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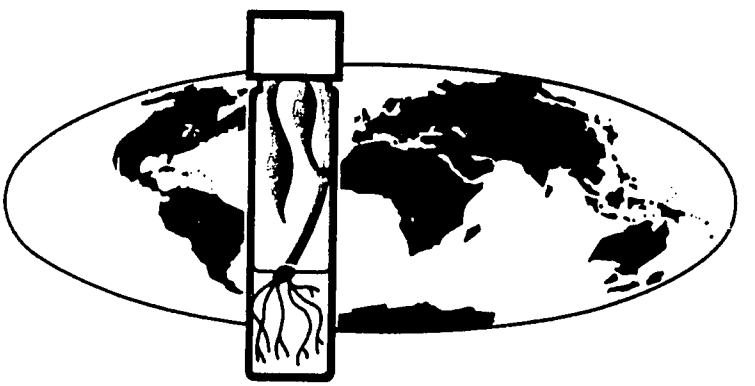
OCTOBER 1988 TO OCTOBER 1989

GRANT YEAR FIVE



Tissue Culture for Crops Project
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PD-AAC

Annual Report of

Agricultural Biotechnology/Plant Tissue Culture

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EXECUTIVE SUMMARY

In 1988 - 89 the Tissue Culture for Crops Project continued to achieve milestones in the areas of research, networking, and training. The primary goal of transferring useful plant biotechnology to developing countries was facilitated in particular by the following important activities:

1. In January 1989, the Third Conference of the International Plant Biotechnology Network (IPBNet) was held in Nairobi, Kenya. Ninety-two scientists, including 50 African participants, attended. As a direct result of the Conference, an all-African network was formed (APBNet) and made enthusiastic plans for a first meeting in 1990.

2. The second IPBNet Directory was published. Four hundred and seventy three scientists interested in plant biotechnology are listed as well as 47 funding sources. To date, approximately 1,700 copies have been distributed to facilitate both research and links between researchers in developing countries.

3. In addition to training at TCCP, two international training courses were co-sponsored. The first, held at IAV Hassan II in Morocco in May 1989, graduated nine African students from an intensive training program conducted in French. The second, held at CATIE in Costa Rica in September 1989, graduated 14 south and central American students from a similar program in Spanish.

4. Field testing of TCCP laboratory-generated germplasm continued at 17 locations including U.S. universities, LDC national programs, and IARC's. Testing for salinity tolerance, drought resistance, and acid tolerance produced statistically analyzed data with yield components. Data strongly support the hypothesis that selected stress-tolerant variants are useful in the field.

5. Final field testing of fall armyworm tolerant sorghum was completed. Arrangements were made for the first Project germplasm release to occur early in 1990. This release will represent the first ever from an AID funded project through the ASA-sanctioned Crop Science. This resistant germplasm will be useful both domestically and worldwide and demonstrates that somaclonal variants generated in the lab have demonstrated field utility.

6. Seven graduate students representing six countries continued pathbreaking research in several areas of plant cell culture while working to complete M.S. and Ph.D. degrees.

7. Active collaborations in both research and field testing continued with IARC's (CIMMYT and CIAT).

8. Cutting edge research continued at TCCP on AID-sponsored and two independently-sponsored research activities.

This brief listing highlights only a selected few of the many Project accomplishments. None of these would have occurred without the continued strong support of the AID Project Monitor, Joel Cohen, and the dedicated hard work of all Project staff and students in Fort Collins and of collaborators and friends worldwide.

EVALUATION OF REGENERATED PLANTS AND PROGENY

Summary

Whole plant evaluation of regenerated material is the critical step in determining the efficiency of *in vitro* selection. In order to statistically verify whether *in vitro* selection can produce lines which have heritable, improved field tolerance for a given stress, it is necessary to evaluate material in multiple replications, environments, and generations. Within these evaluations, three main hypotheses are tested: 1) compare specific stressing agents, stress levels, and culture times for efficiency in producing tolerant lines, 2) compare *in vitro* selected lines to non-selected regenerates for whole plant stress tolerance, and 3) compare individual regenerated lines to non-regenerated checks for superior tolerance or agronomic traits.

Successful testing of the above hypotheses depends upon precise experimental design, good field conditions, and thorough statistical evaluation. The TCCP has contracted with two CSU experts in field testing and statistics to assist with design and analysis of all field experiments. Additionally, TCCP has attempted to work closely with collaborators to ensure field tests achieve the desired objectives. In order to ensure a high degree of precision in field trials, many replications of the check line are included in field tests to more accurately estimate the true mean of check performance. Statistical analyses are conducted by relatively conservative procedures. Mean comparisons for individual lines are made using Dunnett's t-test, which controls type I error rates. *In vitro* treatment means are evaluated using a random model analysis of variance to aid in making inferences about the effectiveness of a given treatment. This model requires a reasonably well

represented sample size for valid comparison, making the establishment of a well balanced treatment grid essential for these tests.

During 1989, sixteen collaborating scientists assisted TCCP in conducting field and greenhouse investigations on regenerated material in 10 international sites and seven domestic sites. Salinity trials in the the Philippines detected nine rice regenerates from in vitro salt selection which performed statistically better than the check. Greenhouse salinity trials in Fort Collins indicated that six regenerated rice lines rated statistically better than the check. In sorghum, two lines from tissue culture were designated for release as fall armyworm-tolerant germplasm. Four elite acid soil-tolerant lines held up for a third year of testing and are being advanced toward germplasm release. Three Hegari regenerates survived a second year on acid soil and will be moved up to elite status next year. Eight $AlCl_3$ in vitro selected sorghum lines from Hegari produced seed on acid soil and will be moved up to confirmation status next year. Salinity screening of spring wheat under field conditions in Mexico and Pakistan yielded 148 R_4 lines from in vitro NaCl selection with some level of salt tolerance. These 148 lines will be confirmed in six test sites next year.

Test results this year indicate that in vitro treatment means are almost always lower than the check mean due to the presence of lines with deleterious mutations. For this reason it is necessary to identify the stressing agent and treatment combination which produces the highest probability of improved lines, regardless of overall treatment mean. Drought testing on sorghum revealed some valuable information on the effect of time in culture, indicating that the longer material is maintained in vitro, the higher the probability of random mutations which generally result in reduced performance. Greenhouse salt screening of

spring wheat produced similar results indicating that both higher passages *in vitro* and higher stress levels increase the probability of deleterious mutations. Additionally, preliminary results indicate that the probability of increasing tolerance in cultivars with high levels of inherent tolerance is low. Examples of these are: salt tolerance in Pokkali rice, acid soil tolerance in SC283 sorghum, and drought tolerance in M-35 sorghum. Conversely, the results of 1989 field tests show that improvement can be made in the stress tolerance of previously non-tolerant but otherwise suitable cultivars.

EVALUATION OF SALT TOLERANCE IN RICE AT PAMPANGA, PHILIPPINES
(Expt. #12, Workplan 5)

Summary

Fifty-nine R_2 and R_4 rice lines derived from IR-36, Mahsuri, and Pokkali were field tested for salinity stress tolerance at Pampanga, Philippines. Significant differences were not detected between Mahsuri and Pokkali regenerated lines for the variables measured. Among the 32 IR36 regenerated lines tested, 9 lines showed significant differences for vegetative salinity rating, reproductive salinity rating, seed yield per plot, or percent stand. One line was significant for all variables and two additional lines were significant for three of the four variables. The best lines from this experiment will be re-tested under both greenhouse and field saline stress conditions to confirm their improved saline tolerance.

Collaborating Scientists

Dr. Joe Hernandez, Plant Breeder, University of the Philippines, Department of Agronomy, Los Banos, Philippines.

Dr. Edward Paningbatan, Soil Scientist, University of the Philippines, Los Banos, Philippines.

Objective

To determine differences between IR36, Mahsuri, and Pokkali regenerated rice lines and their non-regenerated checks for their ability to withstand salinity stress under field conditions.

Research Approach

A total of 59 R_2 and R_4 rice lines (33-IR36, 13-Mahsuri, and 13-Pokkali) were planted at Sto. Domingo, Minalin, Pampanga, under saline conditions. A randomized complete block design was used with 4

replications and randomized at each sub-block (a sub-block represented one cultivar). Twenty days after sowing, the seedlings were transplanted in the field at 25cm spacing between rows and 25cm spacing within rows. Each plot was composed of 4 rows with 17 hills per row. Data was gathered on the inner two rows. Agronomic traits measured were days to heading, days to maturity, height, number of tillers, stand density, dry weight/plot, dry weight/plant, seed yield/plot, and seed yield/plant.

Salinity stress was applied to the Pampanga site by irrigating with salt water from the nearby tidal river and adjusting with fresh water from a ground water well. Salinity was monitored and adjusted accordingly to ensure survival of the seedlings. Saline water was applied approximately 30 days after transplanting. Entries were rated according to their ability to withstand salinity stress at both the vegetative and reproductive stages on a 1 to 9 scale (1 = growth and tillering nearly normal, 9 = almost all plants dead).

All lines included in the Pampanga salinity field were also planted at Los Banos under normal conditions for seed increase (Expt. #5, Workplan 5). Both sites were visited by TCCP personnel from April 25 through April 28, 1989.

Research Output

Due to a problem of inadequate output from the fresh water well, limited fresh water supply only allowed sufficient water to maintain two replications at the Pampanga site.

Electrical conductivities of saturated soil extract increased for the first 10 weeks of plant development and decreased during plant maturation (Table 1).

TABLE 1. Monthly means of electrical conductivities from saturated soil extract for March through September, 1989 at Pampanga, Philippines.

MONTH	E.C. dS/m
March	2.9
April	6.1
May	3.5
June	1.9
July	1.4
August	4.8
September	1.8
Mean	3.2

Significant differences were not detected among Mahsuri and Pokkali regenerated lines for the variables measured. Salinity levels as measured by electrical conductivity may not have been sufficiently high to distinguish performance differences in these two cultivars. Another possible explanation for the lack of statistically detected differences is that Pokkali and Mashuri already have some degree of salt tolerance, making it more difficult to improve the existing level of tolerance through in vitro selection (see description on SC283 WP5 exp #14 under Sorghum Sect. 5). Among IR36 regenerated rice lines, significant differences were found for vegetative salinity rating, reproductive salinity rating, seed yield per plot, and stand survival (Table 2). IR36 lines were compared to the non-regenerated control by using Dunnett's t-test. Lines with significant differences for the 4 variables are shown in Table 3.

TABLE 2. F probabilities for IR36, Mahsuri, and Pokkali regenerated rice lines analyzed for 11 variables at Pampanga, Philippines, 1989.

