the country were invited to participate in the symposium. A thorough review of vegetable development was made with the help of 18 keynote presentations by prominent scientists.

International Symposium on Pulses Research

ARC-AVRDC supported four scientists from Bangladesh to attend the International Symposium on Pulses Research held in New Delhi, India, on 2-6 April 1994. AVRDC sponsored a 1-day mungbean session on 6 April which was chaired by Dr. C.Y. Yang and co-chaired by Dr. M.L. Chada (resident senior horticulturist/agronomist in Bangladesh). Of the 18 papers on mungbean presented, 5 were by AVRDC, 5 by Bangladesh, and 8 by Indian scientists. Most of the participants suggested that AVRDC should coordinate the mungbean program in South Asia.

SAVERNET Midterm Review Meeting

SAVERNET helped facilitate the exchange of germplasm/elite varieties and provided a platform to work on the common problems of vegetable crops in South Asian countries. Five national scientists and Dr. Chadha attended the SAVERNET midterm review meeting on 12-15 September 1994 at Bangalore, India. These scientists also visited the Indo-American Hybrid Seed Company, Bangalore, and the Indian Institute of Horticulture Research at Hessarghatta.

Other Activities

Participation in the 24th International Horticulture Congress

Bangladesh scientists attended the International Horticulture Congress at Kyoto Japan, on 21 to 27 August 1994 and presented one paper and one poster. They also attended sessions on vegetable breeding, protected cultivation, biotechnology, environmental stress, soilless culture, economics, and marketing. They also attended a business session of the International Society for Horticultural Science (IHS) in which a new working group (WG) of IHS on Vegetable Production in the Tropics was established. The main function of this WG will be to promote international exchange of technology, information, and cooperation among the researchers engaged in vegetable production in the tropics. Also, the Bangladesh scientists went on a midcongress tour and visited Kitayamada Vegetable Production Center, Takii Plant Breeding and Experiment Station, and the Dainakanoko Agricultural Cooperative.

Participation in the International Expert Consultation Conference

Dr. Chadha was invited to participate in the international expert consultation conference on Vegetable Production in the Tropics and Subtropics in Peri-urban Areas — Food, Income, and Quality of Life held in Zschortan, Germany, on 14-17 November 1994. The conference was jointly organized by the German Foundation for International Development, Food and Agriculture Development

Center (DSE/ZEL) and the Council of Tropical and Subtropical Agricultural Research (ATSAF). Thirty scientists, experts, and representatives from selected national and international development institutions from various disciplines participated. The participants recommended that strong support for home gardens in urban/peri-urban areas and peri-urban vegetable production programs in developing countries is essential for food security, nutrition, socioeconomic development, sustainability, environmental preservation, and sustainable vegetable production.

Dr. Chadha presented the findings of the Working Group with emphasis on vegetable production systems in Asia. He also acted as rapporteur for the WG on the role of vegetables for food security and income generation.

Regional Programs

Asian Regional Center

Summary

The Asian Regional Center (ARC) has expanded its sphere of activities. A total of 124 experiments on vegetables and legumes were conducted in 1994 by researchers from both ARC and Kasetsart University's Tropical Vegetable Research Center (TVRC). Collaborative research activities, especially on mungbean, with different Asian countries and other international research organizations have been strengthened. Networking with Cambodia, Lao PDR, and Vietnam was initiated. ARC continues to coordinate the ARC-AVRDC/China program. Under this program, the survey and identification work on tomato virus and soybean rust are both nearing completion.

Research

A total of 124 experiments were conducted in the ARC field at Kamphaengsaen from December 1993 until November 1994. These included 23 experiments conducted by training scholars from 10 countries. In addition, experiments on vegetable crops and legumes were conducted by ARC-AVRDC and TVRC staff. Several experiments, including trials under AVNET, were conducted in farmers' fields. Abstracts of the completed research are presented here.

Crucifers

Chinese cabbage variety trial

Twelve entries of Chinese cabbage, six each from AVRDC and from China, were evaluated for their yield and adaptability at the ARC-AVRDC field in the Kamphaengsaen Campus of Kasetsart University from December 1993 to February 1994. There were significant differences in yield and horticultural traits among the 12 entries. Baizao, Fengkang 50, Fengkang 55, Shandong 2 from China, and B 40 from AVRDC were not suitable to the climatic condition of tropical lowland areas. These varieties did not head. Jin Bai 45 from China had the best performance, with the highest unadjusted yield of 31.9 t/ha, and also in most other horticultural traits among the seven varieties which formed heads. The other six varieties which formed heads were also acceptable and suitable to the tropical lowland areas.

Effect of plant population on cauliflower yield

Both plant type and spacing have an interactive effect on cauliflower curd size and weight, plant height, and canopy. The vigorously growing plants in closely spaced treatments overshaded the slow-growing one, which completely stunted the growth of the latter. Strong vegetative growth enhanced favorable conditions for insect infestation, especially diamondback moth (DBM) larvae which inflicted considerable damage to developing curds. Satisfactory results for curd size were obtained from plants spaced at 30 x 60-70 cm. However, plants have lower total yield, compared to 30 x 30-50 cm. For maximum yield, spacing of 40-45 x 50-60 cm for heat-tolerant varieties and 45-50 x 60-70 cm for heat-sensitive types of cauliflower are recommended.

Common cabbage variety trial

Thirteen common cabbage varieties from Japan and Korea were evaluated for their yield and horticultural characters at the ARC-AVRDC field in Kamphaengsaen from November 1993 to February 1994. There were significant differences among the entries evaluated. Except for red cabbage, the other varieties were early maturing and heat tolerant. Variety K.K. Cross had the highest marketable yield of 40.2 t/ha and good horticultural characters. Four varieties, Green Star, Copenhagen, Soyakaze, and Suyeer Green, produced marketable yields ranging from 29.3 to 33.5 t/ha. They also had good horticultural characteristics.

Effect of plant density on Chinese cabbage yield and yield component

An experiment on two Chinese cabbage (*Brassica campestris*, L. ssp. *pekinensis*) varieties, Hybrid 62, and Elephant Brand, with four different spacing was conducted at ARC-AVRDC from November 1993 to January 1994. There were significant differences among the different spacings in mean gross weight and mean head weight per plant, as well as solidity. The highest yield of 43.7 t/ha was obtained from the closest spacing (30 x 50 cm), but the mean gross weight and mean head weight per plant were higher at lower population density or wider spacing. Plants spaced 30 x 40 cm were more solid. No significant differences were found in head shape index (HSI), heading efficiency ratio (HER), and harvest rate. However, there were significant differences between the two varieties in yield, mean gross weight, mean head weight per plant, and HSI. Hybrid 62 had higher yield, mean gross weight, and mean head weight per plant than Elephant Brand.

Tomato

Table tomato variety trial

Sixty varieties/lines of table tomato were evaluated for yield and quality along with Seeda and BC_1F_2-2-6-0 as checks. All entries were almost comparable in terms of days to flowering and maturity. CLN 235 BC_1F_2-26-5-1-3 produced the maximum number of fruit of 80/plant, while Tropic produced the lowest (1.2). Maximum fruit weight of 223.1 g was recorded in CLN 65-349D5-2-0, while L 32 had the lowest mean fruit weight of 16.2 g. The highest marketable yield of 29 g per plant was obtained from CLN 65-349 D5-2-0 and the lowest yield of 200 g from Tropic. Seeda gave the highest marketable yield of 97.6 t/ha, while Fantastic had the lowest (21.6 t/ha). Fruit length was maximum in Floradale (7.4 cm) with the same width, while the lowest (2.8 cm) was in L 232 which had the smallest fruit. The firmest fruit (3.1 kg/cm^2) was obtained from CLN 65 BC_1F_2 -274-0-15-4. Beef Master was the tallest (203 cm), while Patio was the shortest (55 cm) with very compact growth.

Processing tomato variety trial

Fifteen processing tomato varieties were evaluated in comparison with the commercial variety XP 5034 from October 1993 to March 1994 at ARC. PT 4225 and PT 3027 produced the highest yields of 53.1 and 50.4 t/ha and marketable yields of 39.1 and 39.2 t/ha, respectively. These two varieties also had desirable characteristics with fruit-wall thickness of 0.8 and 0.7 cm, firmness of 3.3 and 2.4 kg/cm^2, fruit crack rating of 9.2 and 7.0%, soluble solids content of 4.0 and 4.6%, average fruit weight of 52.3 and 49.4 g, virus-infection ratio of 21.1 and 21.7%, and southern blight rating of 1.4 and 4.2%, respectively. The two varieties showed good heat tolerance and should be suitable for production in the tropics.

Fertilizer rate trial on fresh market tomato

The trial on the effect of 11 different fertilizer rates, ranging from 0-0-0 to 40-40-40, on tomato variety PT 4225 showed that fertilizer rate 20-40-20 gave the highest yield of 54.9 t/ha under Kamphaengsaen field conditions. Fertilizer application also affected fruit quality and horticultural characteristics of PT 4225.

Fertilizer and mulching trial on processing tomato

The effect of mulching and four different rates of NPK (15-15-15) fertilizer was evaluated from November 1993 to March 1994 at ARC. Mulching exerted a significant effect on the total marketable yield (39.5 t/ha), number of marketable fruits per plant (32.4), and marketable yield per plant (2.0 kg). These values are higher compared to those from the unmulched treatment with 23.3 t/ha, 18.1 fruit/plant, and 1.7 kg/plant, respectively. Mulching also resulted in taller plants (84.3 cm) compared to 73.5 cm in the unmulched treatment. Virus and southern blight infection were significantly reduced to 21.3 and 1.3% respectively, in the mulched treatment compared to 56.2 and 4.2% in the unmulched treatments.

The effect of NPK on total yield and other yield components was not significant. The treatment with 200 kg/ha flowered significantly earlier at 24.8 days after transplanting compared to 31.5 days for nonfertilized plants. The sugar content and firmness of processing tomato were negatively correlated to fertilizer rate. Both the mulching and NPK factors had no effect on fruit-wall thickness, pH, number of locules, mean fruit weight, and unmarketable yield.

Hybrid table tomato trial

Fourteen hybrid table tomatoes, 10 from AVRDC and 4 from China, were evaluated at ARC. All entries had higher yield, fruit number, and mean fruit weight than KY 301, the check. FMTT 269, from AVRDC, not only gave the highest marketable yield, but also possessed desirable fruit characteristics indicating its suitability for the tropics.

Pepper

Hot pepper variety trial

The total yield obtained from this trial ranged from 0.7 to 7.3 t/ha. Cultivars Tit, Bangchang, and Paris were the top three yielders with 7.3, 5.9, and 5.4 t/ha, respectively. Tit also had good fruit shape, green color, low rate of disease damage, and other desirable horticultural characters.

Sweet pepper variety trial

Twelve sweet pepper varieties were evaluated for their yield and yield components. The trial results revealed that Danjiao 5 gave the highest yield of 5.8 kg/plot followed by Danjiao 2 and No. 7205 with 5.5 and 4.2 kg/plot, respectively. Temperate varieties Danjiao 5 and Danjiao 2 were adaptable to some extent to the tropical environment of Kamphaengsaen during the dry season. Cayenne variety showed good yield potential with strong growth vigor and a lower incidence of virus infection. Total yield was highly and positively correlated to fruit number and length, to plant height and width, but negatively correlated to fruit thickness.

Detection of plant viruses

A total of 934 pepper samples with virus symptoms were collected. All of the samples were examined using ELISA. Ten viruses, alfalfa mosaic virus (AMV), chili veinal mottle virus (CVMV), cucumber mosaic virus (CMV), potato virus Y (PVY), tobacco etch virus (TEV), tobacco mosaic virus (TMV), tomato mosaic virus (ToMV), pepper mottle virus (PeMV), pepper mild mottle virus (PMMV), and tomato spotted wilt virus (TSWV), were detected and identified. Of these, CVMV, PVY, and CMV were frequently detected either singly or in combination with others. Serological test indicated that the Thai and Taiwan strains of CVMV were related. However, PeMV or PVY and the Thai or Taiwan strain of CVMV were not related.

Screening pepper varieties for anthracnose

The resistance to anthracnose of nine types (42 varieties) of pepper fruit was screened through artificial inoculation. Hlaung Bangbuathong, Yodson Prajoubkerekan, Rachaburi sweet pepper, Nokdang Pissanulok, Yum Lhomsak, Kanchanaburi Red Bird Chili, Sodtai, and Jinda Tai varieties showed the smallest lesions. Varieties Hluang Pakchong, Hom Sujok, Chiangmai Sweet Pepper, Nokdang Nakorn Sawan, Yum Chiangmai, Chiangmai Red Bird Chili, Sod Dumnurn, Sodang Rachaburi, and Jinda Banpaew had the biggest lesions. Nakornpathom Hot Pepper had a lesion size of 1.8 cm.

Mungbean

Yield and quality evaluation of mungbean sprouts

Twelve mungbean varieties from AVRDC, Pakistan, India, Korea, and Japan were evaluated for fresh weight and quality characteristics of sprouts. VC 1973A and VC 3541B obtained the highest fresh weight of 644.3 and 618.9 g/100 g seed, respectively. They also produced good quality sprouts. Temperature significantly affected the hypocotyl length, diameter, and root length of sprouts. Lower temperature (20°C) favored anthocyanin formation in the hook region and cotyledons. Seed size was negatively correlated to fresh weight, but was positively correlated to the hypocotyl diameter.

Mungbean yield trial

Twenty promising mungbean lines from the advanced yield trial compared to two check varieties, KPS 1 and KPS 2, during the dry season at ARC. Five entries, VC 4828-1-4B-1-B, VC 6075-12-1-B, VC 6075-67-1-B, VC 5205-2-3-B-1-B, and VC 6062-1-1-B, outyielded the two check varieties. However, the differences in yield were significant only between KPS 2 and three entries, VC 4828-1-4B-1-B, VC 6075-12-1-B, and VC 6075-67-1-B. These three entries also had desirable agronomic characters and were resistant to cercospora leaf spot (CLS) and lodging, but susceptible to powdery mildew (PM).

Soybean

Evaluation of soybean cultivars from Southern China

Sixteen different ecotype soybean cultivars from China, originally grown at different latitudes as spring or summer soybean, were evaluated for yield and agronomic characters against two Thai cultivars in ARC. All the 16 Chinese cultivars matured uniformly. The three highest grain yielders were CSB 04, CSV 107, and CSB 06 with yields ranging from 2.3 to 2.5 t/ha. CSB 04 had the highest 100-seed weight (25.1 g), with plant height of 35.8 cm at maturity. CSB 05 had the highest pod number per plant of 43.5, with grain weight of 13.9 g/plant.

The Chinese summer soybean cultivars from 30°N and 40°N regions and spring soybean cultivars from 28°N region performed well, characterized by strong growth vigor and a relatively longer growth period of 78 to 84 days similar to that of the checks.

Downy mildew resistance of CM 60 x AGS 129 soybean lines

The resistance to downy mildew of 448 soybean lines selected from CM 60 x AGS 129 was evaluated from November 1993 to February 1994 at ARC. Results showed that all the lines were resistant to downy mildew. All belonged to the medium-duration group which matured in 84 to 97 days. More than 60 lines gave a grain yield of 2.2-3.4 t/ha which is 11-71% higher than that of CM 60.

Vegetable soybean variety trial

The vegetable soybean variety KPS 292 gave the highest yield of 5.4 t/ha, and White Lion the lowest marketable yield of 4.0 t/ha. G 503 had the highest number of marketable pods per 500 g, while TVB 1 had the lowest.