VARIABLE	IR-36	MAHSURI	POKKALI
height	0.51	0.96	0.46
tiller	0.49	0.18	0.05
vegetative salinity rating	0.01*	0.50	0.24
reproductive salinity rating	0.02*	0.16	0.87
dry weight per plot	0.23	0.49	0.88
seed yield per plot	0.01*	0.16	0.42
dry weight per plant	0.41	0.85	0.44
seed yield per plant	0.30	0.39	0.23
days to heading	0.48	0.50	0.50
days to maturity	0.46	0.00	0.50
stand density	0.01*	0.55	0.63

TABLE 3. Differences between IR36 rice lines and non-regenerated controls for vegetative salinity rating, reproductive salinity rating, seed yield per plot, and percent stand at Pampanga, Philippines, 1989.

			DIFFERENCE BETWEEN MEANS			
LINE#	GEN	IN VITRO (NACL) LEVEL	VEGETATIVE SALINITY† —rating—	REPRODUCTIVE SALINITY† —rating—	SEEDYD/PLOT+ —grams—	STAND SURVIVAL$ — % —
3030110	R2	3	-5.167*	-3.5*	90.42*	47.83*
306022	R4	6	-4.167*	-3.5*	65.72	47.83*
300044	R4	0	-4.167*	-3.5*	46.47	52.83*
303142	R4	3	-3.167	-2.5	51.52	47.83*
3001006	R2	6	-3.167	-2.5	111.42*	27.83
303079	R2	3	-3.167	-1.5	30.67	42.83*
303134	R2	3	-2.167	-3.5*	62.72	42.83*
303133	R2	3	-2.167	-2.5	89.07*	22.83
303049	R2	3	-2.167	-1.5	87.97*	23.33

†Values represent differences of line rating minus control rating. Vegetative and reproductive salinity ratings based on a 1 to 9 scale with 1 = normal growth and 9 = nearly all plants dead.

¥Values represent differences of regenerated line yield minus control yield.

$Values represent % stand differences of regenerated line minus control.

*Significantly better than the nonregenerated control at the .05 level by Dunnet's t-test.

Differences between IR36 regenerated rice lines were detected for their ability to withstand salinity stress at Pampanga, Philippines. Among the 32 IR36 regenerated lines screened, 9 lines showed significant differences for vegetative salinity rating, reproductive salinity rating, seed yield per plot, or percent stand. One line (3030110) was significant for all variables and two additional lines (306022 and 300044) were significant for three of the four variables. Of the 9 regenerated IR36 lines showing improved salinity tolerance as measured by these variables, 8 were from *in vitro* salt-selected cultures.

The best lines from this experiment will be re-tested under both greenhouse and field saline stress conditions to confirm their improved saline tolerance.

GREENHOUSE EVALUATION OF SALT TOLERANCE IN IR36 RICE

Summary

The most vigorous 30 lines from previous greenhouse salt evaluations were selected for re-testing. These lines were evaluated for salt tolerance using hydroponic solution containing 3 g/L NaCl. After 48 days of salt stress, the plants were evaluated for height, tiller number, and shoot dry weight. Among the 30 R_2 lines tested, 6 lines (20%) were significantly taller, had more tillers, or had higher shoot dry weight than the non-regenerated control according to Dunnett's t-test procedure. Two of the R_2 lines were significantly better for all three variables.

Collaborating Scientists

Dr. Mark Brick, Legume Breeder, Colorado State University, Agronomy Department, Fort Collins, Colorado.

Dr. Phil Chapman, Statistician, Colorado State University, Department of Statistics, Fort Collins, Colorado.

Objective

To identify IR36 rice regenerates with increased salt tolerance by screening during the vegetative stage in a greenhouse environment.

Research Approach

One hundred and forty-seven R_1 rice lines were chosen from <u>in vitro</u> NaCl treatments and were evaluated for salt tolerance using a hydroponic solution containing 3 g/L NaCl. After four weeks of salt stress during vegetative growth, the plants were evaluated for height, tiller number, and vigor. Thirty R_1 plants were selected from the lines and advanced to the R_2 generation. The R_2 seeds were planted in a 50% perlite and 50% vermiculite mixture in 6.5 cm pots. Pots were randomly placed in watertight trays (42x27x6 cm) and approximately 3 L of de-ionized water

was added. Three R_2 lines and a non-regenerated control, each replicated 8 times, were placed in one tray. After 10 days, a nutrient solution containing 40 ppm N was added. After 7 more days, 3 g/L NaCl was added to the nutrient solution and applied to the trays. This salt solution was applied twice weekly for a period of 48 days. Electrical conductivity (E.C.) values of the salt solution within the trays were recorded at 15, 23, 29, 36, and 48 days after initial salt solution application. Plants were then evaluated for height, tiller number, and shoot dry weight. Analysis of variance was conducted on entries in each tray. Each regenerated line in the tray was compared to the control in that tray using Dunnett's t-test procedure. This method of comparison removed any discrepancy from unequal error variance between trays.

Project Output

Among the 30 R_2 lines tested, 6 lines (20%) were significantly taller, had more tillers, or had higher shoot dry weight than the non-regenerated control according to Dunnett's t-test procedure (Table 4). Two of the R_2 lines (303044-45 and 303030-46) were significantly better for all three variables. Although the experiment was not designed to analyze for the effects of <u>in vitro</u> treatment, these 6 lines were all from the 3 g L^{-1} NaCl <u>in vitro</u> treatment.

Table 4. Differences between 6 IR36 R_2 rice line means and non-regenerated control means for height, number of tillers, and shoot dry weight in a greenhouse hydroponic salt environment.

LINE #	HEIGHT	TILLERS	SHOOT DRY WEIGHT
	—cm—	—no.—	—g—
303044-45	4.8*	1.29*	0.82*
303030-46	4.7*	1.29*	0.55*
303143-45	4.3*	0.00	0.22*
303075-45	1.7	1.25*	0.38
303026-46	-0.1	1.75*	0.62
303049-47	4.0	1.75*	0.46

*Differences significant at the 5% level of probability using Dunnett's t-test procedure.

Electrical conductivity means of the salt solution within the trays increased linearly over time. The E.C. mean at 48 days after initial salt solution application was 17.0 dS/m. This level of salt stress was physiologically damaging to non-tolerant germplasm. The best lines from this experiment will be re-tested in the TCCP greenhouse in early 1990 to confirm their salt tolerance.

EVALUATION OF REGENERATED RICE FROM SUSPENSION CULTURE
(Expt. #1, Workplan 5)

Summary

One hundred and ten lines of suspension culture regenerated Taipei 309 were evaluated for somaclonal variation at Crowley, Louisiana. Variations were observed for plant height, maturity, pubescence, color, disease, and morphology. Yield of regenerates will be compared to the parental check. Greenhouse studies are currently underway on this same material for determining if any variation exists for salt tolerance.

Collaborating Scientist

Dr. Milton Rush, Rice Pathologist, Louisiana State University, Department of Pathology and Crop Physiology, Baton Rouge, Louisiana.

Objectives

1) Observe regenerated rice from suspension culture for incidence of somaclonal variation for agronomic traits under normal field conditions.

2) Screen R_1 and R_2 lines for tolerance to NaCl under greenhouse conditions.

Research Approach and Project Output

Field Study. One-hundred and ten lines of R_1 and R_2 rice (*O. sativa* cv. Taipei 309), from suspension culture, were planted in Crowley, Louisiana at the Louisiana State University Rice Research Station, in collaboration with Dr. M. C. Rush. The lines were observed for somaclonal variation under optimum field conditions. The field was broken into two experiments; the first included 30 R_1 regenerates from suspension cultures with culture times of 4.5, 5, 6, 6.5, and 10 subcultures; the second experiment consisted of R_1 and R_2 regenerates from suspension culture times of 4.5, 5, and 6 subcultures. The field design was a randomized

complete block design with four replicates. The second experiment was designed to conduct t-tests on R_1 lines and R_2 progeny. Data were taken for variations in maturity, height, pubescence, awns, leaf scald, leaf color, type of panicle and brown spot. Most plants were observed to be later in heading than the non-regenerated check. The data taken at harvest included sterility, 100-seed weight and yield.

Observations at heading indicated that a number of variations were present in this material. Most of the regenerated lines were shorter and later in heading than the checks. Some dwarf lines were also present. Variations from the parent genotype were observed for leaf pubescence, leaf color, panicle morphology, and incidence of leaf scald and brown spot diseases. The R_2 somaclones had more variation in height and earliness than the R_1 lines. Some somaclones had a reduction in leaf pubescence compared to checks; others had a reduced tiller number. As of this report, plots have been harvested but seed is still in Louisiana. Sterility, 100-seed weight, and yield will be compared to performance of check line and R_2 progeny. These data will be reported as part of Poungpet Poonsapaya's Ph.D. dissertation.

Greenhouse Study. In conjunction with field evaluations for somaclonal variation, seedling tolerance to NaCl under greenhouse conditions is being evaluated. The greenhouse experiment was designed to address two objectives. The first is comparisons of salt tolerance between R_1 and R_2 suspension lines and the non-regenerated control. These lines were unselected in tissue culture so any statistically superior lines will be variations rather than *in vitro* selection. The second objective is to address which generation (R_1 or R_2) is most appropriate for selection and/or screening. Due to greenhouse space limitations, this

experiment is being replicated over time.

Preliminary results from this experiment indicate that significant variation for agronomic traits can be observed for suspension cultures. Tests have not yet been set-up to confirm differences, but observations at TCCP indicate a greater incidence in variation in suspension culture regenerated material as compared to callus cultured on solid media.

EVALUATION OF SODIC-SALINE TOLERANCE IN RICE AT LAHORE, PAKISTAN
(Expt. #4, Workplan 5)

Summary

Two field trials were conducted in 1988 at Lahore, Pakistan to evaluate for sodic-saline tolerance of tissue culture derived rice. The first trial consisted of 27 R_2 and R_3 lines derived from Calrose, IR-36, Mahsuri and Pokkali which survived the 1987 sodic-saline trial at Lahore. The second trial consisted of 45 R_1 Giza-159 lines. Due to heavy monsoon rains, salts were leached from the field and consequently differences between lines for salt tolerance could not be detected. Seeds were collected and both trials were repeated in 1989.

Collaborating Scientists

Dr. Kauser Malik, Head, Soil Biology Division, NIAB (Nuclear Institute for Agriculture and Biology), Faisalabad, Pakistan.

Dr. Yusuf Zafar, Senior Research Scientist, NIAB, Faisalabad, Pakistan.

Objectives

Expt. 1: To evaluate 27 R_2 and R_3 Calrose, Giza-159, IR-36, Mahsuri and Pokkali lines which survived the 1987 trial for tolerance to sodic/saline-affected soils.

Expt. 2: To evaluate 45 R_1 Giza-159 rice lines for tolerance to sodic/saline-affected soils.