Biological control of seed rot and damping-off in vegetable soybean caused by *Pythium aphanidermatum*

Forty-three isolates of *Trichoderma* spp. were isolated from roots and soil samples collected from vegetable soybean growing fields. All *Trichoderma* isolates inhibited the mycelial growth of *Pythium aphanidermatum* on potato dextrose agar. Twenty-seven inhibitive isolates selected from the laboratory were further tested for their control efficacy against *Pythium* seed rot and damping-off and by coating the seed of variety AGS 292 with spore suspension (10^6 cfu/seed). Coated seeds planted in the soil artificially infected with *Pythium* mycelia (200 cfu/g-soil) or oospores (50 cfu/g soil) and naturally infected soil (150 cfu/g soil) effectively controlled the *Pythium* disease, when compared to an uncoated control treatment. Efficacies of four *Trichoderma* isolates were not significantly different from a mancozeb-benalaxyl treatment when coated seeds were planted in oospore-infected soil. Moreover, coating seeds with either *Trichoderma* isolates or fungicides significantly reduced *Pythium* populations in planting soil.

Effect of temperature and duration on an EC and microwave vigor test of vegetable soybean seeds

The conductivity test for vegetable soybean was performed by soaking seeds in 100-, 150- and 200-ml water at 15, 20, 25, and 30°C for 0 to 48 h. The electrical conductivity (EC) increased as the temperature and the soaking time increased, and decreased as the volume of water increased.

Low germination was obtained in seed aged at 35, 40, 45, or 50°C for 24, 48, 72, and 96 h. Dead seeds (100%) were obtained when the seeds were incubated at 40°C for 72 h or 45-50°C for 48-96 h. There were no significant differences among treatments. Seed germinated after aging at low microwave power level (20%). Seed germination varied among treatments but germination in the treatments were lower than in the control.

Effect of planting time on vegetable soybean seed yield and quality

Vegetable soybean cv. KPS 292 and White Lion were grown in May, September, and December 1991-92 at TVRC, Kasetsart University. Both cultivars grew tall and had the highest number of main stem nodes, days to 50% flowering, days to harvesting, and number of pods per plant when grown in May. Plants grown in December produced the biggest seed and had the highest seed yield and quality. Seeds from plants grown in December also contained high protein and low fat. KPS 292 was better in height, number of nodes, seed yield, and quality, but lower in protein and fat content than White Lion.

Training

The 12th Regional Training Program, with 22 training scholars from 10 countries in Asia and the Pacific, was concluded on 15 March 1994 (table 1). In April 1994, two FAO-sponsored training scholars from Nepal and one with Korean government sponsorship finished their special training course at ARC. The Nepali were at ARC for a 3-month training course on germplasm collection and maintenance. The scholars also spent 15 days in Malaysia visiting various research organizations dealing with vegetable germplasm collection and maintenance. The special student from Korea was in Thailand for 6 months. He conducted his research on corn at the Suwan Farm of Kasetsart University in the eastern part of Thailand.

Twenty agricultural officials from eight Asian countries are attending the 13th Regional Training Program which started on 15 October (table 2). These training scholars were sponsored by different international organizations and by their own governments.

ARC, with the cooperation of headquarters, coordinated the FAO-sponsored study tour of five agricultural officials from Bangladesh on 20-26 November. They visited AVRDC and other organizations in Taiwan dealing with vegetables, especially seed technology.

ARC and TVRC staff also coordinated special seminars and gave lectures on vegetable production and research to several special Japanese exchange students for 2-4 weeks.

Students from several agricultural colleges and the Maejo Institute of Technology conduct their special problems at ARC during summer under the supervision of ARC and TVRC staff. This is a regular summer activity of ARC and TVRC in cooperation with Kasetsart University. For 1994, 10 students from Maejo and 3 students from Kasetsart University attended the summer program. Another 10 students from Kasetsart University are now conducting their thesis or special problems at ARC.

Table 1. 12th Regional Training Program (15 October 1993 - 15 March 1994) participants

Name	Country	Agency
Phoeung Nady	Cambodia	Department of Agronomy
Cai Shuping	China	Oil Crops Res. Inst., CAAS
Chu Jin Ping	China	Ku-Er-Le Fruit Res. Inst. Xinjiang AAS, Urumqi
Chi Dexiang	China	Horticulture Inst., Guizhou AAS, Guiyang
Jiao Zigao	China	Vegetable Res. Inst., Chandong AAS, Jinan
Kang Gaoqiang	China	Agric. Res. Inst., Shanghai AAS, Shanghai
Liu Li	China	Tianjin Veg. Res. Inst., Tianjin
Qian Yongzhong	China	Chinese Academy of Agric. Sciences, Beijing
Rao Zhihong	China	Sciencetech Management Div. Sciencetech Dept., MA
Shi Lin-qi	China	Hebei Veg. Res. Inst., Shijiajung
Xin Huajun	China	Liaoning AAS, Shenyang
Darwin A.	Indonesia	Berastagi Hort. Res. Inst. Harahap
Khamphanh Thommavong	Laos PDR	Agricultural Extension Agency, Vien Xian
Lang Viengasisy	Laos PDR	Agricultural Extension Agency, Vien Xian
Md. Farooq Chaudhary	Pakistan	National Agricultural Res. Centre
Alfred Nongkas	Papua New Guinea	Dept. of Agric. and Livestock
John Yamarak	Papua New Guinea	Dept. of Enga Province
Joselito Antioguia	Philippines	Post-Entry Quarantine Station, Bureau of Plant Industry
Prasarn Vongchalerm	Thailand	Singburi Prov. Agric. Ext. Off.
Somchai Thaisamark	Thailand	Chiangrai Hort. Res. Centre
Nago Quang Vinh	Vietnam	INSA, South Vietnam
Nguyen Ngoc Thanh	Vietnam	Grain Legume Res. and Development Centre, INSA
D.K. Saraf[a]	Nepal	Ministry of Agriculture
Fuleshwar Sing	Nepal	Ministry of Agriculture
Sun Lim Kim[b]	Korea	Central Experiment Station, RDA

[a] Special trainee for 3 months
[b] Special trainee for 6 months

Table 2. 13th Regional Training Program (15 October 1994 - 15 March 1995) participants

Name	Country	Agency
Ya Tek Hour	Cambodia	Kampongchan Agriculture Service
Pang Thyrith	Cambodia	Kbal Koh Vegetable Station
Gao Muqiang	China	Institute of Horticulture,
Guo Xiaoding	China	Xuzhou Sweet Potato Res. Center, Xuzhou
Jiao Huiyan	China	Heilongjiang Academy of Agriculture Sciences
Lei Maoliang	China	Ministry of Agriculture
Wu Yueyong	China	Institute of Horticulture, Guizhou AAS, Guiyang
Xiong Jinqiao	China	Vegetable and Flowers Research Institute, CAAS
Yang Jun	China	Agricultural Environmental Protecting and Monitoring Station of Jilin Prov.
Yang Yuejian	China	Zhejiang Academy of Agric. Sciences, Hanzhou
Yu Qinghui	China	Xinjiang AAS, Urumqi
Zhu Guopeng	China	Horticulture Inst. of Tropical Crops, Hainan
Seon-Woo Cha	Korea	Crop Expt. Station, RDA
Somphet Dokkeo	Laos	Hatdokkeo Agriculture Station
Thavone Saymavong	Laos	Ministry of Agriculture and Forestry
Gul Sanah Shah	Pakistan	NIAB
Angelina Garces	Philippines	PCARRD
Thongchai Roupsouy	Thailand	Srisaket Prov. Agric. Ext. Office.
Vu Thi Tinh	Vietnam	-
Nguyen Thi Hoa	Vietnam	-

Home Garden

A series of demonstration trials were conducted at ARC for Kasetsart University students majoring in horticulture. Different cropping patterns were also tested for adaptation by the Department of Agricultural Extension.

Seeds of several crops such as amaranth, tomato, and pepper were produced in the home garden for distribution to farmers or gardeners who requested small amounts of seeds.

Germplasm Collection, Multiplication, and Exchange

Seeds of pepper, tomato, snap bean, and yard-long bean collected from all over Thailand in 1992 were multiplied and characterized. Crops that have been multiplied, but are still to be characterized are eggplant and bittergourd. Snakegourd, Ceylon spinach, and amaranth have just been planted. Lists of accession numbers of these crops were sent to AVRDC's GRSU. A total of 3000 soybean germplasm accessions have been multiplied, and 1000 are still being multiplied in the field.

A total of more than 500 packets of vegetable and legume seeds were distributed to different institutions in China, Vietnam, Cambodia, and South Asian countries.

Workshops/Seminars

ARC co-coordinated the Agricultural Economics Workshop on Vegetable Production and Consumption Patterns in Asia held in Bangkok on 11-14 October 1994. This workshop explored the state of the art of agricultural economics research, and was attended by leading agricultural economists from 13 countries in Asia and representatives of other international organizations. ARC was also instrumental in bringing together representatives from Cambodia, Lao PDR, and Vietnam to a workshop on vegetable research and development held at AVRDC in Taiwan on 11-14 November. This workshop paved the way for the formation of the Cambodia, Lao PDR, and Vietnam Network (CLVNET) for vegetable research.

ARC also cosponsored national workshops on AVRDC-improved mungbean and Chinese cabbage utilization in China. These activities are part of the ongoing ARC-AVRDC/China program.

The local Thai counterparts have organized several workshops in different parts of Thailand in cooperation with the Departments of Agriculture and Agricultural Extension. These workshops and symposia aimed to promote AVRDC-released materials in Thailand. Staff of ARC and TVRC attended a seminar and a trade fair held at the UN/ESCAP premises last October. This seminar was held to showcase various agricultural technologies.

Networking

ARC maintained its cooperative programs with different countries in Asia and the Pacific. Cooperative research work on mungbean is being conducted jointly with Nuclear Institute of Agriculture and Biology of Pakistan, Bangladesh Agricultural Research Council, and the University of Minnesota in the U.S.

In addition to coordinating the Bangladesh Outreach Program and the regional networks, SAVERNET and AVNET, ARC will also coordinate the newly formed CLVNET, a research network covering Cambodia, Lao PDR, and Vietnam.

ARC continues to collaborate with China through the ARC-AVRDC/China Program. In 1993, more than 30 research trials on AVRDC materials were conducted by nine cooperating stations throughout China. As in previous years, outstanding results have been achieved by cooperating institutions in research on pepper, tomato, Chinese cabbage, mungbean, and sweet potato. Promotion activities for these crops are already at the farmers' level, and many released materials are now being produced in commercial quantity. The research on the survey and identification of tomato viruses and soybean rust now cover more than 80 cities/provinces and counties on both the northern and southern sides of the Yangtze River. Detailed recommendations concerning control of these two major diseases could be drawn up in the middle of 1995.

In addition to cosponsoring the meeting on mungbean held in Beijing and on Chinese cabbage held in Nanjing, ARC is now actively involved in the organization of the International Congress on Postharvest of Horticultural Crops to be held in Beijing in July 1995. The other organizing agencies involved are the Chinese Academy of Agricultural Sciences (CAAS) and the International Horticultural Society of America. An agreement was reached and signed by the Directors of both ARC-AVRDC and the Crop Germplasm Research Institute, CAAS, in Beijing to establish a cooperative mungbean research trial network in China to further test AVRDC-improved mungbean lines. One key junior Chinese scientist working on mungbean will also be trained at ARC for 1 year. The agreement was made effective last October in Beijing.

Other Activities

Trips to Japan and other countries were undertaken to follow up on the proposals made in 1993. The response of the International Cooperation Bureau and the Ministry of Agriculture of Japan was very encouraging. The proposal for the Japan International Cooperation Agency's support of two Japanese scientists to be based at ARC was reactivated.

A collaborative research proposal was jointly developed with Commonwealth Scientific and Industrial Research Organization (CSIRO) researchers for possible Australian Centre for International Agricultural Research funding for 1995. This project proposes to combine the expertise and resources of ARC-AVRDC and the CSIRO Division of Tropical Crops and Pastures. It builds on previous research by both organizations and that of the University of Minnesota in developing a saturated genetic map of mungbean and using RFLP/RAPD markers to select desired characters in breeding populations.

Africa Regional Program

Introduction

The AVRDC Africa Regional Program (ARP) is mandated to conduct vegetable research, training, and information services for the benefit of the African national programs.

The program currently implements two projects, namely (1) Tomato Improvement Program for the African Highlands, and (2) CONVERDS or Collaborative Network for Vegetable Research and Development in Southern Africa.

The goal of the tomato improvement program for the African highlands is to develop improved cultivars with resistance, particularly to tomato yellow leaf curl virus and late blight, two diseases which are among the most important in Africa. In addition, the project aims to introduce, evaluate, and promote the adoption of AVRDC's tropical tomatoes for production under the hot and humid growing conditions in the continent.

The major responsibilities of CONVERDS are research on strategic vegetables, training, and information services for the benefit of the national programs of the Southern African region. Of these mandates, only the training component is currently funded. The activities of the CONVERDS project in 1994 thus, emphasized mainly training of the national research and extension personnel.

Tomato Improvement Program for the African Highlands

The 1994 research activities to improve tomatoes for the African highlands emphasized the continuing evaluation of improved AVRDC germplasm introduced into Tanzania by ARP. Crosses were made to improve the commonly grown local varieties for resistance to tomato mosaic virus (ToMV), root-knot nematode, and Fusarium wilt resistance. Later, derivatives from these crosses will be improved for resistance to tomato yellow leaf curl virus (TYLCV) and late blight. Plant pathology research concentrated on the survey and characterization of the major tomato diseases in Tanzania, strain-typing of ToMV, Fusarium wilt, and root-knot nematode; and identification of sources of resistance to late blight and TYLCV.

Hybridization Program and Segregating Populations

Forty crosses were made in 1993-94 to improve the popular cultivars MoneyMaker, Marglobe, and Roma VF, for resistance to ToMV, root-knot nematode, and Fusarium wilt. The resistance genes will be incorporated into these local cultivars by backcross method. Major sources of resistance to ToMV and nematode in these crosses are AVRDC breeding lines.

Except for the crosses with Roma VF, all F_1 hybrid families were planted in February 19 develop the BC_1 generation. The resulting BC_1 families were planted in July 1994 and later scr for ToMV and nematode resistance. Fifty double-resistant BC_1 plants were selected and are now backcrossed to their respective recurrent parents to produce the BC_2 generation. These BC_1 plant also be crossed with UC 82-L, the Fusarium wilt resistance source carrying the major dominant *I-1*.

Twenty-two F_1 and F_2 lines derived from crosses of AVRDC's heat-tolerant tropical lines Rodade, a South African-bred variety that is reportedly resistant to bacterial wilt and adapted highlands, were evaluated in a 1993 replicated trial. All lines outyielded Rodade, with the bes yielding 20 t more, but were generally still horticulturally variable. These lines were replanted in for further selection. A total of 24 selections were taken from the best lines for multiplicatio further evaluation. Seeds of the best lines have now been distributed to collaborators in M Zimbabwe, and Seychelles.

Adaptation Trials of AVRDC's Fresh Market and Processing Tomato Li

Summary

Two preliminary yield trials (PYTs) conducted in 1993-94 indicated that AVRDC's fresh m hybrids, FMTT 22 and FMTT 138 which yielded consistently well, were resistant to cracking an better fruit firmness than either MoneyMaker or Marglobe, the two popular local checks. Bas these results, these hybrids are proposed to be advanced to the national testing program of Tan and also be included in the Southern African Development Council's regional testing program w will commence in 1995.

The objective of the 1993-94 adaptation trials was to assess the introduced germplasm for field performance in the highland conditions of Tanzania to select the most promising entries for national multilocation trials and for testing in the SADC region already programmed by CONVERDS in 1995.

Materials and Methods

Five adaptation trials were conducted in 1993-94 to evaluate the introduced fresh market and processing tomato germplasm under the highland conditions of Arusha.

The first two trials (PYT 1 of fresh market and processing tomatoes) were planted at the experimental farm of HORTI-Tengeru in September 1993. Spacing was 75 cm between rows and 40 cm between plants within the row using single-row beds.

The other trials (two fresh market and one processing) were planted at Madiira Research and Training Station (MRTS) of ARP. The first two (PYT 2 of fresh market and processing tomatoes) were established in December 1994 and the fifth (PYT 3 of fresh market lines) in June 1994. Spacing in these trials were 75 cm between rows and 50 cm between plants within the row using double-row beds.

All trials conducted at the HORTI-Tengeru Research Farm had 10 t of organic manure incorporated in the trial plots, in addition to 160 kg N and 160 kg P_2O_5/ha. In the MRTS trials, on the other hand, no compost was added. Nitrogen at 160 kg N and phosphorus at 160 kg P_2O_5/ha were added to PYT 2. Nitrogen at about 100 kg N/ha and phosphorus at 80 kg P_2O_5/ha were applied in PYT 3. Irrigation, weeding, and pest control were carried out as needed by the trials.

Days to 50% flowering, fruit-setting rate, disease and pest incidence, yield (marketable and nonmarketable), days to 50% maturity, and fruit traits such as shape, size, color, crack resistance, and others were recorded.

Results and Discussion

AVRDC hybrids FMTT 22 and FMTT 138 performed well in the PYT 1 trial conducted at HORTI-Tengeru although their yields were not statistically different from those of the checks (table 1). In terms of fruit size, FMTT 22 compared well with the large-fruited check, Marglobe, whereas, FMTT 138 compared well with the medium-fruited check, MoneyMaker. The AVRDC hybrids were also more resistant to fruit cracking than the checks, in addition to their having inherent resistance to ToMV (gene *Tm2a*).

In the PYT experiment conducted at the MRTS, the same hybrids also performed well, particularly when compared with the large-fruited check, Marglobe (table 2). Two of AVRDC's open-pollinated lines which had been advanced earlier to the national testing program of Tanzania also did well. Additional advantages of the AVRDC materials are firmer fruit and resistance to ToMV (gene *Tm2a*).

Table 1. Performance of AVRDC's fresh market tomatoes under Arusha conditions, PYT 1, HORTI-Tengeru, 1993-94[a]

Entry	Marketable yield(t/ha)	Mean fruit weight (g)	Culls (t/ha)	Cracking
FMTT 22	70.9 c	134 b-g	2.3 c	slight radial
FMTT 138	64.6 bc	73 a	1.8 bc	none
MoneyMaker (ck 1)	57.3 a-c	84 a-c	1.8 bc	severe radial/concentric
Marglobe (ck 2)	51.9 a-c	141 c-g	1.0 a-c	severe radial/concentric

[a] Mean column separation using Duncan's new multiple range test at P = 5%

Table 2. Performance of AVRDC's fresh market hybrids and open-pollinated lines under Arusha conditions, PYT 2, ARP-MRTS, 1994[a]

Entry	Marketable yield (t/ha)	Mean fruit weight (g)	Culls (% by weight)	Other traits[b]
FMTT 22	86.1 b	105 a-d	1.2 ab	*Tm2a*, F
FMTT 138	71.4 ab	80 ab	1.4 ab	*Tm2a*, MF
MoneyMaker (ck 1)	67.9 ab	72 a	3.8 a-c	+/+, MF
CLN 657-285-0-21-0 (B8)	60.9 ab	90 a-d	1.6 a-c	*Tm2a*, F
CLN 657-285-0-20-0 (B9)	55.0 ab	90 a-d	3.8 a-c	*Tm2a*, F
Marglobe (ck 2)	46.2 a	106.2 a-d	4.7 bc	+/+, S

[a] Mean column separation using Duncan's new multiple range test at P = 5%
[b] *Tm2a* = gene for resistance to strains 0 and 1 of ToMV; +/+ = ToMV susceptible; F = firm fruit; MF = moderately firm; S = soft

Based on these results, two hybrids are considered to be added to the 1995 national testing program of Tanzania and in the SADC regional testing program. FMTT 138 had performed very well in previous tests in Kenya and seeds are now being produced for Kenyan tomato growers, particularly in the coastal region of Mombasa, by the Kenya Seed Company (personal communication with Mr. M. Ngugi, Kenya Seed Company). In the PYT of processing tomatoes at HORTI-Tengeru, two entries outyielded the check, Roma VF, and produced relatively larger fruit (table 3). Additional advantages of these entries over Roma were resistance to ToMV (gene *Tm2a*) and fruit firmness.

In the PYT 2 of processing tomatoes planted at MRTS, Nema 512 gave the highest yield, followed by BL 627-18 (A-18), an AVRDC selection which has been advanced earlier to the national testing program of Tanzania (table 4). Both entries significantly outyielded Roma VF and produced larger fruit. BL 627-18 produced very firm fruit and had jointless pedicel, whereas Roma VF had moderately soft and puffy fruit.

Among the recent introductions from AVRDC which are to be entered in SADC's regional testing program in 1995, two lines had yields that were comparable to that of MoneyMaker, but had significantly larger fruit (table 5). However, fruit of these new lines tended to crack and had severe catfacing problems and, thus, higher percent culls.

While the problems shown by the new AVRDC introductions may be possibly localized under Arusha conditions, it is evident that there is a need to review the list of tomato entries that the AVRDC headquarters intends to enter in the 1995 SADC regional testing program.

Table 3. Performance of AVRDC's determinate processing tomato lines under Arusha conditions, PYT 1, HORTI-Tengeru, 1993-94[a]

Entry	Marketable yield (t/ha)	Mean fruit weight	Culls[b] (t/ha)	Other traits[c] (g)
PT 4176-137-23-1-7-3-0	36.0 e	74 cd	2.4 ab	*Tm2a*, VF
BL 626-72-6	35.3 e	61 a-d	3.2 b	*Tm2a*, VF
Roma VF (ck)	25.6 a-d	41 a	1.1 ab	+/+, MS

[a] Mean column separation using Duncan's new multiple range test at P = 5%
[b] Attributable mainly to sunscald
[c] VF = very firm; MS = moderately soft; +/+ = susceptible to ToMV; *Tm2a* = resistant to ToMV strains 0 and 1

Table 4. Performance of AVRDC's determinate processing tomato lines under Arusha conditions, PYT 2, ARP-MRTS, 1994[a]

Entry	Marketable yield (t/ha)	Mean fruit weight (g)	Culls[b] (t/ha)	Other traits[c] (g)
Nema 512	92.7 c	85 b	12 ab	MF
BL 627-18 (A-18)	70.0 bc	84 b	11 a	VF, jointless
Roma VF (ck)	30.7 a	36 a	16 a-c	MS, puffy fruits

[a] Mean column separation using Duncan's new multiple range test at P = 5%
[b] VF = very firm; MF = moderately firm; MS = moderately soft

Table 5. Performance of AVRDC's new indeterminate fresh market tomato lines under the highland conditions of Arusha, PYT 3, ARP MRTS, 1994[a]

Entry	Marketable yield (t/ha)	Mean fruit weight (g)	Cracking (%)	Catface (%)	Culls (%)
CLN 1462-140-31-60	71.80 c	178.5 e	32.5 d	33.3 d	4.9 b
CLN 1463-160-40-16	71.43 c	132.1 d	0.6 ab	7.7 b	3.6 ab
MoneyMaker (ck)	70.67 c	82.3 ab	0.4 ab	0.4 a	0.6 a

[a] Mean column separation using Duncan's new multiple range test at P = 5%

Tomato Diseases in Tanzania: Identification, Disease Incidence, and Distribution

Summary

Tomato diseases were surveyed and their distribution and incidence in Tanzania described. Among the fungal diseases, late blight and Fusarium wilt cause serious damages in tomato crops. Genetic resistance remains the best strategy for their control. Bacterial spot and bacterial canker are also present. In the farmers' fields, bacterial spot is relatively controlled with copper sprays. Bacterial canker is a newly identified pathogen of the tomato crop in Tanzania. The pathogen is endemic in Iringa, but its incidence is high in this region. Tomato yellow leaf curl virus (TYLCV) and tomato mosaic virus (ToMV) are widespread and are economically important. Cucumber mosaic virus (CMV) and potato virus Y (PVY) have been recorded, but are limited to a few regions. Root-knot nematodes are also a devastating tomato problem.

Introduction

Tomatoes rank first among the vegetables in Tanzania. Although the production has increased over the last 5 years, in the farmer's fields, the average yields of tomato remain low at about 10-14 t/ha. To a great extent, this low productivity is a result of disease problems. Except for the variety Roma VF which is resistant to both Verticillium and Fusarium wilts, the other tomato varieties widely cultivated in Tanzania, Marglobe and MoneyMaker, are highly susceptible to several tomato diseases. Adequate knowledge of the major tomato diseases occurring in Tanzania is a prerequisite for effective control through resistance breeding and/or integrated disease control. Information on disease incidence and distribution are also useful in setting priorities in breeding for disease resistance.

Information on tomato diseases in Tanzania is still scanty (AVRDC 1993 Progress Report). Moreover, the preliminary studies conducted to identify the tomato diseases were mainly based on symptomatology. In certain cases, this has led to erroneous diagnosis. Thus, this study was conducted to identify the most important tomato diseases occurring in Tanzania.

Materials and Methods

Field surveys and collection of isolates. In 1994, extensive surveys and intensive field studies of tomato diseases were conducted in Tanzania. So far, the following tomato growing regions have been visited and surveyed: Arusha, Coast (Dar-es-Salaam), Dodoma, Iringa, Kilimanjaro (Moshi), Mbeya, Morogoro, Mwanza, Rukwa (Mpanda and Sumbawanga), Shinyanga, Tabora and Tanga (fig. 1). During the field trips, samples were collected in polythene bags and stored in a cooler. Detailed field notes were made of the symptoms of each collected sample. The samples were then brought to the laboratory for further analysis. The disease incidence, that is, the proportion of infected plants in the field, was also recorded.

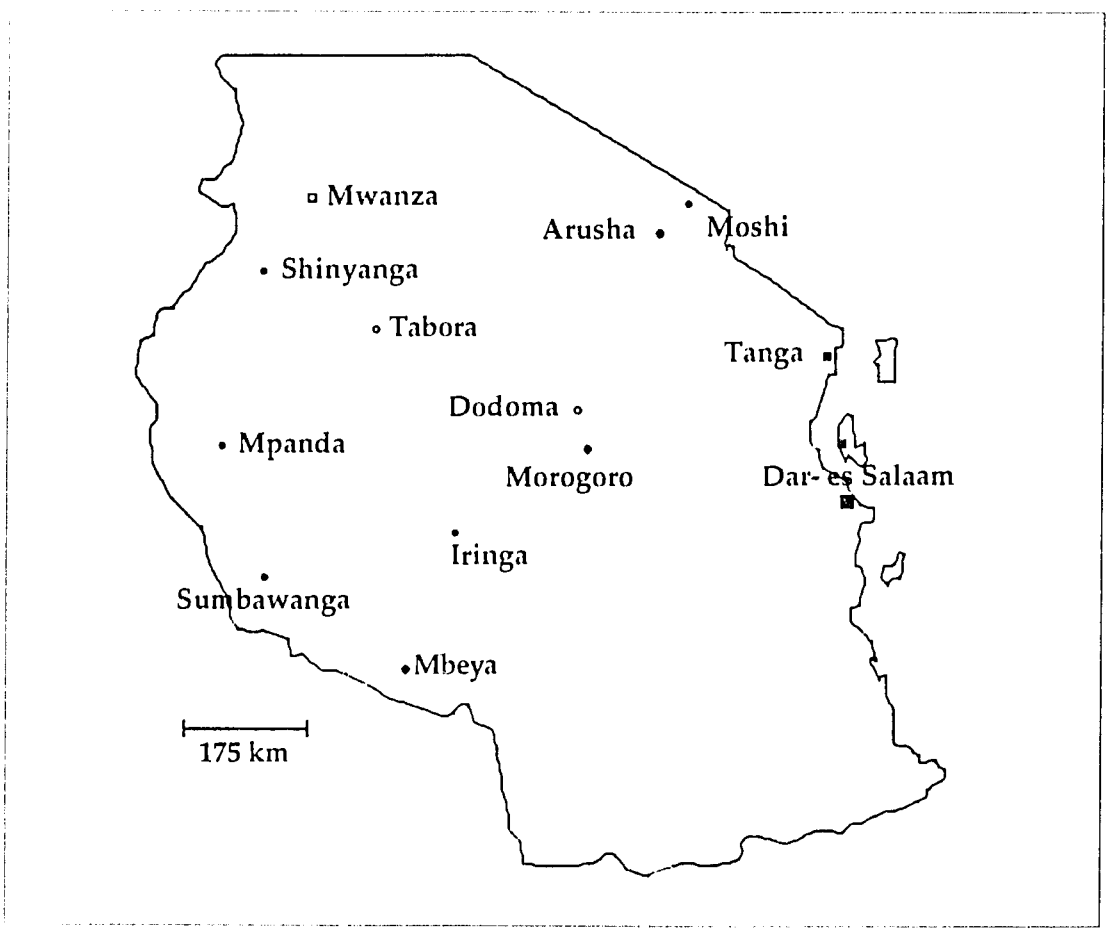

Fig. 1. Map of Tanzania showing the different regions surveyed for tomato diseases

Assay for fungal and bacterial diseases. The identification of foliar fungi was achieved through a study of the spore morphology under the microscope. In the case of vascular wilt, the pathogen was grown on selective medium. A pure culture was subsequently used for the pathogenicity test.

Due to the lack of chemicals, the diagnosis of bacterial diseases was based on the symptoms of the disease and the absence of any other fungal or viral pathogen.

Assay for viral diseases. Upon arrival at the laboratory, the samples from plants showing leaf curl and yellowing symptoms were squashed on duplicate nylon membranes. The membranes were sent to AVRDC headquarters and to Prof. H. Czosnek (The Hebrew University of Jerusalem, Israel) for identification of TYLCV. A few plant materials were also sent to Dr. P. Jones (Rothamstead Experiment Station, U.K.) for electron microscope studies.

The identification of other viruses causing field symptoms was by serological tests using either double immunodiffusion or/and enzyme-linked immunosorbent assay. Samples were tested for the presence of CMV, PVY, pepper veinal mottle virus (PVMV), and ToMV.

Identification of root-knot nematodes. The identification of root-knot nematodes was made by observation of root-knot galls on infected tomato plants. However, for further studies, nematodes were isolated from the roots of infected plants or from the soil surrounding the roots.

Results and Discussion

Fungal and bacterial diseases. Late blight is the most widespread and economically important fungal disease of tomato in Tanzania. It occurs in almost all production areas and its incidence is high (table 6). In certain tomato farms, crop losses of up to 100% are observed. Although late blight is severe during the cool and rainy season, in certain dry regions, the occurrence of dew is sufficient for the development of the disease.

Based on the morphology of spores from pure culture and on pathogenicity tests, *Fusarium oxysporum* f.sp. *lycopersicii* was identified as the causal agent of tomato wilt in four regions (table 6). This result is important because previous reports stated that *Pseudomonas solanacearum* was responsible for much of the tomato wilt in the Mbeya region. During the current survey, bacterial wilt was not recorded in Mbeya. According to its incidence, Fusarium wilt is probably the second most important fungal disease of tomato in Tanzania.