Research Approach

Expt. 1

In July 1987, 76 R_1 and 56 R_2 lines derived from the cultivars Calrose, Giza-159, IR-36, Mahsuri, and Pokkali and their respective checks were planted on a sodic/saline-affected soil in Lahore, Pakistan. Twenty-seven lines and 3 checks survived this trial and were planted again on a sodic/saline-affected soil in August 1988. The 27 R_2 and R_3 lines planted

consisted of 7 Calrose, 6 IR-36, 6 Mahsuri, and 8 Pokkali lines. The following data was collected: 1) survival after transplanting, 2) average height, 3) average number of tillers, 4) total fresh weight per plot, and 5) fresh weight per plant. Electrical conductivity (E.C.) of soil solution and soil pH were also recorded.

Expt. 2

Forty-five R_1 Giza-159 rice lines were sent to NIAB in June 1988. These lines were planted on a sodic/saline-affected soil at Lahore in September 1988. Delays from customs and subsequently from heavy rains resulted in late planting. Data was collected for survival after transplanting, electrical conductivity of soil solution, and soil pH.

Research Output

Expt. 1

Heavy rains resulted in leaching of salts from the field. Electrical conductivity of soil solution was 1.1 dS/m on October 10, 1988. This low level of salinity stress was not adequate to determine salinity stress tolerance.

Among the 27 lines evaluated, 12 lines matured late and had shriveled, infertile, or empty seeds. Seeds were collected from all lines which produced viable seed and were saved for repeating the experiment.

This experiment was repeated in 1989; results will be analyzed when they arrive at TCCP.

Expt. 2

Salinity stress levels were very low due to the heavy rains (E.C. 1.4 dS/m on October 10, 1988). Plant growth was poor due to delayed planting. Seeds were collected from each line and the experiment was repeated in 1989.

EVALUATION OF SPRING WHEAT FOR SALINITY TOLERANCE UNDER GREENHOUSE CONDITIONS

Summary

Fifty-seven R_1 spring wheat lines derived from Pavon were evaluated for salt tolerance in a greenhouse environment (Refer to page 8 of Workplan 5). The effect of in vitro NaCl level and passage time on salinity tolerance was evaluated with a random model analysis of variance. Significant differences for the variable shoot dry weight were found between the 0 and 4 g/l in vitro NaCl treatment. The difference was attributed to a high individual treatment mean at 4 g/l NaCl and 9 total passages. Significant differences for the variable seed yield were found between the 0 and 8 g/l NaCl treatment, between the 4 and 8 g/l NaCl treatment, and between 9 and 13 total passages. These differences were attributed to low individual treatment means within 8 g/l NaCl and low individual treatment means within 13 total passages. A fixed model analysis of variance was conducted to select the lines which performed better than the non-regenerated control. The majority of the best lines for shoot dry weight and seed yield were from the in vitro treatment 0 g/l NaCl and 12 total passages. The line with the highest seed yield was from the treatment 4 g/l NaCl and 9 total passages. These 57 lines will be re-evaluated at La Paz, Mexico under field salinity conditions during Grant Year 6 to verify these results and validate the greenhouse test procedure.

Collaborating Scientists

Dr. Mujeeb Kazi, Wheat Cytogeneticist, CIMMYT, Mexico.

Dr. Mark Brick, Legume Breeder, Colorado State Univeristy, Department of Agronomy, Fort Collins, Colorado.

Dr. Phil Chapman, Statistician, Colorado State University, Department of Statistics, Fort Collins, Colorado.

Objectives

1) Determine the effect of *in vitro* NaCl levels and passage time on R_1 plant salinity tolerance expressed under greenhouse conditions.

2) Identify regenerated lines with significantly improved salt tolerance compared to the parental control line. The top lines among this group will be selected for field testing under saline conditions.

3) Improve the process of greenhouse testing for salinity tolerance in regenerated plants.

Research Approach

Fifty-seven R_1 lines derived from Pavon and selected from 9 *in vitro* treatment levels (Table 5) were evaluated for salt tolerance in a greenhouse environment. A randomized complete block design with 3 replications was used. Each experimental unit consisted of a single 12" pot, filled with 50% vermiculite and 50% perlite, containing 10 plants. Controls consisted of 32 pots of non-regenerated Pavon with salt stress and 12 pots with no salt stress.

Table 5. Number of lines by *in vitro* treatments of Pavon spring wheat used in greenhouse salt evaluation.

		Number of *in vitro* passages total (stress)		
		9 (6)	12 (9)	13 (10)
In vitro NaCl level g/l	0	T=1[+] n= 5	T=4 n=19	T=7 n= 9
	4	T=2 n= 3	T=5 n= 5	T=8 n=11
	8	T=3 n= 1	T=6 n= 2	T=9 n= 2

T=10 (stressed non-regenerated control)
T=11 (non-stressed non-regenerated control)

[+]T = Treatment number
n = number of lines per treatment

The experiment consisted of a total of 214 pots which were contained within 12 large water-tight saucers. Salt stress was applied by pumping 2-3 liters of a saline nutrient solution containing 3 g/L NaCl through the pots twice weekly. After standing in solution for 4 hours, the saucers were drained and the nutrient solution recycled into a storage tank. Solution electrical conductivities (E.C.) were monitored by sampling every 10 days.

Differences between *in vitro* treatments were analyzed using SAS. The model of the analysis of variance table consisted of: 1) treatments, 2) reps, and 3) lines nested within treatments (Table 6). Lines within treatments were designated as a random component; which provided the ability to detect difference between treatments instead of only differences between individual lines within treatments. Variables with significant F values for treatment were then analyzed for *in vitro*

treatment differences by using contrast comparison between treatments. Additionally, the best lines overall were determined by using a fixed model analysis of variance.

Table 6. Analysis of variance model and contrasts used for determining *in vitro* treatment differences.

Source	Degrees of Freedom
Rep	2
Treatment	10
*Lines within treatment	46

* Random component

Contrasts

All *in vitro* treatments (1-9)[+] vrs. stressed control (10)
Stressed control (10) vrs. non-stressed control (11)
0 g/l NaCl (1, 4 & 7) vrs. 4 g/l NaCl (2, 5 & 8)
0 g/l NaCl (1, 4 & 7) vrs. 8 g/l NaCl (3, 6 & 9)
4 g/l NaCl (2, 5 & 8) vrs. 8 g/l NaCl (3, 6 & 9)
9 total passages (1, 2 & 3) vrs. 12 total passages (4, 5 & 6)
9 total passages (1, 2 & 3) vrs. 13 total passages (7, 8 & 9)
12 total passages (4, 5 & 6) vrs. 13 total passages (7, 8 & 9)

[+] designates treatment number (refer to Table 5).

Analysis of variance procedures were performed on the variables: 1) percent germination, 2) survival at 50 days, 3) height, 4) shoot dry weight, 5) seed yield, and 6) weight of 100 seeds.

Research Output

Salinity levels increased linearly over time. Mean soil solution electrical conductivities (E.C.'s) of randomly selected stressed control pots were: 1) 3.6 dS/m at 19 days after planting, 2) 9.3 dS/m at 53 days after planting, and 3) 14.35 dS/m at harvest (110 days after planting).

In vitro treatment results of the analysis of variance for the primary variables shoot dry weight and seed yield are shown in Table 7. Overall treatment effects were highly significant for both variables.

Table 7. Shoot dry weight and seed yield F probabilities for treatment contrast comparisons.

CONTRAST	Shoot Dry Weight	Seed Yield
OVERALL TREATMENT EFFECT	.01*	.01*
All *in vitro* trts. vs. stressed control	.02*	.01*
Stressed control vs. non-stressed control	.01*	.01*
0 g/l NaCl vs. 4 g/l NaCl	.05*	.28
0 g/l NaCl vs. 8 g/l NaCl	.70	.01*
4 g/l NaCl vs. 8 g/l NaCl	.09	.01*
9 total passages vs. 12 total passages	.76	.20
9 total passages vs. 13 total passages	.81	.03*
12 total passages vs. 13 total passages	.51	.25

* Significantly different at the 0.05 level of probability.

Shoot Dry Weight. Contrast comparisons between *in vitro* treatments for the variable shoot dry weight show significant differences between 0 g/l NaCl and 4 g/l NaCl (Table 7). Individual treatment means for shoot dry weight are shown in Table 8. It is evident that treatment #2 (4 g/l NaCl at 9 total and 6 stress passages) was responsible for the significant difference between 0 g/l NaCl and 4 g/l NaCl. The treatment mean of 122.6 g. for treatment #2 ranked 2nd of all treatments; the only treatment mean with higher shoot dry weight was treatment 11 (non-stressed control).

Table 8. Individual treatment means for shoot dry weight.

In vitro NaCl level g/l	Number of in vitro passages total (stress): 9 (6)	12 (9)	13 (10)
0	T=1[†] 99.3[¥]	T=4 113.8	T=7 98.5
4	T=2 122.6	T=5 105.2	T=8 106.8
8	T=3 91.7	T=6 99.4	T=9 102.7

T = 10-stressed control 117.2
T = 11-non-stressed control 150.9

[†]treatment number

[¥]treatment mean (g)

Seed Yield. Contrast comparisons between in vitro treatments for the variable seed yield show significant differences between 0 g/l NaCl and 8 g/l NaCl, between 4 g/l NaCl and 8 g/l NaCl, and between 9 total passages and 13 total passages (Table 7). Individual treatment means for seed yield are shown in Table 9. The significant differences between 0 g/l NaCl and 8 g/l NaCl and between 4 g/l NaCl and 8 g/l NaCl can be attributed to low individual treatment means within 8 g/l NaCl. Furthermore the significant difference between 9 total passages and 13 total passages can be attributed to low individual treatment means within 13 total passages.

Table 9. Individual treatment means for seed yield.

In vitro NaCl level g/l	Number of in vitro passages total (stress) 9 (6)	12 (9)	13 (10)
0	T=1[†] 45.4[¥]	T=4 49.1	T=7 39.6
4	T=2 56.1	T=5 42.3	T=8 39.9
8	T=3 36.2	T=6 30.8	T=9 34.0

T = 10-stressed control 57.1
T = 11-non-stressed control 68.9

[†]treatment number

[¥]treatment mean (g)

Best lines. The fixed model analysis of variance indicated that several lines performed better than the stressed control (Table 10). Twelve lines had higher shoot dry weight than the stressed control. The top two lines (1061715 and 1061117) were from treatment 8 (4 g/L NaCl and 13 total passages) and treatment 2 (4 g/L NaCl and 9 total passages), respectively. However, 8 of the 12 lines with higher shoot dry weight were from treatment 4 (0 g/L NaCl and 12 total passages). Six lines had higher seed yield than the stressed control. The top line (1061117) was from treatment 2 (4 g/L NaCl and 9 total passages); however, three of the 6 lines with highest seed yield were from treatment 4 (0 g/L NaCl and 12 total passages).

Table 10. Pavon lines with higher shoot dry weight and seed yield than the stressed control.