Powdery mildew is also widespread in Tanzania (table 6). It causes defoliation of tomato plants and occurs year-round. A study of spore morphology showed that *Erysiphe* sp. was the causal agent of tomato powdery mildew. Thus far, *Leveillula taurica*, another causal agent of tomato powdery mildew, has not yet been isolated.

Leaf mold (*Fulvia fulvum*) was found in three regions (table 6). The pathogen also causes defoliation of tomato plants. Very often, leaf mold is found in mixed infections with powdery mildew.

Early blight caused by *Alternaria solani* occurs in different regions (table 6). According to its incidence, early blight is less important than late blight in Tanzania.

Two distinct bacterial diseases were recorded during this study. Bacterial spot occurs in four regions. In Tanzania, almost all tomato growers spray copper fungicide so that the general incidence of bacterial spot is low. Bacterial canker occurs only in the Iringa region. It is widespread and causes serious losses on tomato crops in the region. A study of the disease distribution in several tomato farms seems to show that *Clavibacter michiganense* was introduced in the region with the seeds. Thus, to control the spread of this disease, we hope to develop an improved seed disinfection method which can be applied by tomato growers.

Table 6. Distribution and incidence of fungal and bacterial diseases of tomato crops in Tanzania

Region	Disease[a]						
	Late blight	Early blight	Fusarium wilt	Powdery mildew	Leaf mold	Bacterial spot	Bacterial canker
Arusha	+++	++	+++	+++	+++	++	
Dodoma		++	++	+	+++		
Iringa	+++	+				++	+++
Kilimanjaro	+++						
Mbeya	+++		+++			+	
Morogoro	++			++	++	++	
Mwanza		++	+++				
Rukwa	+++						
Shinyanga							
Tabora	++	++					
Tanga	+++						

[a] +++ = high incidence; ++ = moderate incidence; + = low incidence

Viral diseases. Several tomato plants with symptoms similar to those induced by TYLCV were recorded in different regions (table 7). The symptoms are variable and consist of curling, yellowing, or stunting. In a few cases, the leaf sizes were reduced. Populations of whitefly (*Bemisia tabaci*) were often associated with the symptomatic plants. In Arusha, Coast, Dodoma, and Shinyanga regions, the disease incidence was 70-100 % in several tomato farms. The virus is not sap-transmitted. The results of tests on the membranes sent to the AVRDC headquarters to identify TYLCV are reported separately under the Tomato Pathology section.

Table 7. Distribution and incidence of viruses and root-knot nematodes of tomato crops in Tanzania

Region	Disease				
	CMV	PVY	ToMV	TYLCV[a]	Root-knot nematode
Arusha	+	++	+++	+++	+++
Coast				+++	+++
Dodoma				+++	+++
Iringa			+++	+	+++
Kilimanjaro			+++	++	+++
Mbeya			+++		+++
Morogoro			++	+	+++
Mwanza			+	++	+++
Rukwa	+	+	+	+	++
Shinyanga				+++	+++
Tabora			+	+	+++
Tanga	++	++	+	+	+++

[a] TYLCV and TYLCV-like disease; +++ = high incidence; ++ = moderate incidence; + = low incidence

Recently, Prof. Czosnek communicated the results of two of three membranes sent to him earlier. The results showed that the TYLCV-Israel DNA probe hybridized strongly with several tomato samples from Arusha, Coast, and Morogoro. The incidence of TYLCV is high during the dry season. A few bell pepper samples showing leaf curl and interveinal chlorosis also gave positive signals with the TYLCV-Israel probe. These results were confirmed by in situ studies. Gemini particles were observed under electron microscope in sections of both tomato and pepper samples sent to Dr. Jones (U.K.).

Although the different results reported confirm the importance of TYLC, results are still expected from Dodoma, Mwanza, and Shinyanga to make sure that TYLCV is the only geminivirus causing leaf curl and yellowing disease of tomato in Tanzania.

TYLCV isolates from Arusha were grafted to four seedlings of tomato cv. MoneyMaker and *Lycopersicon chilense* LA 1969 in the screenhouse. Three seedlings of MoneyMaker developed symptoms, whereas *L. chilense* plants did not. Samples were taken from both tomato species to check whether they harbor the virus or not. If the resistance of LA 1969 is confirmed, populations derived from crosses between *L. chilense* and *L. esculentum* will be tested for resistance to the TYLCV isolates of Tanzania.

A few weed samples showing virus symptoms were collected from infected tomato farms and spotted onto nylon membrane. *Achyranthes aspera* (*Amaranthaceae*), *Euphorbia heterophylla* (*Euphorbiaceae*), and *Nicandra physalodes* (*Solanaceae*) were positive when tested with the TYLCV-Israel probe. This is believed to be the first report of these weeds as alternate hosts of TYLCV.

Like TYLCV, ToMV is widespread in Tanzania (table 7). It causes important losses in tomato crops. In Tanzania, tomatoes are grown year-round in many regions and crops often overlap. The local tomato cultivars are susceptible to ToMV and, moreover, several tomato growers produce their own seeds, thus accounting for the high incidence of this virus in the country.

CMV and PVY were isolated from tomatoes in different localities. Unlike TYLCV and ToMV, CMV and PVY pose only a limited threat to tomato production.

PVMV was not observed in tomato crops during our survey. However, a distinct potyvirus serologically related to PVMV was detected in several tomato samples. Further studies will allow the complete characterization of this potyvirus.

Root-knot nematodes. Root-knot nematodes are widespread in Tanzania (table 7). The incidence of this pest is high in all regions where it is found. Moreover, it is of great economic importance. In addition to galled roots, nematodes cause wilting of tomato crops. Yield losses may reach 100%. A study to characterize the nematode species occurring on tomato crops was initiated.

Characterization of Some Pathogens Infecting Tomatoes in Tanzania

Summary

A study was carried out to characterize a few isolates of ToMV, Fusarium wilt, and root-knot nematodes occurring in Tanzania. Both ToMV pathotypes 0 and 1 are present. Thus far, only Fusarium race 1 has been characterized. Preliminary typing of *Meloidogyne* spp. showed that *M. javanica* is widespread.

Introduction

ToMV, Fusarium wilt, and root-knot nematodes are among the most widespread and economically important tomato diseases in Tanzania. Sources of resistance to each of these pathogens are readily available.

With a view to incorporate resistance genes into the widely grown local cultivars of tomato, a study to characterize the pathotypes, races, and species of ToMV, Fusarium, and root-knot nematodes, respectively, was initiated.

Materials and Methods

ToMV. Several ToMV samples from different localities were multiplied on tomato cv. MoneyMaker. The following tomato differential hosts were used: GCR 26 (susceptible), CSTM 18 (*Tm-1/Tm-1*), Peru 2 (*Tm-2/Tm-2*), Mocimor (*Tm-1/Tm-2*^{2}), and Delisa (*Tm-2*^{2}*/Tm-2*^{2}). Prior to inoculation, the inoculum was heated at 70°C for 5 min.

Fusarium wilt. Three isolates from Iduda and Uyole (Mbeya), and from Oleigenuro (Arusha) were cultured on PDA for 7 days. Three-week-old seedlings of the following tomato differentials were inoculated: Fantastic (susceptible to all races), UC 82-L (resistant to race 1), Florida MH-1 (resistant to races 1 and 2), and I3R-1 (resistant to races 1, 2, and 3). The plants were maintained in the screenhouse for disease development.

Root-knot nematodes. The characterization of root-knot nematode species was carried out according to the North Carolina State University chart, using the differential host reactions on the following: cotton cv. Delta Pine, tobacco cv. NC 95, bell pepper cv. California Wonder, watermelon cv. Charleston Grey, peanut cv. Florrunner, and tomato cv. Rutgers.

Results and Discussion

ToMV. Isolates from Arusha and Iringa were characterized (table 8). ToMV pathotype 0 is present in Arusha and Iringa regions. ToMV pathotype 1 is also found in Arusha region. The two isolates from Zimbabwe were found to be of ToMV pathotype 1.

The tomato disease resistance breeding program of ARP aims to incorporate the *Tm-2*^{2} gene into the local varieties, MoneyMaker, Marglobe, and Roma VF, which are widely grown in Tanzania and neighboring African countries. It appears that we are on the right track as this resistance gene is effective against the different ToMV pathotypes that have been identified so far in Tanzania and, to some extent, in Zimbabwe.

Table 8. Reactions of tomato lines to distinct ToMV isolates[a]

Tomato differential	ToMV isolate				
	Tengeru (Arusha)	Pole (Arusha)	Iringa	Zimbabwe 1	Zimbabwe 2
GCR 26	M, +	M, +	M, +	M, +	M, +
CSTM 18	M, +	-	-	M, +	M, +
Peru 2	-	-	-	-	-
Mocimor	-	-	-	-	-
Delisa	-	-	-	-	-

[a] M = systemic mosaic; + = apical leaves showed positive reaction for ToMV in DAS-ELISA test; - = no systemic symptoms and apical leaves tested negatively for ToMV in DAS-ELISA

Fusarium wilt. According to the reaction of differential hosts, Fusarium isolates from Arusha and Mbeya belong to race 1 (table 9). The characterization of additional isolates from Dodoma, Tengeru (Arusha), and Mwanza is still in progress.

To avoid virulent strains evolving quickly, incorporation of the gene *I-1* for resistance to Fusarium race 1 into breeding materials is considered.

Root-knot nematodes. Preliminary typing of *Meloidogyne* species showed that *M. javanica* is present in Arusha, Iringa, and Mbeya (table 10). The characterization of isolates from the regions of the Coast, Dodoma, Kilimanjaro, Morogoro, Mwanza, Rukwa, Shinyanga, and Tabora has not yet been completed. The *Mi* gene that is now being incorporated into local tomato cultivars is effective against *M. arenaria*, *M. incognita*, and *M. javanica*.

Pyrethrum from Igawilo (Mbeya) was found to be infected with *M. hapla*. Though *M. hapla* is present in Tanzania, it occurs only at high altitude (2400 m asl) where tomato is not normally grown.

Table 9. Reaction of tomato differentials to *Fusarium oxysporum* f. sp. *lycopersicii* isolates[a]

Tomato differential	Fusarium isolate Iduda (Mbeya)	Uyole (Mbeya)	Oleigenuro (Arusha)
Fantastic	W, Y, +	W, Y, +	W, Y, +
UC 82-L	-	-	-
Florida MH-1	-	-	-
I3R-1	-	-	-

[a] W : wilting; Y : yellowing; + : observation of *Fusarium* conidia in pure culture - : no disease symptoms

Table 10. Characterization of several isolates of root-knot nematodes from Tanzania[a]

Host	Isolate Manyire (Arusha)	Tengeru (Arusha)	USA River (Arusha)	Ilula (Iringa)	Kidamali (Iringa)	Iringa town	Iduda (Mbeya)	Izumbwe (Mbeya)
Cotton cv. Deltapine	-	-	-	-	-	-	-	-
Peanut cv. Florrunner	-	-	-	-	-	-	-	-
Pepper cv. California Wonder	+	-	+	-	-	-	-	-
Tobacco cv. NC 95	+	+	+	+	+	+	+	+
Tomato cv. Rutgers	+	+	+	+	+	+	+	+
Watermelon cv. Charleston Gray	+	+	+	+	+	+	+	+

[a] + = infection; - = no infection

Screening *Lycopersicon* Accessions for Resistance to Late Blight

Summary

The genetic approach of breeding resistant plants is the long-term solution to control late blight. Twenty-six *Lycopersicon* accessions were screened for their reaction to late blight under field conditions in Iringa. Seven accessions were highly resistant, namely LO 3707, LA 1511, LO 3684, LO 0901, LO 3683, WV 36, and WV 106. One accession, L 1395, was rated moderately resistant.

Introduction

Late blight caused by *Phytophthora infestans* is a devastating tomato disease particularly during the cool, wet season. In 1994, 50-100% incidence of late blight was recorded in various farmers' fields visited.

All tomato varieties grown in Tanzania are susceptible to late blight. Consequently, fungicides are widely sprayed to control the disease in the field.

The purpose of this study is to evaluate several *Lycopersicon* accessions for reaction to late blight to identify sources of resistance for use in breeding.

Materials and Methods

In collaboration with Mrs. Ruth Gerson Minja of the Seatondale Research Station, 26 tomato accessions were screened for their reaction to late blight under field conditions in Iringa.

Four-week-old seedlings of each accession were transplanted in the field in single-row plots, with 10 plants to each plot. Susceptible rows were planted every four test rows. The experimental design was a randomized complete block design (RCBD) with two replications.

Disease severity was estimated as follows: 0 (no symptoms); 1 (1-10% leaf area affected); 2 (11-20% leaf area affected); 3 (21-40% leaf area affected); 4 (41-70% leaf area affected); 5 (71-90% leaf area affected); and 6 (91-100% leaf area affected).

Results and Discussion

Accessions LO 3707, LA 1511, LO 3684, LO 0901, LO 3683, WV 36, and WV 106 were rated as highly resistant (table 11). L 1395 was rated as moderately resistant.

These resistant accessions will be tested again next year in two different locations to ascertain their late blight resistance.

Table 11. Reactions of tomato accessions to late blight under natural field epiphytotics (*Phytophthora infestans*)

Accession name or number	Source	Disease severity rating[a]
Highly resistant		
LO 3707 (PI 365951)	AVRDC	0
LA 1511	Bangor	0.5
LO 3684 (PI 365905)	AVRDC	0.5
LO 0901 (PI 128664)	AVRDC	1
LO 3683	AVRDC	1
WV 36	Mike Coffey	1
WV 106	Mike Coffey	1
Moderately resistant		
L 1395	AVRDC	3.7

[a] Disease severity rating on a scale of 0 to 6; 0 = no symptoms and 6 = 91 to 100% leaf area affected

Research on Other Strategically Important Vegetables of Southern Africa

A number of experiments on other major vegetable species of the SADC region were carried out in 1994. Some of these experiments were conducted in collaboration with the SADC training scholars who participated in the First Regional Vegetable Production Course in Southern Africa that was conducted from May to September 1994. These experiments are described below.

Selection Experiment with Ethiopian Mustard

Twenty-nine selections varying in levels of turnip mosaic virus (TuMV) tolerance and stem color were made in 1993 and selfed to produce the S_1 generation to be tested for TuMV tolerance and segregation for stem color (green vs. purple). Thirteen S_1 lines with sufficient seeds were planted to observe the segregation for stem color. Of these, two were rated uniform for green stem and one for purple stem. These pure S_1 lines were intercrossed within the line to reconstitute new populations which are uniform for stem color for testing of TuMV tolerance. Additional S_1 lines will be tested in 1995 to recover several uniform lines (for stem color) for TuMV tolerance testing. Future TuMV-tolerant populations will subsequently be reconstituted from several subpopulations that are uniform for stem color and tolerant to TuMV.

Effect of Nitrogen Level and Spacing on Bulb Splitting in Onion cv. Bombay Red

Summary

The effect of in-row spacing (10 and 20 cm), and nitrogen levels (0, 80, and 160 kg N/ha) on bulb doubling (or splitting) of onion cv. Bombay Red was studied at MRTS, Arusha, Tanzania. Wide spacing significantly increased bulb doubling, but nitrogen level had no effect. On the other hand, the no-nitrogen plot had significantly higher bolting rate relative to the fertilized plots. Spacing did not affect bolting rate. Increasing levels of nitrogen did not increase yield, indicating that the native soil at Madiira may be already highly fertile.

Introduction

The common onion (*Allium cepa* var. *cepa*) is a major vegetable crop in many tropical countries and is cultivated for its bulb as an annual. Demand for onion has increased considerably as a result of diversification of eating habits, urbanization, and growth in population. Consumers generally prefer onions with good bulb size and appearance, and nonsplitting (nondoubling), i.e., whole single bulb.

Cultivars with large bulbs like Texas Early Grano are prone to bulb doubling than cultivars with smaller bulbs. Bulb splitting has no effect on onion taste, but has adverse effects on the market value of the crop.

Most countries, including Kenya and Tanzania, growing red-skinned onions such as Red Creole or Bombay Red, experience bulb splitting (or doubling) problems. There is, therefore, a need to find solutions to this problem.

Spacing and nitrogen have been implicated to influence bulb doubling. This is not surprising since closer spacing is known to reduce bulb doubling in susceptible varieties. This is perhaps due to the reduction of the bulb sizes at higher population densities.

The apparent causes of this physiological disorder are not well-defined, but it is claimed that horticultural and agronomic factors, including genotype, soil fertility, soil moisture, and age at transplanting might be involved.

A greater understanding of the major factors which induce bulb doubling will provide a suitable screening environment for germplasm screening to select cultivars that are tolerant to splitting, and will also make it possible to alleviate their impact on bulb quality in the farmers' production fields.

The objective of this study is to determine the effects of nitrogen and spacing on bulb splitting in onions under Arusha conditions.

Materials and Methods

This field experiment was conducted at MRTS, Arusha, Tanzania, which is situated 1400 m above sea level. The cultivar used was Bombay Red.

Seeds were sown in wooden flat trays on 11 May 1994 and transplanted 42 days later. Treatments were factorially arranged in RCBD with three replications. Plot size was 0.75 x 4 m.

Treatments included three nitrogen levels namely, 0, 80, and 160 kg N/ha, and two in-row spacings of 10 and 20 cm. The nitrogen in ammonium sulfate form was split-applied in three proportions: 1/2, 1/4, 1/4. The first application was basal. The first and second topdressing were done 30 and 45 days after transplanting.

Irrigation, weeding, pest and disease control, and other cultural practices were done as recommended. Data on neck thickness (girth), number of doubled bulbs, and bolting rate were recorded.

Results and Discussion

Wider in-row spacing significantly increased the rate of bulb splitting, but had no effect on bolting rate and thickness of the neck (table 12; fig. 2). As expected, per unit area yield was higher at closer spacing.

Table 12. Influence of spacing and nitrogen levels on frequency of bulb doubling (splitting) and bolting in onion, cv. Bombay Red, ARP-MRTS, 1994[a]

Treatment	% Doubling	% Bolting	Neck girth[b] (cm)	Yield (t/ha)
Spacing				
10 x 10 cm	10.9 a	18.9 a	1.63 a	29.4 b
10 x 20 cm	25.8 b	14.8 a	1.60 a	22.8 a
Nitrogen level				
0 kg N/ha	17.1 a	25.3 b	1.65 a	24.4 a
80 kg N/ha	21.8 a	12.8 a	1.62 a	27.3 a
160 kg N/ha	16.3 a	12.3 a	1.58 a	26.6 a

[a] Mean column separation using Duncan's new multiple range test at P = 5%
[b] Taken 74 days after transplanting

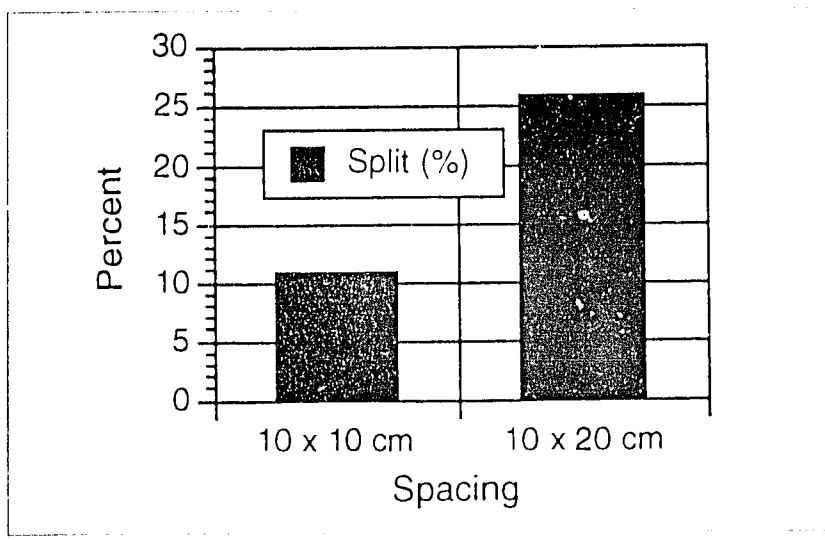

Fig. 2. Rate of bulb splitting in onion cv. Bombay Red as influenced by spacing

Contrary to previous reports, increasing nitrogen level did not influence the rate of bulb doubling in this experiment (table 12). However, rate of bolting was highest under the no-nitrogen plot (fig. 3; table 12). It appears that the native fertility at the MRTS site is already high as evidenced by the absence of nitrogen effect on yield.

This study is being repeated by the HORTI-Tengeru collaborators to look also at the effect of irrigation, seedling age, and date of planting. Subsequently, it will be possible to identify the combination of the most important factors which maximize bulb doubling and to use it in selecting for genotypes that are resistant to this physiological disorder.

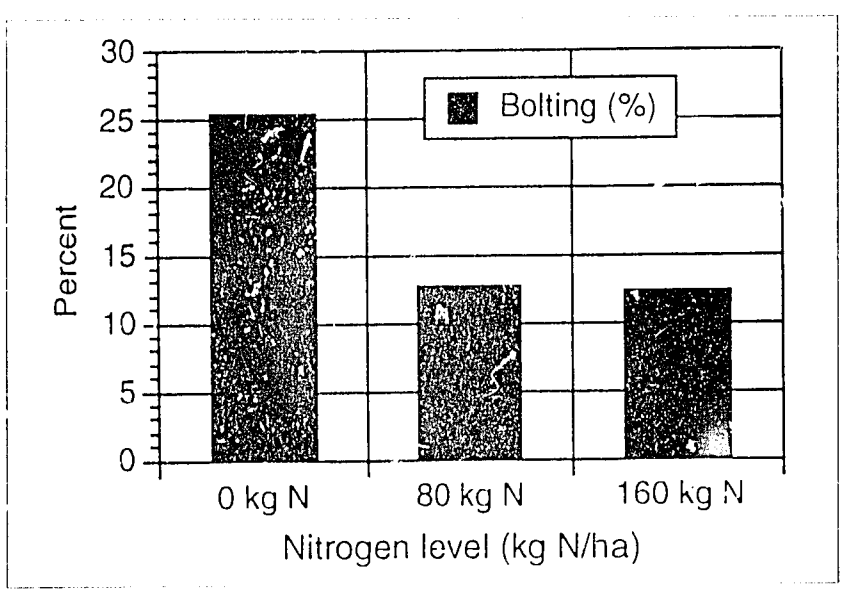

Fig. 3. Rate of bolting in onion cv. Bombay Red as influenced by nitrogen level

Effect of Early Loss of Apical Dominance on Yield and Other Characters of African Eggplant (*Solanum aethiopicum*)

Summary

Pinching (or topping) of African eggplant did not influence the yield and other characters of African eggplant regardless of cultivar. The variety Tengeru White was found to be better than Manyire Green in yield, fruit size, and plant canopy. It also tended to have taller plants and more fruit clusters than Manyire Green. Topping of African eggplant is not a worthwhile cultural practice to recommend to vegetable producers.

Introduction

The African eggplant, *Solanum aethiopicum*, locally known in Tanzania as "ngogwe," is one of the most important indigenous vegetables grown in many parts of Eastern and Northern Tanzania, especially around the urban centers. This vegetable resembles eggplant in general growth and characteristics, but with slightly bitter fruits.

In recent years, consumption of the African eggplant, especially in urban areas of Tanzania, has grown tremendously and, thus, there is a need to increase production to supply the market demand.

There is little information on the improvement of productivity. Information on improved cultural practices are direly lacking.

This experiment was conducted to study the effect of topping to induce loss of apical dominance on the performance of African eggplant cultivars.

Materials and Methods

This experiment was conducted from May to September 1994 at MRTS of AKP, Tengeru, Arusha, Tanzania, which is situated 1300 m above sea level.

Seeds of two varieties, Manyire Green and Tengeru White, provided by HORTI-Tengeru, were sown in wooden flats on 18 May 1994. Seedlings were thinned 4 weeks after sowing to one plant per hill. One week before transplanting, the seedlings were hardened by reducing the frequency of irrigation. Bacterial spot disease was noted in the nursery and was controlled by spraying Kocide.

Before transplanting the seedlings, nitrogen fertilizer in the form of urea was basally applied in the planting holes at the rate of 25 kg N/ha. The same amount was sidedressed 4 weeks later.

Seedlings were transplanted on 30 June at a spacing of 90 cm between rows and 75 cm between plants. Each plot had an area of 1.5 x 6.0 m, with two rows of 14 plants each. A 2 x 4 factorial arrangement of treatments in RCBD was used. Variety served as one factor and age of pinching (or topping) as the other factor, with four levels namely, pinching at 3, 4, and 5 weeks after transplanting, and no pinching as control.

Furrow irrigation was done every 3 days. Weeding was done as needed. Furadan was applied to control cutworms which attacked the plants at seedling stage. Actellic was sprayed to control ladybird beetles which attacked the leaves. Ridomil fungicide was sprayed to control the fungal diseases.

Yield and other horticultural characters were recorded.

Results and Discussion

Pinching (or topping) of African eggplant at various stages did not affect yield and other characters of African eggplant, regardless of cultivar.

The variety Tengeru White had a higher marketable yield of 19.4 t/ha than Manyire Green with 16.2 t/ha. Tengeru White also had bigger fruit, produced bigger canopy, and tended to have taller plants and more fruit clusters than Manyire Green.

It appears from this experiment that topping of African eggplant is not a worthwhile cultural practice to follow. In a 1993 study, a similar result was obtained from a topping experiment in another traditional vegetable, black nightshade (*Solanum nigrum*).

Comparative Performance of Introduced Amaranth Germplasm vs. Local Cultivars of Tanzania

Summary

In a replicated field trial comparing the Taiwan commercial amaranth cultivars with the local cultivars which are widely grown in Tanzania, the Taiwan varieties proved to be weaker, slower growing, and lower yielding than the latter. Tanzania Red (*Amaranthus hypochondriacus*) grew the fastest and yielded the highest among the four varieties tested. Generally, the growth rates of all cultivars were comparable during the first 2 weeks after seeding, but afterwards, the Tanzanian varieties grew much faster. The Taiwan commercial amaranth varieties may not be acceptable to the Tanzanians and, perhaps, even the rest of Africa, where most of the commonly grown cultivars are the rapidly growing types.

The observational trial of 82 amaranth accessions from the AVRDC collection indicated a wide diversity of characteristics such as stem and leaf color, vigor, size, and shape of the leaves, flowering behavior, plant height and weight, growth rate, and leaf/stem yield. Bimodal distribution was evident for growth rate. Fastest growth was observed in the local cultivar, Tanzania Red (*Amaranthus hypochondriacus*). Distribution for plant weight was noticeably skewed, with most accessions falling under low weight classes. Heaviest plants were produced by the local cultivar, Tanzania Red. Histogram for yield largely reflected that for plant weight. Again, the best yielder was the local cultivar, Tanzania Red. Thirteen accessions that performed relatively well were selected and will need to be multiplied for larger field trials in Tanzania and other African countries.

Introduction

Leafy amaranth is widely grown and is a very popular vegetable in Tanzania. It is consumed largely as a relish and goes traditionally with "ugali" or cooked corn flour. In destitute regions of Tanzania, it is often the major source of proteins, vitamins, and minerals for the local people. Amaranth is grown both in the home and urban gardens, and is consumed, as well as sold in the local markets.

In recent years, consumption of vegetables like amaranth, especially in urban areas of Tanzania, has grown tremendously. There is, thus, a need to increase production to supply the market demand. However, there is scanty information related to the improvement of productivity, improved cultivars, and cultural practices.

Two trials to evaluate the newly introduced amaranth germplasm were carried out in 1994. These experiments were conducted to compare the yield and other characteristics of the introduced amaranth accessions with the commonly grown local cultivars of Tanzania.

Materials and Methods

The first trial compared four varieties: Taiwan White Leaf, Taiwan Edible Red, Tanzania Green (*Amaranthus cruentus*), and Tanzania Red (*Amaranthus hypochondriacus*). Seeds of the Taiwan varieties are sold commercially by Taiwan seed companies and were collected and sent to ARP by AVRDC's GRSU.

About 5.45 g seeds of each entry was mixed with an equal amount of white sand as seed carrier. The seed-sand mixture was direct seeded on 10 October 1994, on 3-m-long beds, spaced 90 cm apart. The experiment was laid out using an RCBD with four replications.

Germination was observed on 13 October. The trial was irrigated twice a day. Starter solution consisting of dissolved urea at 3% was applied twice (2 and 3 weeks after seeding) during the duration of the experiment.

Data on growth rate (measured as progressive seedling height) using five samples in each plot were recorded beginning 22 October and repeated at 3-day intervals three times thereafter. A final plant height measurement was made at harvest on 9 November. Plant yield (stem and leaves) was recorded for each plot.

The second trial was carried out as an observational trial of 80 amaranth accessions that were provided earlier by GRSU from the center's collection. As the amount of seeds of each accession was not adequate, this experiment was not replicated.

About 1 g seeds of each accession was mixed with white sand as seed carrier. The seed-sand mixtures were direct seeded 14 October 1994, on 1-m-long and 90-cm-wide beds.

Germination was observed 3 days later on most accessions. The trial was irrigated twice a day. Starter solution consisting of dissolved urea at 3% was applied twice (at 2 and 3 weeks after seeding) during the course of the experiment. In addition, urea and triple superphosphate were applied basally at the rate of 10 kg N/ha and 10 kg P_2O_5/ha by incorporating the fertilizer into the soil prior to planting.

Data on growth rate (measured as progressive seedling height) using five samples in each plot was recorded beginning 25 October and repeated at 3-day intervals three times thereafter. A final plant height measurement was made at harvest on 16 November. Total plant yield (stem and leaves) was recorded for each plot.

Results and Discussion

Table 13 shows the results of the first trial comparing the commercial amaranth cultivars introduced from Taiwan with the local cultivars of Tanzania. The Taiwan cultivars were weaker, slower growing, and lower yielding than the Tanzanian varieties. In particular, Tanzania Red (*Amaranthus hypochondriacus*) had the fastest growth rate and the highest yield among the four varieties tested.

Generally, the growth rate of all cultivars was comparable during the first 2 weeks after seeding (D_1 and D_2), but afterwards (D_3, D_4, and at harvest) the Tanzanian varieties grew much faster (fig. 4).

It does not appear from the results of this field trial that the Taiwan commercial amaranth varieties will be acceptable to the Tanzanians and, perhaps, even the rest of Africa, where most of the commonly grown cultivars are the rapidly growing types.