LINE #	TOTAL (STRESS) PASSAGES	NaCl LEVEL	TRT#	SHOOT DRY WEIGHT	SEED YIELD
		—g/L—		----g----	----g----
Non-stressed control		-	11	150.9	69.0
1061715	13 (10)	4	8	143.6	57.4
1061117	9 (6)	4	2	141.9	63.9
1061306	12 (0)	0	4	137.1	56.8
1061323	12 (0)	0	4	136.2	58.8
1061304	12 (0)	0	4	126.3	57.8
1051021	9 (0)	0	1	126.2	55.8
1061326	12 (0)	0	4	124.1	53.4
1061302	12 (0)	0	4	123.2	57.2
1061314	12 (0)	0	4	119.8	48.9
1061105	9 (0)	0	1	119.7	57.4
1061318	12 (0)	0	4	118.1	51.2
1061317	12 (0)	0	4	118.0	52.7
Stressed control		-	10	117.2	57.1

FIELD EVALUATION OF REGENERATED SPRING WHEAT
(EXPT. #'s 10 AND 11, WORKPLAN 5)

Summary

Field evaluation of spring wheat regenerates from in vitro salt selection continued this year with cooperation from CIMMYT scientists. Wheat lines were evaluated for disease resistance at Poza Rico, uniformity and somaclonal variation at Obregon, and salinity tolerance at La Paz, Mexico, Faisalabad and Tandojam, Pakistan. Results from these screens indicate that 245 lines performed well enough to warrant further testing under more rigidly controlled saline stress.

Collaborators

Dr. Mujeeb Kazi, Wheat Cytogeneticist, CIMMYT, Mexico.

Dr. R. Qureshi, Professor of Soil Science, University of Faisalabad, Pakistan.

Dr. Azmi, Director, Atomic Energy Agricultural Research Centre, Tandojam, Pakistan.

Dr. Fraga, Professor, University of La Paz, La Paz, Mexico.

Dr. Rajaram, Wheat Breeder, CIMMYT, Mexico

Research Approach

Four hundred and sixty-three R_3 lines derived from Pavon and Glennson were planted November 15, 1989 at La Paz, Mexico to evaluate for salt tolerance. Salinity stress was applied by irrigating from two wells: one well containing 3000 ppm NaCl and a second well containing 5000 ppm NaCl. A split plot design was used with 1 replication per irrigation treatment. Each plot consisted of a single row 3 m long.

Data was collected for days to emergence, days to tillering, days to boot, days to spike emergence, days to flowering, grain development, physiological maturity, harvest date, height, size of spike, seeds/spike,

and yield. Electrical conductivity and soil pH were measured for each block.

Plots were harvested on April 7, 1989 and selections for further testing were made. Lines were selected based on: 1) uniformity, 2) growth development, 3) days to heading, 4) days to physiological maturity, and 5) overall tolerance to saline conditions.

All lines planted at La Paz were also planted at Obregon, Mexico to increase seed. Yield data was collected for each plot at Obregon. These lines were also sent to two sites in Pakistan: Faisalabad and Tandojam. They were increased there and selected on the basis of local adaptation and overall uniformity (height, maturity, disease resistance).

Research Output

At the time of this report, data is available only on the Obregon seed increase and selections made at La Paz and Pakistan. Of the 461 lines screened on salt at La Paz, 142 regenerates were selected for further testing in replicated plots next year. Table 11 summarizes the yields of all lines at Obregon and the La Paz selected lines. The yield data at Obregon is not equivalent to La Paz as the site provides no salt stress. Table 12 shows the *in vitro* treatment grid for all lines and the individuals selected from La Paz. Grain yield by treatment is also included but is difficult to analyze since plots were not replicated.

Table 11. Number and mean yield for all lines and selected lines of Glennson regenerates, Glennson checks, Pavon regenerates, and Pavon checks at Obregon, Mexico, 1989.

	Total Lines		Selected Lines+	
	Lines	Mean Yield	Lines	Mean Yield
	--no.--	--g--	--no.--	--g--
Glennson regenerates	57	504.4	0	-
Glennson checks	5	659.2	0	-
Pavon regenerates	381	663.5	142	642.7
Pavon checks	18	736.2	1	791.0
Total lines	461		143	

+ Lines selected under salt stress conditions at La Paz, Mexico.

Table 12. Number lines selected per treatment, percentage of lines selected per treatment, and mean yield of lines selected at La Paz.

Total Passages	0	g/l NaCl 3	6	7	8
0	1+ .06++ 791$				
3	1 .33 551	X	X	X	X
5	21 .33 633	60 .32 686	5 .56 597	X	X
6	10 .77 649	2 .11 689	0 0 -	X	X
7	2 1.00 444	3 .19 721	X	X	X
8	9 .47 679	0 0 -	1 1.00 511	9 .43 583	X
9	14 .88 536	X	1 1.00 682	X	1 .33 562
10	2 .50 621	X	X	X	X
11	1 .50 493	X	X	X	X

+ number lines selected
++ percentage of lines selected from original population
$ mean yield of selected lines (g)

In Pakistan 138 lines were selected for salt screening in the R_4 generation at both Tandojam and Faisalabad. Thirteen additional lines were selected at Tandojam and 29 additional lines were selected at Faisalabad. Selections were based on adaptation and uniformity. Table 13 summarizes the in vitro background of selected lines to be further tested in Pakistan next year.

Table 13. Number of lines selected from various *in vitro* treatments at Tandojam (T) and Faisalabad (F), Pakistan for future testing.

Total Passages	0	3	6	7	8
3	T=2 F=2				
5	T=25 F=27	T=58 F=63	T=2 F=5		
6	T=10 F=11	T=6 F=7	T=1 F=0		
7	T=2 F=3	T=4 F=5			
8	T=14 F=14	T=1 F=1	T=2 F=2	T=6 F=8	
9	T=13 F=15				T=0 F=2
10	T=2 F=2				
11					

REGENERATED MILLET EVALUATION
(Expt. #'s 6 and 7, Workplan 5)

Summary

Millet field testing for salinity tolerance was initiated in Pakistan during 1988. Due to heavy rains, planting was delayed until spring of 1989. Excessively high salinity and sodicity resulted in failed germination of all regenerates and checks.

During 1988 millet tissue culture was terminated at TCCP due to budgetary constraints. In January 1989, millet field testing and all remaining germplasm was subsequently turned over to Bill Stegmeier, Kansas State University and INTSORMIL Millet Breeder, for incorporation into his breeding program. As a result of field performance under drought conditions in 1989, Mr. Stegmeier selected 25 regenerates for inclusion into his breeding program. Additionally, selected R_2 lines were backcrossed to parent lines and will be evaluated as hybrid parents with disease resistance.

Collaborating Scientists

Dr. Kauser A. Malik, Soil Biology Division, NIAB, Faisalabad, Pakistan.

Mr. William D. Stegmeier, Pearl Millet Breeder, Kansas State University, Fort Hays Experiment Station, Hays, Kansas.

Dr. Yusuf Zafar, Senior Research Scientist, NIAB, Faisalabad, Pakistan.

Objectives

1) To establish linkages with a Pearl Millet breeding program for testing of regenerated millet germplasm from salt, PEG, and aluminum <u>in vitro</u> selections. The criteria for this program was that it had the capability to field test large amounts of millet germplasm and incorporate useful material into an internationally linked breeding program.

2) To identify R_1 and R_2 millet lines with increased tolerance to sodic-saline affected soils in Pakistan.

Research Approach and Project Output

Kansas Drought Evaluation. Field evaluation of regenerated millet was turned over to Mr. Bill Stegmeier for this year. In January 1989, approximately 1800 lines of regenerated millet were sent to Mr. Stegmeier with the agreement that he would incorporate the material into his breeding program (Table 14). It was stipulated that TCCP would receive notice of any useful material and recognition in any releases. Prior to turning this germplasm over to Mr. Stegmeier, seed increases were successfully conducted in Yuma, Arizona (Workplan 5, Expt. #7).

Table 14. Number of regenerated Millet lines sent to Kansas State University.

In vitro stress	Cultivar				
	HMP 559	SB 7203	Tift 23	2069	7911
none	361	54	713	21	15
NaCl	109	85	-	-	-
$AlCl_3$	55	22	-	-	-
PEG	240	258	-	-	-
Total	765	319	713	21	15

The 1989 field season began with extremely dry conditions and poor subsoil moisture in western Kansas. Due to these conditions, Mr. Stegmeier elected to evaluate 492 lines which had sufficient seed to ensure that a poor field season would not cause the loss of these lines. Late season rains relieved drought stress and allowed the millet to mature normally. However, early season drought stress facilitated selection of lines which performed better than the check. Lines which matured early and showed no saddle effect or other drought symptoms were selected for advance. Out of

492 regenerated lines, approximately 25 were selected for inclusion into Mr. Stegmeier's on-going breeding program. Additionally, selected R_2 lines were backcrossed to parent lines and will be sent to ICRISAT and evaluated for downy mildew resistance. The remainder of the regenerated material which has not yet been evaluated will be field tested in the ensuing seasons. Mr. Stegmeier is linked with INTSORMIL and ICRISAT and can use these resources to evaluate and disseminate useful germplasm.

Pakistan Salinity Evaluation. Fifty-nine R_1 and R_2 lines derived from HMP 559 and SB 7703 and their respective checks were sent to Faisalabad, Pakistan in June 1988 (Workplan 5, Expt. # 6). The material was sent with three replications, randomized, and sorted back to planting order. A field map of randomized complete block design was also sent. The expected planting date was September 1988 on a sodic-saline soil.

Heavy rains during the fall of 1988 and the spring of 1989 delayed planting. On April 12, 1989 the experiment was planted on a highly sodic-saline area at Lahore. All entries failed to germinate due to either the inability of millet seed to germinate under these conditions or loss of germination potential and seedling vigor during shipping and storage.

EVALUATION OF REGENERATED TEPARY BEANS FOR TOLERANCE TO ACID SOIL AND DROUGHT CONDITIONS. (PALMIRA AND QUILACHAO, COLOMBIA)

Summary

Fifteen R_3 tepary bean (Phaseolus acutifolius) lines were field tested at two environments (acid soil and drought) in Colombia during 1989. Data was collected for number of plants, days to flowering, days to maturity, and yield. Among the 15 lines tested, none were statistically better than their check for the variables measured. Four lines which showed improved yields are presently being increased in the TCCP greenhouse. They will be evaluated at two drought sites in Colorado in 1990.

Collaborating Scientist

Dr. Jeffrey White, Legume Physiologist, CIAT (Centro Internacional de Agricultura Tropical), Cali, Colombia.

Objective

To evaluate R_3 15 tepary bean lines for tolerance to acid soil and drought conditions in comparison to their non-regenerated checks.

Research Approach

Fifteen R_2 tepary bean lines were sent to Dr. Jeffrey White at CIAT (Centro Internacional de Agricultura Tropical) in Cali, Colombia. The seed was increased to the R_3 generation in early 1989 and planted under drought and acid soil conditions at Palmira and Quilachao, Colombia in June 1989. A lattice design with 2 reps and 5 blocks was used to test these lines at both locations. Five checks were included in the trials: 2 parental tepary checks from TCCP (1 white-seeded and 1 brown-seeded), 1 tepary check from CIAT, and 2 common bean checks. Data was collected for number of plants, days to flowering, days to maturity, and yield. Soil pH values were 7.7 at the Palmira site and 5.0 at the Quilachao site.

Data was analyzed by Dr. White using Genstat; results were sent to TCCP. Additional analysis was conducted at TCCP with SAS (Statistical Analysis System) using analysis of variance and least-square mean separation.

Research Output

None of the R_3 tepary bean lines were significantly better than their check for yield, early flowering, early maturity, or number of plants. Although regenerated lines were not significantly better than their check for yield, four lines showed improved yields. These lines are shown in Table 15. Three of these lines (1041003-1, 1041417-1, and 1020001-1) had improved yields at both sites. Line 1041601-1 did not yield better at Quilachao, but was the highest yielding at Palmira.

These four lines are presently being increased in the TCCP greenhouse. They will be evaluated at two drought sites in Colorado by Dr. Mark Brick in 1990.

Table 15. Yield means of four R_3 tepary bean lines and two non-regenerated checks at Palmira and Quilachao, Colombia in 1989.

Line Number	Grain Yield	
	Palmira	Quilachao
	— kg/ha —	— kg ha —
1041003-1	2687.4	846.7
1041417-1	2348.7	837.9
1041601-1	2736.6	660.8
104check+	2183.6	731.2
102001-1	2601.7	720.4
102check++	2372.6	651.9

+ 104 lines and check are brown-seeded.

++ 102 lines and check are white-seeded.

EVALUATION FOR FALL ARMYWORM TOLERANCE
(Expt. #13, Workplan 5)

Summary

Data from field and laboratory studies conducted from 1986 through 1989 indicate that tissue culture regenerated sorghum line 2442 has statistically verified resistance to fall armyworm feeding. Two selections from this line hold the most promise as sources of genetic resistance to leaf feeding by the fall armyworm. Based on the laboratory studies conducted, it appears that the resistance observed is due to a combination of antibiosis and non-preference mechanisms. The results of this study indicate that tissue culture can be employed to generate new sources of genetic diversity for use in crop improvement with application in both industrialized and developing nations. The two selections identified from this research are slated for sorghum germplasm release in early 1990.

Collaborating Scientists

Dr. David Isenhour, Entomologist, University of Georgia, Entomology Department, Tifton, Georgia.

Dr. Ronny Duncan, Sorghum Breeder, University of Georgia, Georgia Experiment Station, Griffin, Georgia.

Objectives

1) To statistically verify superior leaf feeding tolerance to fall armyworm that regenerated line 2442 has demonstrated in previous years.

2) To make selections of this material which could be released as fall armyworm-tolerant sorghum germplasm.

Research Approach

Line 2442 was regenerated in 1986 from Hegari callus which had been subcultured a total of 17 times. Five of these subcultures were in the presence of 0.9% NaCl. Line 2442 was selected in 1986 under a naturally occurring infestation of fall armyworm. During 1987 and 1988, 2442 was studied under artificial infestation and further selections were made. In 1989, three tests were conducted to verify tolerance: 1) an artificially infested field study at Tifton, GA, 2) a laboratory fall armyworm developmental study in which larvae were fed a diet incorporating regenerated sorghum tissue, and 3) a laboratory free-choice test which allowed larvae to feed on the diet of choice.

Field Study. The field studies were conducted at the Coastal Plain Experiment Station using standard agronomic practices for sorghum in south Georgia. A randomized complete block design with nine replications was used to test severity of fall armyworm feeding in 30 R_3 and R_4 selections and the non-regenerated check. Each plant was artificially infested with 35 fall armyworm neonates and scored for damage at 7 and 14 days after infestation.

Developmental Laboratory Study. The developmental studies incorporated whorl leaf tissue from regenerated lines chosen randomly from field studies into an insect diet at a concentration of 50 mg dried foliage/ml of diet. Neonate fall armyworms were placed singly in 30 ml diet cups containing 20 ml of diet and maintained in an environmental growth chamber at 26°C with a 14L:10D photoperiod. The study was arranged in randomized complete block design with 11 treatments and 25 replications. Data were taken on larval weights at 8 and 12 days of age, days to pupation, pupal weight, and days to adult eclosion.

Free Choice Laboratory Study. The free-choice tests used the same diets as the developmental study but lines were chosen on the basis of field performance rather than at random. The two best regenerate lines and two which were not significantly different were included with the non-regenerated control in this experiment. The test arena consisted of a plastic container which had sections holding each diet. Approximately 250 fall armyworm neonates were placed in the center of the container and allowed to migrate to the diet of choice. A randomized complete block design was used with 10 replications. Data was collected on the number of larvae per diet after 72 hours.

Project Output

Field Results. In 1989, 30 R_3 and R_4 selections from line 2442 were compared for resistance to leaf feeding by fall armyworm (Table 16). Four lines had significantly lower damage ratings than non-regenerated Hegari at 7 days after infestation. Two of these lines, 2442-12-14 and 2442-12-18, had significantly lower ratings than the other 28 selections. Extremely high infestation rates (35 larvae/plant) precluded detection of significant differences at 14 days after infestation. Plant height scores are also presented in Table 16. Significant variation in height was observed between R_3 lines, R_4 lines, and Hegari check. Line 2442-1-14 had the lowest damage rating and was also among the shortest entries screened.

Table 16. Fall armyworm leaf damage ratings at 7 days post infestation and mean height for R_3 and R_4 sorghum selections, Tifton, GA 1989.

Sorghum entry	7 day damage rating[+]	Plant height (cm)
2442-12-14	6.0 d[¥]	165 h-i
2442-12-15	8.0 a	193 a
2442-12-16	8.0 a	188 a-d
2442-12-17	8.0 a	187 a-f
2442-12-18	6.5 cd	191 a-b
2442-12-19	8.0 a	176 f-g
2442-12-20	8.0 a	187 a-f
2442-12-21	7.9 ab	180 d-g
2442-12-22	7.9 ab	182 b-g
2442-12-23	7.9 ab	188 a-d
2442-12-24	7.5 ab	191 a-b
2442-12-25	7.0 bc	184 a-g
2442-12-26	7.8 ab	186 a-f
2442-12-27	8.0 a	189 a-c
2442-12-28	8.0 a	178 d-g
2442-12-29	7.9 ab	182 b-g
2442-12-4-1	8.0 a	185 a-g
2442-12-4-2	7.9 a	183 a-g
2442-12-4-3	8.0 a	185 a-g
2442-12-4-4	8.0 a	180 d-g
2442-12-4-5	8.0 a	187 a-f
2442-12-4-6	7.9 a	174 g-h
2442-12-4-7	8.0 a	174 g-h
2442-12-4-8	7.8 ab	161 i
2442-12-4-10	7.0 bc	183 a-g
2442-12-4-11	8.0 a	176 e-g
2442-12-4-12	8.0 a	183 a-g
2442-12-4-30	8.0 a	178 e-g
Hegari check	8.0 a	193 a

[+]Visual observations to assess damage were made using a scale where 1=resistant and 9=susceptible.

[¥]Means within a column followed by the same letter do not differ significantly at the .05 level; Waller-Duncan K-ratio t-test. Values represent the mean of nine replications.

Laboratory Results. The effects of randomly selected entries from line 2442 in meridic diets on growth and development of fall armyworms are presented in Table 17. Significantly lower larval weights at 8 and 12 days of age were recorded for selections 2442-12-18 and 2442-12-24 as compared to the non-regenerated check suggesting an antibiosis mechanism

of resistance. Although not statistically significant, it is interesting that these two lines also resulted in a two day increase in time to pupation and adult eclosion as compared to the check.

Table 17. Weight and development of fall armyworm larvae fed diets containing dried sorghum leaf tissue from randomly picked R_3 and R_4 2442 selections and non-regenerated controls, 1989.

Sorghum	Larval weights (ug)		Days to:	
Entry	8 day	12 day	Pupation	Adult Eclosion
2442-12-16	66 ab	363 cd+	19.2 a	29.2 a
2442-12-18	26 c	204 e	19.5 a	29.7 a
2442-12-20	65 ab	391 abcd	18.2 a	28.6 a
2442-12-24	52 bc	325 de	19.2 a	29.1 a
2442-12-4-2	64 ab	389 bcd	18.4 a	28.6 a
2442-12-4-4	67 ab	394 abcd	18.7 a	30.1 a
2442-12-4-6	83 a	400 abc	17.9 a	27.7 a
2442-12-4-8	74 a	382 bcd	18.2 a	29.4 a
Hegari	83 a	448 abc	17.5 a	28.5 a
Savannah-5	90 a	512 a	17.2 a	28.5 a
Diet-Check	90 a	489 ab	17.2 a	27.9 a

+ Means within a column followed by the same letter do not differ significantly at the .05 level; Waller-Duncan k-ratio t-test.

Table 18 summarizes the results of the free-choice feeding test for fall armyworm larvae exposed to diets containing selected sorghum entries. A clear and significant preference was shown for the diet-check, which lacked any sorghum leaf tissue, but significant differences were also detected between the diets that incorporated sorghum tissue. Entry 2442-12-18 had significantly fewer larvae than Hegari or the two R_4 selections evaluated. Entry 2442-12-24 was intermediate in the number of fall armyworms feeding upon it.

Table 18. Larval feeding preference as indicated by mean number of fall armyworms per diet containing dried sorghum whorl tissue from selected entries.

Sorghum Entry	No. of Fall Armyworms per diet	
2442-12-18	15.0	b +
2442-12-24	20.9	ab
2442-12-4-4	26.9	a
2442-12-4-6	26.1	a
Hegari	26.1	a
Diet-Check	41.6	c

+ Means within a column followed by the same letter do not differ significantly at the .05 level; Waller Duncan R-ratio t-test.

DROUGHT TOLERANCE SCREENING OF SORGHUM REGENERATES
(Expt. #'s 19 and 20, Workplan 5)

Summary

Evaluation of drought tolerance was conducted on two populations of regenerated material at Tucson, Arizona in 1989. Experiment 19 included a population of 153 R_1 M35-1 lines from in vitro PEG screening. Eleven lines yielded higher than the check but differences could not be declared significant. In vitro treatment means for yield were not greater than the check mean due to high variability between regenerated lines. The low passage, no stress treatment was the best in vitro treatment overall. PEG selected treatments did not perform better than the non-selected treatments under drought conditions. Neither were there any individual lines from PEG which out yielded the check. Poor quality of R_1 seed and limited plot size reduced the accuracy of these comparisons and necessitates retesting in the R_2 generation.