Table 13. Comparative horticultural traits of Taiwan and local amaranth cultivars of Tanzania, ARP-MRTS, 1994[a]

Cultivars	Plant height at harvest (cm)	Mean plant weight (g)	Mean growth rate (cm/day)	Unadjusted plant yield (t/ha)	Adjusted plant yield (t/ha)
Taiwan White Leaf	4.2 a	4.9 b	0.23 a	4.4 a	4.6 a
Taiwan Edible Red	8.6 b	2.5 a	0.47 b	4.5 a	9.1 b
Tanzania Green	29.8 c	6.8 b	1.65 c	12.1 b	12.6 b
Tanzania Red	43.0 d	11.5 c	2.39 d	16.0 c	21.3 c

[a] Mean column separation by Duncan's multiple range test at P = 5%
Note: Approximate population density = 1,850,000 plants/ha

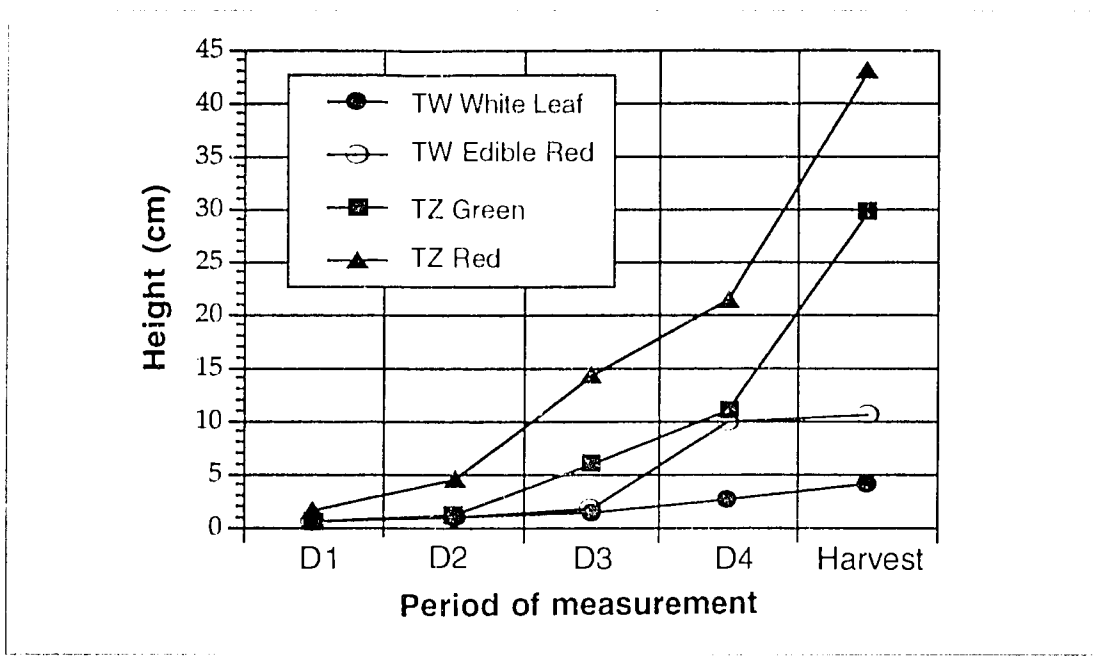

Fig. 4. Comparative growth rate of introduced leafy amaranth cultivars from Taiwan and popularly grown local cultivars in Tanzania

The observational trial of 82 amaranth accessions from the AVRDC collection indicated a wide diversity in stem and leaf color, vigor, size, and shape of the leaves, flowering behavior, plant height and weight, growth rate, and leaf/stem yield. Fig. 5-7 show histograms of the 82 accessions for characters such as growth rate, plant weight, and estimated yield.

The fastest growth was observed in the local cultivar, Tanzania Red (*Amaranthus hypochondriacus*), followed by accession A 86 from India (fig. 5). Bimodal distribution for mean growth rate is evident.

The distribution for mean plant weight of the 82 accessions is noticeably skewed (fig. 6), with most accessions falling under the relatively low plant weight classes. Only three accessions exhibited mean plant weights over 25 g. Again, the heaviest plants were produced by the local cultivar, Tanzania Red.

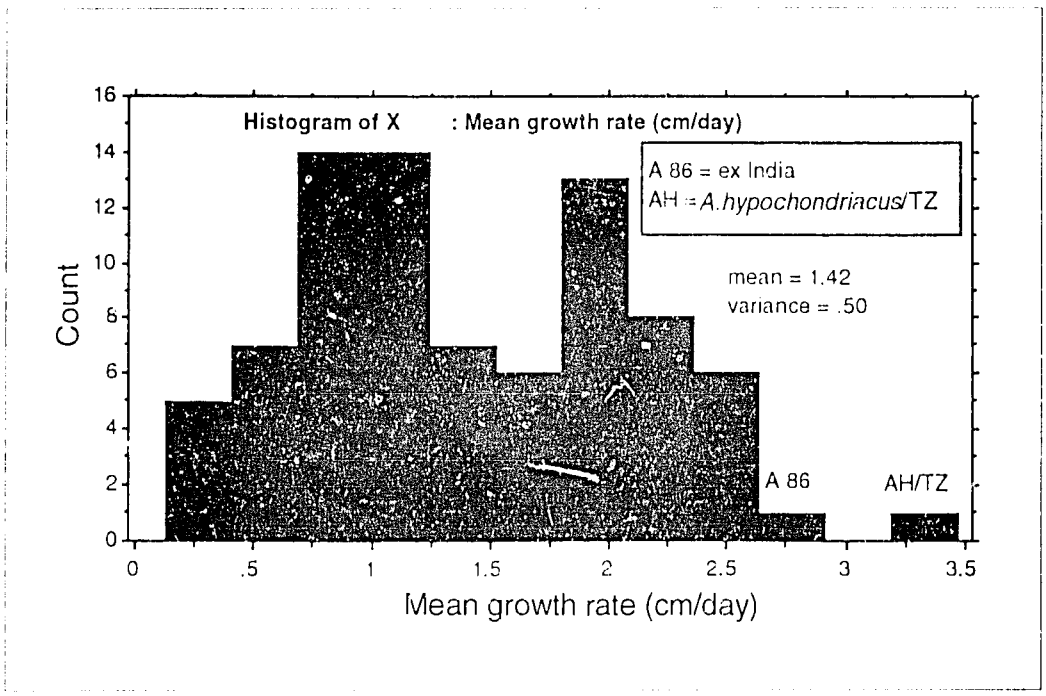

Fig. 5. Frequency distribution of mean growth rate (cm/day) of 82 amaranth accessions tested in ARP-MRTS, 1994

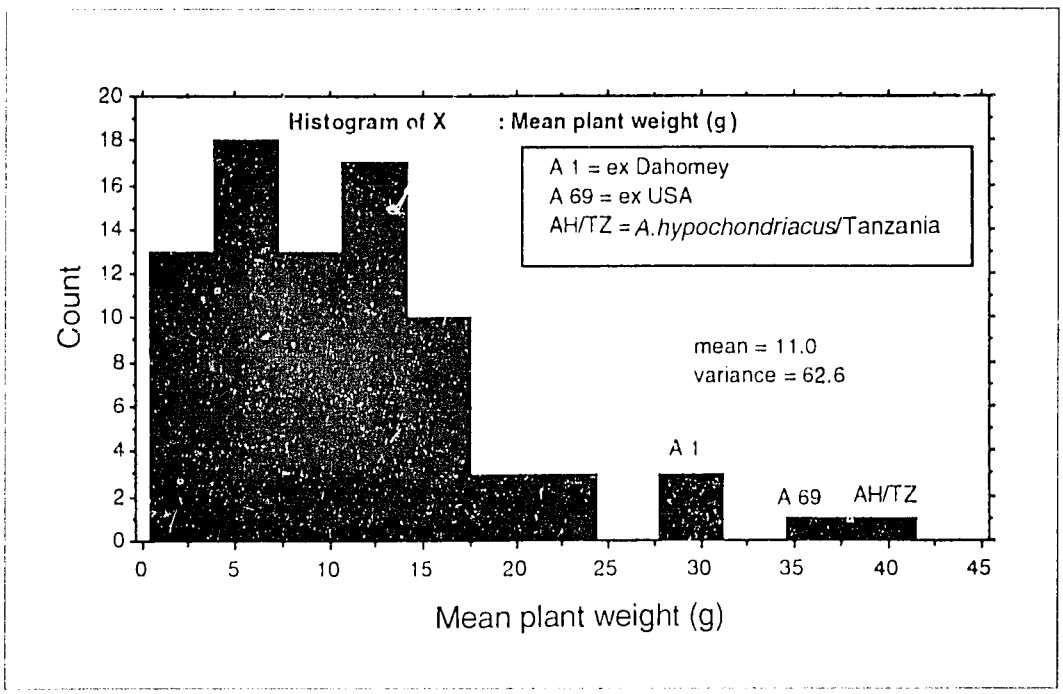

Fig. 6. Frequency distribution of mean plant weight (g) of 82 amaranth accessions tested in ARP-MRTS, 1994

The histogram for estimated yield largely mirrored that for mean plant weight, both in nature of distribution and in the identity of the promising accessions (fig. 7). Again, the best yielder was Tanzania Red, followed by A 69.

From this observational trial, we selected 13 accessions that performed relatively well in terms of general vigor, plant weight, and yield. These accessions need to be multiplied in larger quantity for larger field trials in Tanzania and other African countries.

In terms of overall vigor and yield, the Tanzania Red variety is still the best cultivar.

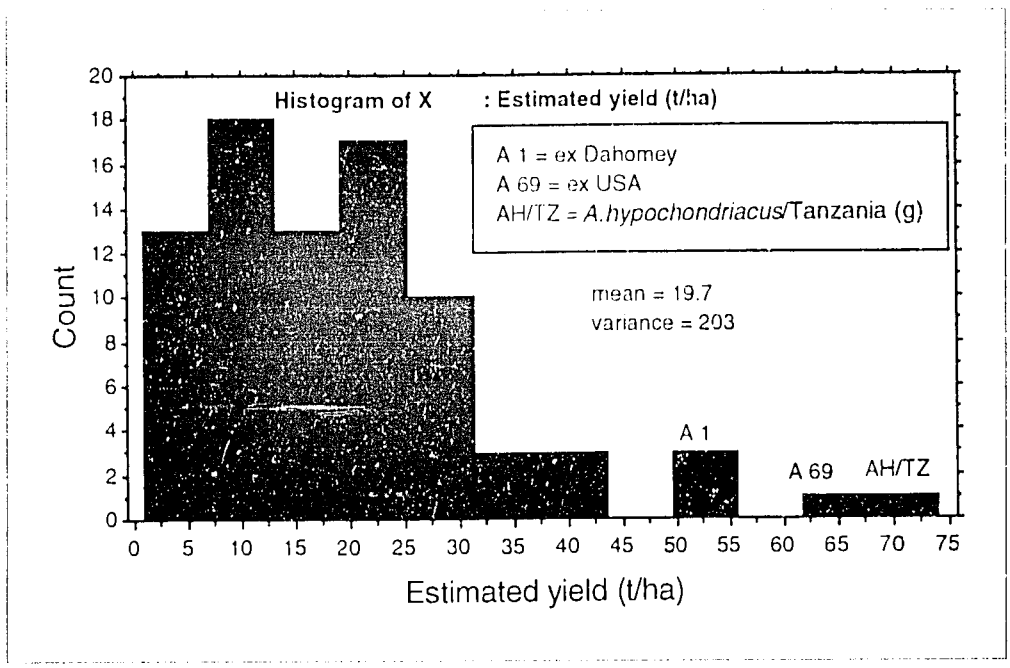

Fig. 7. Frequency distribution of approximate yield (t/ha) of 82 amaranth accessions tested in ARP-MRTS, 1994

Training Activities

The training component of CONVERDS was funded by the German Ministry for Economic Cooperation/German Agency for Technical Cooperation (BMZ/GTZ) beginning in March 1994. Following that funding commitment, the ARP staff began preparation for the First Regional Vegetable Production Course which began in May 1994.

A total of 16 training scholars participated in this 5-month course. Of these, 13 came from the SADC countries and three from neighboring African countries (Kenya and the Seychelles). In addition, three special-purpose trainees participated; two from the SADC countries, and one student from Holland who joined on part-time research internship for 6 months.

At the end of the training program, the participants were asked to score the potential usefulness of the course on a scale of 1 (= not useful) to 5 (= very useful). Of 16 students who submitted their evaluation sheets, 15 rated the course as very useful and 1, useful.

General comments raised by the students to improve the course were as follows: accommodation problems, particularly lack of electricity and water; lack of in-depth and quality lectures in some subject matter areas of the curriculum; insufficient practical sessions in some disciplines, e.g., statistics, plant breeding, cultural practices, and others; poor field trip exposures in Tanzania; and limited leisure and extracurricular activities in Arusha.

The first batch of vegetable production trainees graduated on 28 September. The commencement exercise was officiated by the principal secretary of the Ministry of Agriculture (represented by the commissioner for research and training), and attended by the SACCAR board chairman and SACCAR director, and by the AVRDC director general, who came also to serve as one of the last resource persons in the course.

A 3-week short-term course on vegetable diseases, with emphasis on disease diagnostics, distribution, and strain-typing, was also conducted from 2 to 22 October. The aim of this course was to build a capable subnetwork of national personnel working on vegetable diseases in the SADC region and neighboring East African countries. These NARS scientists, in close collaboration with ARP plant pathologists, are expected to generate more knowledge on vegetable diseases than had been possible in the past, following the intensive short course.

A total of 16 NARS personnel, 13 from SADC countries, and 3 from neighboring African countries (Kenya and Uganda), participated in this course.

As in the earlier vegetable production course, the participants were asked to evaluate the usefulness of the course. Of 14 who turned in their evaluation sheets, 11 rated the course as very useful and 3 as useful. Although the ratio of hours of practicals to hours of lectures in this short course was already about 2:1, 10 of 16 participants suggested that more practicals should have been programmed. The most glaring complaint had been the inadequacy of laboratory equipment, particularly microscopes for practical work.

Donors and/or sponsors who supported the participants in these two courses were BMZ/GTZ, AVRDC, FAO, German Agency for International Development (DSE), and USAID.

Networking and Priority Setting Activities in the NARS

A tomato network planning workshop was coorganized with Zimbabwe, the lead NARS for tomato research in the SADC region, on 7-11 February 1994, and attended by the national vegetable research team leaders from seven SADC countries. A total of 16 participants and 5 invited speakers from various sectors of Zimbabwe's tomato industry participated.

In this meeting, high priority was accorded to the assembly, distribution, and testing of advanced tomato germplasm from seed companies and public research organizations in Africa and other parts of the world, based on the major requirements of the crop in the region. Zimbabwe will assume the role of assembling the improved germplasm and will provide a master list of assembled cultivars, complete with their major traits, to the various NARS so that each could select the most appropriate cultivars to include in its national testing schemes. This regional tomato germplasm testing activity will commence in 1995.

In 1993, ARP assisted in the formation of the national vegetable research team of Tanzania and in the prioritization of the vegetable research program of the country. The national vegetable research team has since then embarked on pursuing its research priorities. For example, the national tomato germplasm testing program has been resuscitated in 1994. Four AVRDC breeding lines, two indeterminate and two determinate lines, have been entered in the national trials. Based on the 1993

and 1994 trial results, the national vegetable research team has communicated that at least two of the AVRDC breeding lines shall be entered in the 1995 on-farm trials.

Three national priority setting workshops were explored with the other lead NARS of CONVERDS, Zambia, Malawi, and Zimbabwe. Zimbabwe declined to carry out the exercise, rationalizing that this process is already built in their annual research planning exercise. The proposed meetings in Zambia and Malawi did not materialize as planned. The meetings have now been reprogrammed with the national vegetable research team leaders of the two countries and hopefully, will be rescheduled sometime in 1995.