Experiment 20 included 12 Hegari and 3 RTx430 selections from previous drought tolerance studies at Yuma. Five Hegari lines were better than check but differences could not be declared significant. Lower stress levels at this site may have obscured the differences observed last year. Selections were made from this population for a final test under more extreme conditions next year.

Collaborating Scientists

Dr. Robert Voigt, Sorghum Breeder, University of Arizona, Department of Plant Sciences, Tucson, Arizona.

Dr. Albert Dobrenz, Stress Physiologist, University of Arizona, Department of Plant Sciences, Tucson, Arizona.

Dr. Robert Kuehl, Statistician, University of Arizona, Department of Agricultural Economics, Tucson, Arizona.

Dr. Phil Chapman, Statistician, Colorado State University, Department of Statistics, Fort Collins, Colorado.

Dr. Mark Brick, Legume Breeder, Colorado State University, Department of Agronomy, Fort Collins, Colorado.

Experiment 19, Workplan 5

Objectives

1) Determine the effect of PEG in vitro treatment on whole plant drought tolerance observed under field conditions.

2) Determine effect of duration in tissue culture on the performance of regenerated lines.

3) Identify any superior regenerated lines of M35-1 as compared to the parental checks.

Research Approach

Selection on a cellular level for a complex trait such as drought tolerance is considered a difficult objective by many plant scientists. It is known that drought tolerance at the whole plant level is a multigenic trait involving many levels of plant organization. However, it is also known that a cellular-level component of drought tolerance exists and involves osmoregulation as well as other metabolic events. The use of polyethylene glycol (PEG) and other similar osmoticum as a drought simulator or selection tool has long been a subject of controversy, but scientists have reported favorable results on both a whole plant and cellular level (R.H. Smith, et. al., 1985 and Sullivan and Ross, 1979). This research project seeks to identify if any improvement in drought tolerance can be affected on a whole plant level by in vitro selection with PEG. A single cultivar, M35-1, was selected for testing this objective. M35-1 is known to be a reasonably drought-tolerant line with good heat and desiccation tolerance. It was selected for this study on

the hypothesis that the effect of in vitro treatment could be more easily detected in a line which already had some inherent tolerance rather than using a susceptible parent line as explant.

As M35-1 had not previously been examined in our tissue culture program, it was necessary to spend some time working out optimal media requirements, regeneration procedures, and appropriate levels of PEG for screening. For this reason, no germplasm was regenerated from the stress treatments until eleven total passages in vitro. A total of 153 R_1 lines of M35 were selected for this field study on the basis of having adequate seed to plant 4 replications. These 153 lines were regenerated from 7 different in vitro treatments ranging from 0 to 15% PEG and culture times from 0 to 12 total passages (Table 19).

Field plots were established near Tucson, Arizona at the University of Arizona, Marana Agricultural Center. Dr. Robert Voigt, was our primary collaborator on this study, with Drs. Dobrenz and Kuehl providing support. The test was planted May 30, 1989 in a randomized complete block design with four blocks. Individual plots were 3 meters long with 1 meter centers and .75 meter alleys. Each block included all 153 R_1 lines and 16 non-regenerated M35-1 check lines. Drought stress was controlled by applying irrigation as needed to keep the checks maturing normally. A total of four irrigations were necessary. Adjacent to the drought plots were 20 plots of M35-1 check under an optimal irrigation regime. These were included to quantify the relative level of drought stress on the main plots.

Three site visits were made by TCCP personnel to evaluate plots and record notes. Data taken included plant stand, flowering data, and grain yield as well as notes on variants. Grain was harvested October 1 with a

plot combine and yield was adjusted by factoring in stand gaps and bird damage to give more precision to the test. All data were analyzed at Colorado State University using SAS procedures for ANOVA with fixed and random models, linear regression, linear contrasts, and mean separation.

Research Output

Experiment 19 was designed as a two year test to investigate the effect of in vitro selection. As such, it was handled in a fashion unique to the normal field testing procedure. The best possible treatment grid was produced and field tested as R_1 material (Table 19). No attempt was made to increase R_1 seed of other lines to fill in the treatment grid in the ensuing R_2 test. Rather, this test was set-up to run the exact same material over two generations. Although much variation was apparent both within and between entries, selections were not made for any of these characters. Seed was advanced for R_2 testing by bagging the predominant phenotype of given lines. This way the treatment grid can again be analyzed in the second year by a random model analysis of variance.

In general, most of the lines exhibited a phenotype much like M35-1. However, variations observed in the 1989 test included differences in sterility, panicle exertion, panicle morphology, height, leaf color, and overall vigor and uniformity. The 1990 evaluation should indicate whether these are fixed traits or a residual of the culture process.

Table 19. *In vitro* treatment grid including number of lines per treatment (n), mean yield per plot in grams of all lines under drought conditions (y), and rank based on treatment yield (r).

% PEG	Total Passages *in vitro* 0	7	9	12
0	n = 64 y = 1138.9 (Dry) 4068.8 (Wet) r = 1	n = 47 y = 984.0 r = 2	n=5 y = 847.5 r = 3	n=48 y = 698.4 r = 5
9	X	X	X	n = 28 y = 548.0 r = 7
12	X	X	X	n = 7 y = 722.4 r = 4
15	X	X	X	n = 18 y = 557.4 r = 6

One fact learned in field testing regenerated crops is the desirability of testing in the R_2 or later generations. R_0 performance is confounded by its adaptation to the culture process and selection media. R_1 seed is usually too limited for adequate testing and never equivalent in quality to the non-regenerated check. It is therefore preferable to simultaneously increase R_1 seed and check seed in the same environment for screening and selection as R_2. Confirmation and advance towards uniformity should continue in R_3 and R_4 generations. This experiment was initially screened as R_1 due to limited time in which to satisfy the objective of the Cooperative Agreement. Problems occurred as expected in both plot size and stands. Plots were limited to single rows 3 meters long. This small size creates some difficulties in detecting true differences in grain yield. The variable stand was significant and 55 of the R_1 lines had significantly poorer stands than the non-regenerated check. Two lines failed to germinate altogether. The rep effect was not

significant for stand which indicated that treatment differences were more important than location in the field. Stands ranged from 68.25 plants per plot to less than 1 plant per plot, but stand was not highly correlated to final grain yield (Table 20). Except in cases of very poor stands, sorghum is generally capable of compensating for high or low stands. The observed difference between the mean of the check (54.5) and the mean of all treated lines (45.4) is undesirable and will be corrected next year by virtue of using R_2 seed.

Table 20. Statistical analysis of plant stand at Marana, 1989.

Variable: Stand

Source	DF	P>F
Rep	3	0.078
Line	153	0.001

C.V.	Overall $\bar{x}$	$\bar{x}$ of check
15.96	45.4 plants/plot	54.5 plants/plot

Simple correlation coefficient for stand x yield r = 0.105

Maturity of individual lines was observed to be variable and mean days to 50% anthesis ranged from 56 to 69 days after planting. M35-1 check reached half bloom in 57 days on average. Analysis of variance for this trait indicated that differences were not significant even though some lines were up to 12 days later than the check.

Data on grain yield was planned as the major criteria for detecting enhanced drought tolerance in the regenerated M35-1 material. For this reason, subjective ratings for vigor and drought tolerance were not included in this study. As grain yield is the final product of grain

sorghum cultivation, improved drought resistance must translate into improved grain production to be useful to farmers. Despite limitations in plot size, every effort was made to get good data on grain yield. Notes were taken on stand gap and bird damage and were factored in to a correct grain yield.

Table 19 lists the mean grain yield per plot by in vitro treatment and their relative ranking. The control treatment ranked first over all in vitro treatments. The degree of drought stress imposed on the treated material is evident when the grain yield from the full irrigated check is compared to the "dry" check. The dry check yielded 28% of the irrigated check, indicating that stress levels were severe but not lethal. Previous tests have shown that treatment means rarely, if ever, are superior to the mean of the check. This is due to the wide range of performance of tissue culture derived lines for any given treatment.

The treatment of 7 passages, 0% PEG (7°, 0) included eleven lines which yielded better than check, although not significantly different by Dunnett's procedure (Table 21). It also included the two poorest lines in the test (lines 2060038 and 2060040). The extremely poor lines which are commonly seen in most in vitro experiments are often sterile or partially sterile, indicating some type of chromosomal aberration. These low performing lines make comparison of treatment means to the non-regenerated check difficult to interpret correctly. For this reason it is generally necessary to begin field testing with a generation of screening and selection.

Table 21. R_1 M35-1 lines which yielded better than check under drought conditions.

Line Number	In vitro background Total Passages	Stress Level	Yield g/plot
2060008	7	0	1485.7
2060071	7	0	1449.2
2060023	7	0	1412.3
2060036	7	0	1376.5
2060024	7	0	1325.4
2060022	7	0	1315.0
2060047	7	0	1276.0
2060018	7	0	1253.6
2060027	7	0	1213.2
2060011	7	0	1202.4
2060006	7	0	1196.3
M35-1	0	0	1183.9

A major objective of this test was to examine the effect of *in vitro* selection with PEG on field performance under drought conditions. Table 19 shows the mean yield for each *in vitro* treatment level. Linear contrasts indicated that there was no significant difference between yield for the treatments with 9, 12, or 15% PEG in selection media. However, all stress selected material considered as a single treatment was significantly poorer than the equivalent treatment (12°, 0 vs. 12°, stress) under no stress (Table 22). This type of analysis does not compare the performance level of individual lines and so cannot rule out the effectiveness of selecting with PEG. It only indicates that on average there are more inferior plants produced when PEG is used as a

stressing agent. However, as no lines from PEG selection proved superior to the non-regenerated check, it is difficult to conclude that PEG is an effective *in vitro* selection agent for enhancing drought tolerance in M35-1. It may be that other genotypes would respond differently and this must be tested before ruling out PEG.