Program Staffing

The senior plant pathologist funded under the Africa tomato breeding project was hired in January 1994 and joined the project in February 1994.

Following negotiations with the Ministry of Agriculture, Livestock and Development Cooperatives of Tanzania (MALDC), two staff members of HORTI-Tengeru, one tutor (trainer), and one laboratory technician, were seconded to work with ARP.

One field assistant, a second driver, and a watchman were also hired on full-time basis by the project during the year.

In April, the research assistant in vegetable breeding resigned from secondment duties with ARP and rejoined HORTI-Tengeru as coordinator of the national seed production program. To date, this post has not yet been filled up.

In August, the project secretary also resigned. This post has not been filled.

Facility Development

The development of the 14-acre site at the Madiira Farm of HORTI-Tengeru is now complete, except for facilities such as screenhouses. Research and training activities are now fully concentrated at MRTS. This facility has a field house, irrigation and drainage canals, water reservoir, and guard houses, and is fully secured by a fence.

At HORTI-Tengeru, renovation of the offices of three senior staff members, one secretary, and one general-purpose storage space has been completed. The plant pathology laboratory was also renovated. A screenhouse for plant pathology research is yet to be constructed.

Renovation of the library for research and training purposes is also complete. The soils laboratory adjoining the library was converted into a training classroom and was used in the last two courses.

The guest dining room at the Livestock Training Institute was also renovated and equipped with appliances, kitchen utensils, and other accessories for use by ARP training scholars.

The development of the regional research and training facilities at HORTI-Tengeru and at the Madiira farm, except for hostel facilities for training and screenhouses for research, has been completed.

Appendices

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Chief of Agricultural Affairs
Taipei Office, Interchange Association
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Taipei, Taiwan, ROC

Dr. Te-yeh Ku
Director, Food and Agriculture Department
Council of Agriculture, Executive Yuan
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[a] **Resigned chairmanship effective April 1994**
[b] **Effective April 1994**

Staff

Administration

Samson C.S. Tsou, Ph.D., Director General
Tung-hai Huang, MBA, Director of Administration
Nancy C. Chao, Assistant Comptroller
Jeng-hua Chen, B.S., Superintendent, Buildings and Maintenance
Sui-fang Wu, B.A., Manager, Food and Dormitory Services

Crop Improvement Program

Program Director: C. George Kuo, Ph.D.

Breeding

Crucifer
Lien-chung Chang, B.S., Associate Specialist

Pepper
Jean M. Poulos, Ph.D., Associate Plant Breeder
Ren-chieh Jin, B.S., Senior Research Assistant
Chien-rong Ho, B.S., Research Assistant[c]
Yayeh Zewdie, M.S., Research Fellow

Soybean
S. Shanmugasundaram, Ph.D., Plant Breeder
Miaw-rong Yan, B.S., Research Assistant

Tomato
Peter H. Hanson, Ph.D., Associate Plant Breeder[1]
Jen-tzu Chen, B.S., Associate Specialist

Allium
C.S. Pathak, Ph.D., Scientist
Shin-jiun Cherng, M.S., Principal Research Assistant[bd]
Swee Suak Ko, M.S., Principal Research Assistant

Entomology

N.S. Talekar, Ph.D., Entomologist[b]
Wen-jin Hu, M.S., Principal Research Assistant
Mei-yin Lin, M.S., Principal Research Assistant
Fu-cheng Su, B.S., Senior Research Assistant
Bruce L. Parker, Ph.D., Visiting Scientist[c]
(8 January-22 December)

Plant Pathology

Lowell L. Black, Ph.D., Plant Pathologist (Mycology)
Sylvia K. Green, Ph.D., Plant Pathologist (Virology)
Jaw-fen Wang, Ph.D., Associate Plant Pathologist[d]
(Bacteriology)
Tien-cheng Wang, M.S., Associate Specialist
Yi-hsiou Huang, M.S., Principal Research Assistant[a]
Ber-tsu Chiange, M.S., Principal Research Assistant
Su-ling Shih, M.S., Principal Research Assistant
Jung-chin Chou, B.S., Electron Microscope Technician
Tsai-hsia Li, M.S., Principal Research Assistant
Yuan-chuen Lin, M.S., Principal Research Assistant[c]
Rodel Maghirang, Ph.D., Visiting Scientist
(4-29 September)

Plant Physiology

C. George Kuo, Ph.D., Plant Physiologist
Huei-mei Chen, M.S., Assistant Specialist
Tyng-shyang Lai, M.S., Principal Research Assistant
An-ni Tsai, M.S., Principal Research Assistant
Jinq-chyi Chang, M.S., Principal Research Assistant
Pi-er Huang, B.S., Research Assistant
Wen-Ju Yang, Ph.D., Research Associate[c]

Genetic Resources and Seed Unit

Liwayway M. Engle, Ph.D., Geneticist and Head
Yeong-ho Lee, Ph.D., Visiting Associate Scientist
Yung-kuang Huang, M.S., Principal Research Assistant
Jia-chain Shieh, B.S., Research Assistant
Ching-huan Chang, B.S., Research Assistant
Elizabeth M. Libas, M.S., Research Fellow
Ann Inez N. Gironella, Ph.D., Visiting Resource Scientist (18 October-18 November)
Mae P. Cababasay, B.S., Research Fellow
(18 October-18 November)

Production Systems Program

Program Director: David J. Midmore, Ph.D.

Crop Management

David J. Midmore, Ph.D., Crop Physiologist
Takeo Koyama, Ph.D., Environmental Physiologist
Yu-chi Roan, B.S., Associate Specialist
Mei-huey Wu, B.S., Principal Research Assistant
Feei-yu Jiang, M.S., Principal Research Assistant

Soil Science

Richard A. Morris, Ph.D., Soil Scientist
Chin-hua Ma, M.S., Associate Specialist
Chun-jen Chen, M.S., Principal Research Assistant
Shu-chun Tseng, M.S., Principal Research Asistant
(August-September)

Chien-ling Chen, B.S., Research Assistant[a]
Clarita Aganon, Ph.D., Postdoctoral Fellow (4 July-2 October)

Socioeconomics

Hans G.P. Jansen, Ph.D., Associate Agricultural Economist[f]
Mubarik Ali, Ph.D., Associate Agricultural Economist
Robin Marsh, Ph.D., Postdoctoral Fellow (located at IICA, Costa Rica)
Huei-lin Huang, M.S., Principal Research Assistant[f]
Mong-chun Hu, M.S., Principal Research Assistant
Deng-fang Sheu, M.S., Principal Research Assistant (January-November)
Sunkara Valasayya, Research Fellow[f]
Durga Dutta Poudel, M.S., Research Fellow[f]
Muthiah Asokan, M.S., Research Fellow

International Cooperation Program

Program Director: S. Shanmugasundaram, Ph.D.

Training

Yi-ming Chen, M.S., Principal Training Assistant

Publications and Communications

Jack W. Reeves, J.D., Communications Specialist and Head
Sylvia Katherine S. Lopez, M.S., Research Fellow
Cristina P. Bejosano, B.S., Research Fellow[a]
Ma. Victoria Dinnah F. Cabangbang, B.S., Research Fellow

Information and Documentation

Teng-hui Hwang, B.A., Senior Librarian
Fang-chin Chen, B.A., Catalog Librarian
Shwu-min Hsu, B.A., Documents Librarian
Huei-hua Wang, B.A., Acquisition Librarian[a]
Wen-yu Yeh, B.A., Acquisition Librarian[c]
Jin-tien Hu, Senior Library Clerk

Cooperative Programs

Country Program

Nung-che Chen, Ph.D., Horticulturist (also OIC of Crucifer Breeding Group)
Huei-mei Li, M.S., Principal Research Assistant
V. Ponnuswami, Ph.D., Visiting Scientist[c]

Indonesia-AVRDC Vegetable Research Program

Farid A. Bahar, Ph.D., Director, Central Research Institute for Horticulture, Jalan Ragunan 29, Pasar Minggu, Jakarta, Indonesia

Malaysia-AVRDC Vegetable Research Program

Mohammed Yusof bin Hashim, Ph.D., Director General, Malaysian Agricultural Research and Development Institute, Kuala Lumpur, Malaysia

Philippines-AVRDC Outreach Program

Benjamin M. Legaspi, B.S., Project Director, Los Baños National Crop Research and Development Center, Bureau of Plant Industry, Department of Agriculture, Laguna, Philippines

Korea-AVRDC Outreach Program

Ju-ho Chung, Ph.D., Director, Vegetable Cultivation Division, Horticultural Experiment Station, Rural Development Administration, Imok Dong, Suweon, Rep. of Korea

Bangladesh-AVRDC Agricultural Research Program

Madan Mohan Lal Chadha, Ph.D., Senior Horticulturist/Agronomist, Dhaka-1212, Bangladesh

Nepal-AVRDC Vegetable Research Program

R.K. Raut, Ph.D., Chief, Vegetable Development Division, Nepal Agricultural Research Council, Khumaltar Agricultural Research Station, Lalitpur, Nepal

Regional Programs

Asian Regional Center, Bangkok, Thailand

Charles Y. Yang, Ph.D., Director
Doo-hwan Kim, Ph.D., Associate Plant Breeder

Africa Regional Program, SADCC-AVRDC-CONVERDS, Arusha, Tanzania

Romeo T. Opeña, Ph.D., Director
Remi Nono-Womdim, Ph.D., Associate Plant Pathologist[c]

Research Support Services

Analytical Laboratory

Samson C.S. Tsou, Ph.D., Biochemist
Tuan-liang Hong, Ph.D., Principal Research Assistant
Ray-yui Yang, M.S., Principal Research Assistant
Mong-hsin Ouyang, B.S., Research Assistant

Statistics and Computer Services

Hsien-yang Tien, B.S., Assistant Computer Specialist
Yuh-ling Chen, B.S., Computer Assistant

Farm Operations

Teng-sheng Tu, B.S., Farm Superintendent

[a] Left during 1994
[b] Reinstated in 1994
[c] Arrived during 1994
[d] Promoted in 1994
[e] On sabbatical
[f] Change of title

Publications

Symposium Proceedings

AVRDC. 1993. Germplasm collection, evaluation, documentation, and conservation. (A compilation of lecture materials of a training course held in BARI, Joydebpur, Gazipur, Bangladesh, 4-6 May 1992). AVRDC, Shanhua, Tainan, Taiwan. Publication no. 93-398. 95 p.

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Custodio, H.M. III, Engle, L.M., and Laude, R.P. 1994. Genetic diversity of seed proteins in populations of mungbean *Vigna radiata* (L.) Wilczek from the Philippines. Abstract. The Philippine Journal of Crop Science 19(Supplement No. 1):2

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Midmore, D.J. and Poudel, D.D. 1994. Asian vegetable production systems for the future. Agricultural Systems (in press).

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Oswald, A., Alkamper, J., and Midmore, D.J. 1994. The effect of different shade levels on growth and tuber yield of sweet potato: I. Plant Development. J. Agron. and Crop Sci. (in press).

Pathak, C.S. 1993. Flowering and fertility studies in garlic (*Allium sativum* L.) Allium Improvement Newsletter: 3: 15-16.

Pathak, C.S. 1994. A possible new source of male sterility in onion. The 1st International Symposium on Edible Alliaceae, 14-18 March 1994, Mendoza, Argentina. Abstract p. 48.

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Poulos, J.M. 1993. Germplasm evaluation and utilization. pp. 69-74 In: AVRDC. 1993. Germplasm collection, evaluation, documentation, and conservation. (A compilation of lecture materials of a training course held in BARI, Joydebpur, Gazipur, Bangladesh, 4-6 May 1992). AVRDC, Shanhua, Tainan, Taiwan. Publication no. 93-398, 95 p.

Poulos, J.M. 1994. Pepper breeding (*Capsicum* spp.): achievements, challenges, and possibilities. [invited review article] Plant Breeding Abstracts Vol. 64 (2):143-155.

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Roberts, E.H., Qi, A., Ellis, R.H., Summerfield, R.J., Lawn, R.J., and Shanmugasundaram, S. 1994. AVRDC's activities related to soybean. Report of the Regional Expert Consultation on The Asian Soybean Network. Bangkok and Chiang Mai, Thailand, 20-26 February 1994. RAPA Publication: 1994/15.

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Shanmugasundaram, S. 1994. Strengthening Asian countries' efforts to improve vegetable production. Paper presented in the XXIV International Horticultural Congress, August 1994, Kyoto, Japan.

Shanmugasundaram, S. 1994. Use of field observations to characterize genotypic flowering responses to photoperiod and temperature: a soybean exemplar. Expt. Agrl. (in press).

Shanmugasundaram, S., Tsou, S.C.S., and Hong, T.L. 1994. Vegetable soybeans production and research. Paper presented in the World Soybean Research Conference, 21-27 February 1994, Chiang Mai, Thailand.

Shanmugasundaram, S. and Yan, M.R. 1993. Institutional Report 1 - AVRDC. p. 151-157. In: Proceedings of the Planning Workshop for the Establishment of the Asian Component of a Global Network on Tropical and Subtropical Soybeans. 2-7 March 1992. Chiang Mai, Thailand. RAPA Publication: 1993/6.

Talekar, N.S. 1993. Biological studies with *Diadegma semiclausum* (Hymenoptera: Ichneumonidae), a parasite of diamondback moth. Entomophaga 38: 579-586.

Talekar, N.S. and Yang, J.C. 1993. Effect of crucifer cropping system on the parasitism of *Plutella xylostella* (Lepidoptera, Yponomeutidae) by *Cotesia plutellae* (Hymenoptera, Braconidae) and *Diadegma semiclausum* (Hymenoptera, Ichneumonidae). Entomophaga 38: 541-550.

Talekar, N.S. and Lin., C.P. 1994. Characterization of resistance to limabean pod borer (Lepidoptera: Pyralidae) in soybean. J. Econ. Entomol. 87: 821-825.

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Valverde, R.A., Black, L.L., and Dufresne, D.J. 1994. A comovirus from tabasco pepper, *Capsicum frutescens*. Plant Dis. 78:0000-0000 (accepted for publication).

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Wang, J.F., Jone, J.B., Scott, J.W. and Stall, R.E. 1994. Several genes in *Lycopersicon esculentum* control hypersensitivity to *Xanthomonas campestris* pv. *vesicatoria*. Phytopathology 84: 702-706.

Yang, J.C., and Chu, Y.I., and Talekar, N.S. 1993. Biological studies with *Diadegma semiclausum* (Hymenoptera: Ichneumonidae), a parasite of diamondback moth. Entomophaga 38:579-586.

Financial Statements

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The Board of Directors
The Asian Vegetable Research and
Development Center

We have examined the statements of assets, liabilities and fund balances of The Asian Vegetable Research and Development Center as of December 31, 1994 and 1993, and the related statements of changes in core fund, working capital fund, restricted core fund, special projects fund, and self-sustaining operation fund for the years then ended. Our examinations were made in accordance with generally accepted auditing standards and, accordingly, included such tests of the accounting records and such other auditing procedures as we considered necessary in the circumstances.

As mentioned in Note 2 to the financial statements:

1. The Center maintains its accounts on a cash basis, except for the inclusion of provisions and reserves for certain employee benefits.

2. Capital expenditures are accounted for as deductions from funds and, accordingly, are not included in assets.

3. The Center records its transactions in the currencies in which these are denominated. The accompanying statements reflect the actual U.S. dollar amounts of transactions in U.S. dollars, and the U.S. dollar equivalents of transactions in other currencies based on year-end bank buying exchange rates, except contributions and grants which are translated at the exchange rates in effect when these were received.

In our opinion, the financial statements referred to in the first paragraph present fairly the assets, liabilities and fund balances of The Asian Vegetable Research and Development Center as of December 31, 1994 and 1993, and the changes in its funds for the years then ended, on the basis of accounting described in the second paragraph, which basis has been applied on a consistent manner.

In connection with our examinations, we noted no indication that the Center was not in compliance with any of the terms, conditions, or provisions of its grant agreements. It should be noted, however, that our examinations were not directed primarily toward obtaining knowledge of such non-compliance.

T.N. Soong & Co

March 14, 1995

THE ASIAN VEGETABLE RESEARCH AND DEVELOPMENT CENTER

STATEMENTS OF ASSETS, LIABILITIES AND FUNDS
(Prepared on a Modified Cash Basis and
Expressed in U.S. Dollars - Note 2)

	December 31	
ASSETS	1994	1993
CASH	$3,405,112	$4,400,223
ADVANCES AND REFUNDABLE DEPOSITS (Note 3)	363,011	442,946
PREPAYMENTS	27,887	17,183
TOTAL ASSETS	$3,796,010	$4,860,352
LIABILITIES AND FUND BALANCES		
RECEIPTS FOR CUSTODY (Note 4)	$ 271,025	$ 168,281
RESERVES FOR EMPLOYEE BENEFITS (Note 5)	1,218,654	1,167,582
FUNDS		
Core fund	934,898	1,000,110
Working capital fund	900,000	800,000
Restricted core fund	142,328	478,935
Special projects fund	220,576	1,133,402
Self-sustaining operation fund	108,529	112,042
Total Funds	2,306,331	3,524,489
TOTAL LIABILITIES AND FUNDS	$3,796,010	$4,860,352

The accompanying notes are an integral part of the financial statements.

(With T N Soong & Co report dated March 14, 1995)

THE ASIAN VEGETABLE RESEARCH AND DEVELOPMENT CENTER

STATEMENTS OF CHANGES IN CORE FUND (Note 6)
(Prepared on a Modified Cash Basis and Expressed in U.S. Dollars - Note 2)

	Years Ended December 31	
	1994	1993
ADDITIONS		
Contributions		
Republic of China	$5,337,614	$5,386,901
Japan	733,000	233,000
Federal Republic of Germany (from restricted core fund)	712,461	439,827
United States of America	675,000	1,000,000
France	296,049	320,331
Republic of Korea	150,000	183,527
Australia	144,390	126,575
Philippines	100,000	248,592
Thailand	-	209,888
World Bank	-	550,000
Total contributions	8,148,514	8,698,641
Grants from Japan International Cooperation Agency	85,839	60,431
Translation adjustment (Note 2)	19,971	(90,512)
Other (Note 6)	710,711	630,667
Total Additions	8,965,035	9,299,227
DEDUCTIONS		
Capital expenditures (Notes 2 and 6)	291,699	581,556
Operating expenditures (Note 6)	8,671,306	8,191,550
Transfer to working capital fund	100,000	300,000
Total Deductions	9,063,005	9,073,106
NET INCREASE (DECREASE) IN FUND	(97,970)	226,121
BALANCE, BEGINNING OF YEAR		
As previously reported	1,000,110	841,864
Translation adjustment (Note 2)	32,758	(67,875)
As restated	1,032,868	773,989
BALANCE, END OF YEAR	$ 934,898	$1,000,110

The accompanying notes are an integral part of the financial statements.

(With T N Soong & Co report dated March 14, 1995)

THE ASIAN VEGETABLE RESEARCH AND DEVELOPMENT CENTER

STATEMENTS OF CHANGES IN WORKING CAPITAL FUND (Note 7)
(Prepared on a Modified Cash Basis and Expressed in
U.S. Dollars - Note 2)

	Years Ended December 31	
	1994	1993
ADDITIONS		
Transfer from Core Fund	$100,000	$300,000
DEDUCTIONS	-	-
NET INCREASE IN FUND	100,000	300,000
BALANCE, BEGINNING OF YEAR	800,000	500,000
BALANCE, END OF YEAR	$900,000	$800,000

The accompanying notes are an integral part of the financial statements.

(With T N Soong & Co report dated March 14, 1995)

THE ASIAN VEGETABLE RESEARCH AND DEVELOPMENT CENTER

STATEMENTS OF CHANGES IN RESTRICTED CORE FUND (Note 8)
(Prepared on a Modified Cash Basis and Expressed in U.S. Dollars - Note 2)

	Years Ended December 31	
	1994	1993
ADDITIONS		
From German Agency for Technical Cooperation	$320,554	$546,008
DEDUCTIONS		
Transfer to Core Fund	(712,461)	(439,827)
NET INCREASE (DECREASE) IN FUND	(391,907)	106,181
BALANCE, BEGINNING OF YEAR		
As previously reported	478,935	400,145
Translation adjustment (Note 2)	55,300	(27,391)
As restated	534,235	372,754
BALANCE, END OF YEAR	$142,328	$478,935

The accompanying notes are an integral part of the financial statements.

(With T N Soong & Co report dated March 14, 1995)

THE ASIAN VEGETABLE RESEARCH AND DEVELOPMENT CENTER

STATEMENTS OF CHANGES IN SPECIAL PROJECTS FUND (Note 9)
(Prepared on a Modified Cash Basis and Expressed in
U.S. Dollars - Note 2)

	Year Ended December 31, 1993					Year Ended December 31, 1994			
Sponsors	Balance, Beginning of Year	Translation Adjustment	Additions	Deductions	Balance, End of Year	Translation Adjustment	Additions	Deductions	Balance, End of Year
Council of Agriculture/ROC	$197,558	($ 9,426)	$1,414,172	$ 810,277	$ 792,027	$ 8,723	$ 799,973	$1,492,408	$ 108,315
Asian Development Bank	(4,065)	-	524,939	457,022	63,852	-	40,000	317,624	(213,772)
Japan Government	61,974	-	310,000	199,342	172,632	-	310,000	311,434	171,198
U.S. AID	(65,599)	-	227,794	268,012	(105,817)	-	306,349	195,190	5,342
International Development Research Center	-	-	130,962	125,500	5,462	-	103,276	105,462	3,276
National Science Council/ROC	9,875	(471)	13,422	22,826	-	-	20,737	-	20,737
Potash and Phasthate Institute of Canada	1,047	-	12,000	9,828	3,219	-	11,995	9,744	5,470
Rural Development Administration/Korea	15,722	-	19,982	12,582	23,122	-	19,985	19,003	24,104
Federal Republic of Germany	-	-	-	-	-	-	387,338	336,579	50,759
Swiss Development Cooperation	28,262	-	87,615	86,057	29,820	-	95,649	100,975	24,494
Others	373,051	(1,546)	335,963	558,383	149,085	(372)	85,670	213,730	20,653
	$617,825	($ 11,443)	$3,076,849	$2,549,829	$1,133,402	$ 8,351	$2,180,972	$3,102,149	$ 220,576

The accompanying notes are an integral part of the financial statements.

(With T N Soong & Co report dated March 14, 1995)

THE ASIAN VEGETABLE RESEARCH AND DEVELOPMENT CENTER

STATEMENTS OF CHANGES IN SELF-SUSTAINING OPERATION FUND (Note 10)
(Prepared on a Modified Cash Basis and Expressed in
U.S. Dollars - Note 2)

	Years Ended December 31	
	1994	1993
ADDITIONS (Note 10)		
Dormitory rentals	$ 56,572	$ 53,372
DEDUCTIONS (Note 10)		
Dormitory expenses	(60,085)	(27,916)
NET INCREASE (DECREASE) IN FUND	(3,513)	25,456
BALANCE, BEGINNING OF YEAR	112,042	86,586
BALANCE, END OF YEAR	$108,529	$112,042

The accompanying notes are an integral part of the financial statements.

(With T N Soong & Co report dated March 14, 1995)

THE ASIAN VEGETABLE RESEARCH AND DEVELOPMENT CENTER

NOTES TO FINANCIAL STATEMENTS
(Amounts Expressed in U.S. Dollars Unless Otherwise Stated)

1. ORGANIZATION AND OPERATIONS

The Center was established in the Republic of China as an autonomous, philanthropic, and non-profit research and development organization for promoting production, marketing and utilization of vegetables in Asia. Its members consist of the original signatories to the Center's Charter and other countries or organizations approved for membership by the Center's Board of Directors.

The financial requirements of the Center are funded mainly by contributions and grants or donations from member and non-member countries or organizations.

The Center may terminate its operations by a resolution adopted unanimously by all members of the Board of Directors. In case the Center terminates its operations, all buildings, equipment and other assets belonging to the Center (and/or affiliated sub-centers) will be transferred, upon the concurrence and approval of the Board of Directors and host country, to organizations in the host country which were formed and are operated exclusively for scientific or educational purposes and which meet certain conditions prescribed in the Center's Charter.

2. BASIS OF ACCOUNTING

The Center maintains its accounts on a cash basis, except for the inclusion of provisions and reserves for employee benefits, namely, unused compensated annual leave and repatriation expenses.

The Center records its transactions in the currencies in which these are denominated. The accompanying statements reflect the actual U.S. dollar amounts of transactions in U.S. dollars, and the U.S. dollar equivalents of transactions in other currencies based on year-end bank buying exchange rates, except contributions and grants which are translated at the exchange rates in effect when these are received. Translation adjustments are reflected as additions to or deductions from the funds.

Capital expenditures are accounted for as deductions from funds and, accordingly, are not included in assets.

3. ADVANCES AND REFUNDABLE DEPOSITS

	December 31	
	1994	1993
Advances to Asia Regional Program	$ 80,917	$201,465
Advances to Africa Regional Program	211,368	182,800
Refundable deposits and other advances	70,726	58,661
	$363,011	$442,946

4. RECEIPTS FOR CUSTODY

These include amounts received from trainees for medical, Vitamin A Gardening Project, and field operations, less related cost and expenses.

5. RESERVES FOR EMPLOYEE BENEFITS

	December 31	
	1994	1993
Unused compensated annual leaves	$ 864,020	$ 815,722
Repatriation expenses	354,634	351,860
	$1,218,654	$1,167,582

Local employees and international staff are entitled to annual leaves of 44 days and 48 days, respectively. The Center shall compensate an employee for accumulated unused annual leaves upon resignation or termination.

The Center shall bear the transportation and relocation costs of the international staff and their immediate family upon termination of such staff employment.

6. CORE FUND

The core fund is used exclusively in support of the Center's overall operations. Contributions and grants are subject to certain terms and conditions specified in the relevant agreements.

The details of certain changes in this fund are as follows:

	1994	1993
Other additions		
Fees for various services rendered	$372,579	$307,175
Interest earned from funds other than the contribution from U.S.A.	171,389	197,073
Food and dormitory services (net of employees' salaries and benefits of $224,037 in 1994 and $216,361 in 1993)	74,996	13,263
Training fees	10,108	23,573
Refunds of various expenditures incurred in previous years	56,995	56,524
Miscellaneous	24,644	33,059
	$710,711	$630,667

Capital expenditures

Buildings and improvements	$ 75,959	$110,883
Equipment and furniture	91,544	153,410
Vehicles	84,807	285,563
Books and journals	39,389	31,700
	$291,699	$581,556

Operating expenditures

Salaries	$3,952,227	$3,563,310
Regular wages	1,159,547	1,116,400
Employee benefits	1,722,720	1,534,242
Supplies	667,062	603,845
Travel	416,775	362,927
Utilities	271,096	253,822
20th anniversary celebration	1,481	169,723
Training scholarships	111,195	90,711
Postage, telephone and cable	88,604	85,131
Moving expenses	46,244	74,701
Collaborative research	15,673	67,131
International conference	23,049	66,757
Other	195,633	202,850
	$8,671,306	$8,191,550

Employee benefits include payments for the follows:

	1994	1993
Contributions to retirement and group insurance plans for international staff	$ 197,981	$ 182,776
Contributions to retirement savings fund for local employees	475,054	443,778

The Center has made arrangements for its international staff members to be hired by the Association of International Agricultural Research Centers (AIARC) so that they may participate in suitable retirement and group insurance plans. The Center pays the entire cost of participation in these plans.

The Center has a retirement savings plan covering all local employees. The Center deposits 1/12 to 2/12 of each employee's monthly salaries, depending on service period, to a savings fund. Payments from the fund are governed by the provisions of the plan. The changes in the fund are summarized as follows:

	1994	1993
Balance, beginning of year	$2,584,483	$2,223,161
Translation adjustment	28,465	(106,064)
Contributions	475,054	433,778
Interest income	213,442	176,101
Payments	(70,088)	(142,493)
Balance, end of year	$3,231,356	$2,584,483

7. WORKING CAPITAL FUND

This fund has been established, following a common practice among international agriculture research centers. Yearly transfers are made to this fund from the core fund.

8. RESTRICTED CORE FUND

This fund is contributed by the German Agency for Technical Cooperation, and its use is restricted. The transfer from this fund to the core fund is reported as a contribution.

9. SPECIAL PROJECTS FUND

Special projects are normally based on agreed-upon budgets. Accordingly, some projects may have negative year-end balances, which shall be reimbursed in the following year.

Expenditures from this fund are subject to certain conditions and terms as set forth in agreements with the sponsors.

10. SELF-SUSTAINING OPERATION FUND

This represents the operating fund of the Center's dormitory.

11. CONTINGENCY

In 1989, the U.S. AID-supported AVRDC Vitamin A Gardening Project in Nigeria was sued by some Nigerian merchants for recovery of merchandise ordered by a U.S. Embassy employee using the Project's stationery. Most of the merchandise has been recovered by the merchants, but the lawsuit is still pending. The possible outcome of this litigation is not determinable but the Center's management believes this would not have a significant adverse effect on the Center.

Meteorological Information for 1994

Monthly mean metrorological data collected at AVRDC weather station, 1994

		January	February	March	April	May	June	July	August	September	October	November	December
Humidity	Max.	95	97	96	94	93	93	93	94	93	94	97	97
(%)	Min.	49	55	52	50	54	57	58	65	50	44	45	51
Air temp.	Max.	23.6	24.3	24.0	31.3	31.8	32.4	33.3	31.7	32.4	30.9	29.3	27.3
(°C)	Min.	13.5	15.0	14.6	21.4	23.5	24.7	25.5	25.1	23.9	20.6	18	18.2
Soil temp. (10 cm)	Max.	21.5	21.9	22.2	29.8	30.5	30.3	30.8	30.0	31.6	29.8	28.6	27.3
(°C)	Min.	19.0	19.6	19.5	25.4	27.2	28.1	29.0	27.4	28.8	26.5	24.9	24.1
Soil temp. (30 cm)	Max.	20.6	21.1	21.0	26.4	28.5	29.3	30.5	29.6	31.0	29.2	27.6	26.7
(°C)	Min.	20.2	20.6	20.5	25.8	28.0	28.8	30.1	29.0	30.4	28.5	26.9	26.2
Wind (km)	Daily	89	101	103	76	67	80	74	87	55	77	56	83
Solar radiation (Watt-h/m²)	Daily	3455	3459	4021	5454	5274	5215	5201	4013	4926	4451	3888	2920
Precipitation (mm)	Total	6.5	78.5	19.0	12.0	169.0	227.5	348.0	1433.5	23.0	1.0	0.0	8.0
Evaporation (mm)	Daily	3.3	3.6	4.1	6.4	6.2	5.7	5.8	4.1	5.2	5.4	4.2	3.6