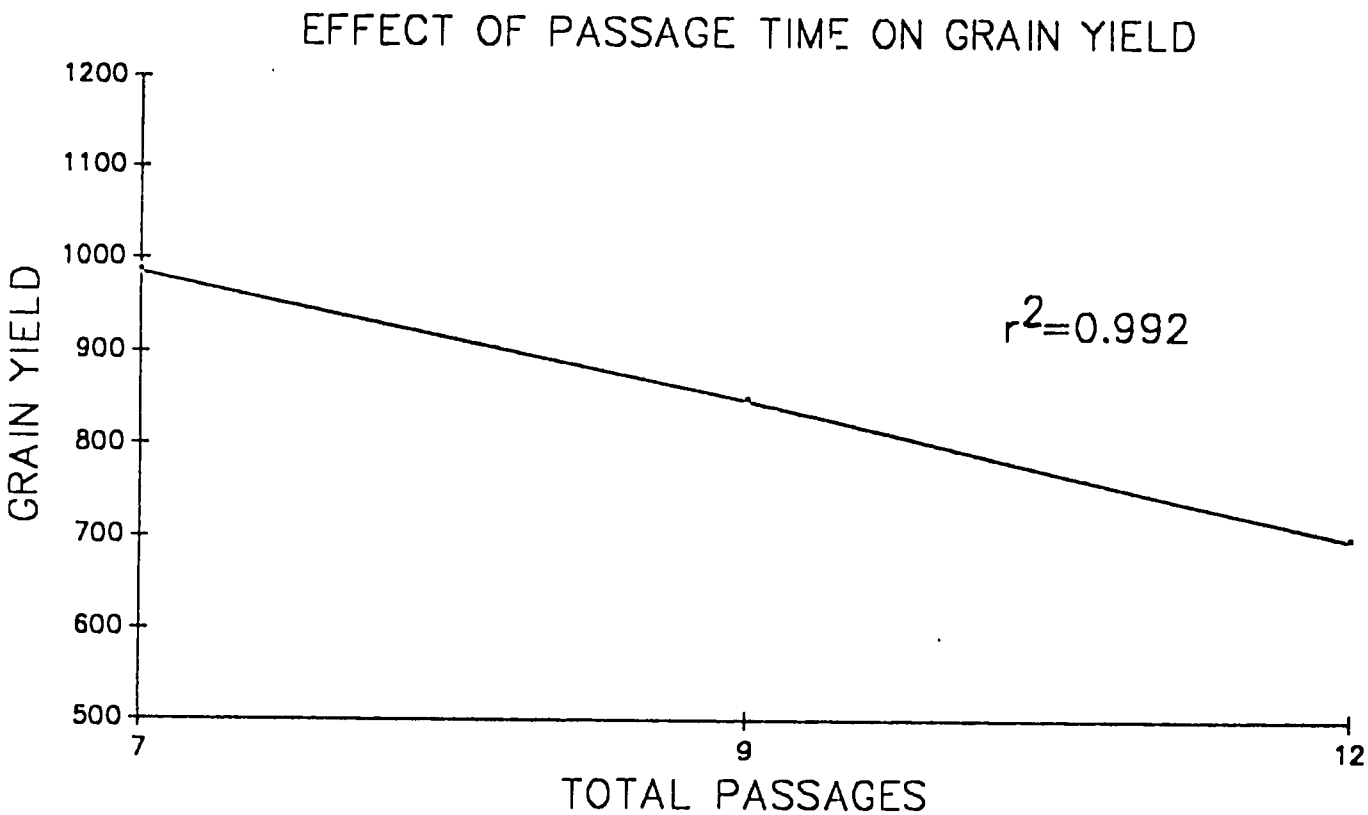
Table 22. Linear contrasts for the variable yield computed using a random AOV model with lines nested within treatments.

Contrast[+]	Probability of > F value
M35 -1 vs. 7°, 0 stress	.005
7°, 0 stress vs. 12°, 0 stress	.001
7°, 0 stress vs. all stress selected (12°)	.001
12°, 0 stress vs. all stress selected (12°)	.012

[+] Treatment with higher yield is listed first.

A final comparison that this test was designed to examine was the effect of passage time. Table 22 indicates that the 7°, 0 treatment was statistically superior to the 12°, 0 treatment. The 9°, 0 treatment was intermediate and not significant. A strong linear relationship between total time *in vitro* and treatment yield is apparent in this material (Figure 1). This has been observed before in other crops and *in vitro* treatments and is possibly due to the higher probability of an event which alters genetic stability in culture. The longer a cell remains in culture and the more times it divides, the more likely some mutation will occur. Commonly, these changes are manifested as lethals or steriles but in some cases only suppress growth or vigor. The poorer performance of the 12°, 0 treatment compared to the 7°, 0 treatment suggest the need to investigate the effect of PEG selection under shorter duration in tissue culture.

Figure 1. Linear regression of total passage or grain yield in R_1 M35-1 under drought stress.



Experiment 20, Workplan 5

Objective

To confirm stress tolerance of sorghum lines previously selected under the arid, saline conditions in Yuma, Arizona.

Research Approach

Five R_3 Hegari, seven R_4 Hegari, and three R_4 RTx 430 selections were evaluated under drought stress conditions at the University of Arizona research farm near Marana, Arizona during the 1989 field season (Table 23). These 15 regenerated lines and their respective checks were maintained under two irrigation regimes; a full irrigated plot where moisture was provided as needed to maintain optimum growth and a second plot where irrigation was limited to impose drought stress. Both plots were planted in a randomized complete block design with four complete blocks. Individual lines were evaluated on two row plots, 3 meters long. The 15 lines included in this study had been selected under two cycles of drought and saline soils at Yuma, Arizona during 1987 and 1988. These lines were originally selected on the basis of vigor and ability to set seed under drought/saline conditions and had not been previously compared for grain yield. This test was set-up to facilitate yield comparison between regenerated and the parental check on a dry non-saline field. Three site visits were made by TCCP personnel to observe the progress of this study during the growing season.

Table 23. Regenerate sorghum lines included in the 1989 Marana drought test.

TCCP Line #	Generation	Parent	Stress Level (g/l NaCl)	Stress Passages	Total Passages	$\bar{x}$ Grain Yield/Dry Plot (g)	$\bar{x}$ Grain Yield/Wet Plot (g)
Hegari Ck	-	Hegari	-	-	-	1314.6	4558.7
862168-22	R_3	Hegari	0	0	4	1409.6	-
862218-24	R_3	Hegari	0	0	12	1549.0	-
864012-22	R_3	Hegari	0	0	4	1069.4	-
862436-32	R_3	Hegari	9	5	11	325.5	-
862509-36	R_3	Hegari	9	7	13	1597.8	-
862021-4-1	R_4	Hegari	0	0	6	1290.5	-
862442-7-21	R_4	Hegari	9	5	11	1098.8	-
862443-1-21	R_4	Hegari	12	4	10	638.4	-
862644-2-22	R_4	Hegari	12	4	10	844.8	-
862644-7-25	R_4	Hegari	12	4	10	1409.6	-
862650-2-21	R_4	Hegari	12	4	10	1365.3	-
862650-7-21	R_4	Hegari	12	4	10	42.0	-
RTx430 Ck	-	RTx430	-	-	-	3114.0	5321.8
862075-46-1-24	R_4	RTx430	0	0	3	2503.9	4895.5
862075-46-1-26	R_4	RTx430	0	0	3	2647.9	4707.2
862075-46-1-30	R_4	RTx430	0	0	3	1617.1	4334.1

Research Output

The Tucson field site proved a much more moderate environment than the previous trials at Yuma. Soil pH and E.C. averaged 7.5 and 2.4 dS/cm respectively compared to a pH of 7.9 and E.C. up to 12.0 dS/cm at Yuma.

Drought and heat stress were also more moderate at Marana. Both Hegari and RTx430 regenerates appeared to tolerate the minimum irrigation regime fairly well. However, grain yields were reduced 70% in Hegari controls and 43% in RTx430 controls as compared to the yield in the full irrigated field.

The results of this test were primarily based on plot yields of the regenerated lines compared to their unselected controls. One problem with basing results solely on the yield of these plots is that the plots were only 3 meters long, which is shorter than desired to give a high degree of precision. Another problem encountered was that some of the entries were still segregating into three to five different phenotypes. This made yield comparisons more difficult. One final difficulty was that the full irrigated Hegari lines grew too tall to mechanically harvest. It was therefore only possible to hand harvest the irrigated Hegari checks for yield comparison to the "dry" checks. The full irrigated Hegari regenerates were not harvested or used for comparison.

All three RTx430 selections out of the line 862075-46-1 were found to be distinctly different from each other and the parent line. Selection 30, which performed much better than check under the harsh conditions at Yuma, was significantly poorer for yield than the check at Marana. The phenotype of this selection was quite different than the parent, with a very upright leaf architecture. The other two selections, numbers 24 and 26, were more similar to the parent and had grain yields which were not significantly different than the check by Dunnett's t-test. Selections of a single phenotype were made from each of the three lines. These will be observed one more season under more severe drought pressure.

The Hegari regenerates appeared to handle the drought stress well based upon vegetative characteristics. However, grain yields were

variable and some entries were partially sterile. Three R_3 lines and two R_4 lines yielded more grain per plot than the check but these differences were not significant by Dunnett's t-test. Larger test plots and higher stress levels may be necessary to detect significance. Four lines performed very poorly under these conditions. These lines were segregating for phenotype and sterility. The top five Hegari lines will be retested next year along with other advanced material under greater drought stress.

EVALUATION OF ACID SOIL TOLERANCE IN $AlCl_3$ *IN VITRO* SELECTED LINES DERIVED FROM HEGARI AND SC283.
(Expt. # 14, Workplan 5)

Summary

Aluminum chloride *in vitro* selected sorghum lines derived from Hegari and SC283 were field tested for acid soil tolerance at Griffin, Georgia in 1989. Statistical analysis was conducted to detect differences between *in vitro* treatments by using a random model analysis of variance with contrast comparisons between treatments.

Results for Hegari lines showed no significant differences between *in vitro* treatments, and a higher mean for the non-regenerated Hegari check than for all *in vitro* treatments. Chi-square analyses were conducted on plots which produced heads at 108 days after planting and heads with harvestable seed. These results indicated that the *in vitro* treatments had lower frequencies than the non-regenerated checks for survival and seed production under acid stress conditions. Although over-all *in vitro* means were lower than the check, a small frequency of individual lines survived and produced seed. These lines will be re-tested in 1990.

Results for SC283 lines showed no significant differences between *in vitro* treatments and no significant difference between *in vitro* treatments and the non-regenerated checks. Differences between individual lines were detected; however, this was attributed to lines which were significantly worse than the non-regenerated check according to Dunnett's t-test. A possible explanation for these results is that the level of acid soil tolerance is already high in SC283, making it difficult to improve the genotype. However, performance of some lines may be lower due to occurance of deleterious mutations.

Collaborating Scientists

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Dr. Mark Brick, Legume Breeder, Colorado State University, Department of Agronomy, Fort Collins, Colorado.

Objectives

1) To determine differences between in vitro treatments of R_2 lines derived from Hegari for acid soil tolerance and to harvest seed from surviving lines for re-testing.

2) To determine differences between in vitro treatments of R_1 and R_2 lines derived from SC283 for acid soil tolerance.

Research Approach (Hegari lines)

Aluminum chloride in vitro selected sorghum lines derived from Hegari were field tested for acid soil tolerance at Griffin, Georgia in 1989. One hundred twenty-two R_2 lines representing in vitro treatments of 0, 100, 300, 400, and 700 ppm $AlCl_3$ and from 8 to 17 total passages (Table 24) were included. A randomized complete block design with 6 replications was used. Non-regenerated Hegari (acid soil-susceptible) and SC283 (acid soil-tolerant) checks were randomized within each block. Plots were 3.0 m long with 0.75 m spacing between rows.

Table 24. Number of R_2 Hegari lines by *in vitro* treatment tested for acid soil tolerance at Griffin, GA 1989.

		In vitro stress level (ppm $AlCl_3$)				
		0	100	300	400	700
Total passages *in vitro*	low (8, 9, & 10)	7	27	16	4	4
	medium (11,12,13,&14)	8	26	1	3	0
	high (15,16,17,&18)	7	15	0	4	0

Three site visits were conducted by TCCP personnel: 1) at 16 days after planting to evaluate stand conditions, 2) at 44 days after planting to rate for vigor and to record the number of surviving plants, and 3) at 108 days after planting to rate for vigor, to record the number of surviving plants, and to record and bag heads capable of producing seed. Vigor ratings were based on a 1 to 5 scale with: 1 = healthy plants, 2 = slight nutritional deficiencies, 3 = plants stunted with some nutritional deficiencies, 4 = plants barely alive with severe nutritional deficiencies, and 5 = dead.

Statistical analysis was conducted with SAS, (Statistical Analysis System), by using analysis of variance with a random model and contrast comparisons to detect differences between *in vitro* treatments. Square root transformations were used on variables with high levels of heterogeneity of error variance. Data was analyzed for surviving plants with heads and heads which produced seed by using chi-square analysis.

Seed was collected from all lines which produced seed. These lines

were also planted at Griffin on a normal pH soil for seed increase (Expt. #17, Workplan 5).

Research Output (Hegari lines)

In vitro treatment F probabilities for the variables vigor at 44 days, number of plants at 44 days, vigor at 108 days, number of plants at 108 days, and number of heads at 108 days are shown in Table 25.

Table 25. In vitro treatment contrasts F probabilities for vigor at 44 days, number of plants at 44 days, vigor at 108 days, number of plants at 108 days, and number heads at 108 days at Griffin, GA 1989.

In Vitro Treatments	Vigor (44 d.)	Plants (44 d.)	Vigor (108 d.)	Plants (108 d.)	Heads (108 d.)
OVERALL TREATMENT EFFECT	.01*	.01*	.01*	.01*	.01*
All trts. vs Hegari	.01*	.02*	.01*	.01*	.01*
0 ppm vs 100 ppm $AlCL_3$	.76	.30	.82	.96	.76
100 ppm vs 3, 4 & 700 ppm $AlCL_3$	.28	.68	.95	.98	.92
0 ppm vs 3, 4 & 700 ppm $AlCL_3$	.50	.65	.82	.95	.75
low pass. vs high pass.	.20	.77	.31	.82	.92

Overall treatment effects were highly significant for all variables. Contrast comparisons for all treatments versus Hegari check were also significant; however, all other contrasts between in vitro treatments were non-significant. This can be attributed to higher mean values for the Hegari check than for in vitro treatments. Means for lines non-stressed in vitro, lines stressed in vitro, Hegari checks, and SC283 checks are shown in Table 26.

Table 26. Means for vigor at 44 days, number of plants at 44 days, vigor at 108 days, number of plants at 108 days, and number of heads at 108 days at Griffin, Georgia, 1989.

	44 days		108 days		
Class	Vigor+	Plants/Plot	Vigor+	Plants/Plot	Heads/Plot
	—rating—	—no.—	—rating—	—no.—	—no.—
Non-stressed *in vitro*	4.80 c*	0.36 b	4.83 c	0.19 b	0.06 b
Stressed *in vitro*	4.81 c	0.52 b	4.85 c	0.17 b	0.08 b
Hegari checks	4.66 b	0.83 b	4.68 b	0.46 b	0.29 b
SC283 checks	2.71 a	26.82 a	2.69 a	21.31 a	17.11 a

* Values within the column followed by a different letter are significantly different at the 5% level of probability using Duncan's multiple range test.

+ Vigor ratings based on a 1 to 5 scale with 1 = healthy and 5 = dead.

Among the 122 R_2 lines tested, 31 lines produced heads at 108 days after planting. A 2x2 chi-square analysis conducted on 1) plots which produced heads 108 days after planting versus plots not producing heads, and 2) regenerates versus Hegari checks is shown in Table 27. Seventeen of 126 Hegari check plots produced heads (13.5%), while only 34 of 732 regenerate plots produced heads (4.6%). These frequencies are significantly different at the .001 level of probability by chi-square analysis. Among the 32 lines which produced heads at 108 days, 9 lines produced heads with harvestable seed. A 2x2 chi-square analysis conducted on 1) plots with harvested heads versus plots without harvested heads, and 2) regenerates versus Hegari checks is shown in Table 28. Six of 126 Hegari check plots were harvested (4.8%), while 9 of 732 regenerate plots were harvested (1.2%). These frequencies are significantly different at the .005 level of probability by chi-square analysis.

Table 27. Frequencies of regenerated Hegari lines and Hegari checks by plots with heads and plots without heads at 108 days after planting.

	Plots with heads (108 d. after planting)	Plots without heads (108 d. after planting)	Total
Hegari checks	17	109	126
All regenerates	34	698	732
Total	51	807	858

Chi-square value P = .001

Table 28. Frequencies of regenerated Hegari lines and Hegari checks by plots with harvested heads and plots without harvested heads.

	Plots with harvested heads	Plots without harvested heads	Total
Hegari checks	6	120	126
All regenerates	9	723	732
Total	15	843	858

Chi-square value P = .005

These results indicated that the *in vitro* treatments had lower probabilities than the non-regenerated checks for the ability to survive and produce seed under acid stress conditions. Although, over-all *in vitro* treatment means were not improved over the mean of the check, a small frequency of individual lines survived and produced seed. Six of the nine lines which set seed were from the low level of *in vitro* $AlCl_3$. These lines are shown in Table 29. To confirm their ability to withstand acid soil stress conditions these lines will be re-tested in 1990 at Griffin, Georgia.

Table 29. Line number, *in vitro* history, and seed harvested from 9 R_2 Hegari lines which survived acid soil conditions and produced seed at Griffin, GA 1989.

Line Number	Total Passages *in vitro*	$AlCl_3$ level	Seed harvested
	—no.—	—ppm—	—g—
02301032-41	13	100	13.6
2031006-48	11	0	11.6
2000110-1	15	100	2.9
02301001-41	8	100	14.8
02301008-41	10	700	0.2
02301012-41	10	100	9.9
2030203-1	8	100	0.1
2030204-1	8	100	2.8
2030301-1	8	300	3.0

Research Approach (SC283 lines)

Aluminum chloride *in vitro* selected lines derived from SC283 were field tested for acid soil tolerance at Griffin, Georgia in 1989. Forty-three R_1 lines and 15 R_2 lines representing *in vitro* treatments of 0, 75, 150, and 225 ppm $AlCl_3$ and from 7 to 10 total passages were included. A randomized complete block design with 6 replications was used. Non-regenerated SC283 was used as a check and randomized within each block. Plots were 3.0 m long with 0.75 m spacing between rows. Site visits and data collection were identical to the Hegari lines previously mentioned.

Statistical analysis was conducted with SAS, using analysis of variance with a random model and contrast comparisons to detect differences between *in vitro* treatments. Individual lines were analyzed

with a fixed model analysis of variance and Dunnett's t-test comparisons were made between lines and the non-regenerated check.

Seed was harvested from each line. These lines were also planted at Griffin on a normal pH soil for seed increase (Expt. #17, Workplan 5).

Research Output (SC283 lines)

In vitro treatment differences were non-significant for all variables (vigor at 44 days, number of plants at 44 days, vigor at 108 days, number of plants at 108 days, and seed weight/head). Means for vigor at 108 days, number of plants at 108 days, and seed weight/head by *in vitro* treatment are shown in Table 30.

Table 30. Number of SC283 R_1 and R_2 lines, mean vigor rating at 108 days after planting, mean number of plants 108 days after planting, and mean seed weight/head by *in vitro* treatment at Griffin, Georgia, 1989.

		In vitro stress level (ppm $AlCl_3$)			
		0	75	150	225
Total Passages *in vitro*	7	n=37* vig = 3.13+ plts = 18.16^ seed = 5.67$	n=3 vig = 3.22 plts = 18.89 seed = 7.93	n=3 vig = 3.06 plts = 18.61 seed = 8.27	n=1 vig = 3.00 plts = 22.50 seed = 3.95
	8 & 9	n=5 vig = 2.90 plts = 22.83 seed = 5.61	X	X	X
	10	n=9 vig = 3.17 plts = 18.06 seed = 5.50	X	X	X

SC283 check - n = 11
vig = 2.86
plts = 20.02
seed = 7.22

* Number of lines

\+ Mean vigor rating 108 days after planting (1 = healthy, 5 = dead)

^ Mean number of plants/plot 108 days after planting

$ Mean seed weight/head (g)

Differences between lines were significant ($P < .05$) for stand, vigor at 44 days, number of plants at 44 days, vigor at 108 days, and number of plants at 108 days. These differences were due to lines which were significantly worse than the check according to Dunnett's t-test. Differences between lines were non-significant for the variable seed weight/head.

These results confirm visual observations made at 108 days after planting. Little variation in plant performance could be visually detected at that time. A possible explanation for these results is that the level of acid soil tolerance is already high in SC283 making it difficult to improve the performance under these conditions. However, one R_1 lines (2040009) had the top grain yield per plot and highest rating for vigor at 108 days. This line was regenerated after seven total passages with no *in vitro* stress. Although this line was not declared statistically superior to the check, its performance was good enough to warrant another generation of testing on acid soil.

CONFIRMATION OF ACID SOIL TOLERANCE IN R_2 AND R_4 LINES DERIVED FROM HEGARI
(Expt. # 15, Workplan 5)

Summary

Twenty-two R_2 and R_4 Hegari lines were tested for acid soil tolerance at Griffin, Georgia in 1989. These lines survived and produced seed under acid soil stress conditions at Griffin in 1988. Among the 22 lines tested, 5 lines produced seed. Two of these 5 lines were uncharacteristic of the Hegari phenotype and will not be evaluated in future generations. The other three lines will be increased in the TCCP greenhouse and re-tested at Griffin in 1990.

Collaborating Scientists

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Dr. Phil Chapman, Statistician, Colorado State University, Department of Statistics, Fort Collins, Colorado.

Dr. Mark Brick, Legume Breeder, Colorado State University, Department of Agronomy, Fort Collins, Colorado.

Objective

To confirm acid soil tolerance of R_2 and R_4 Hegari lines which survived acid soil stress conditions in 1988.

Research Approach

Twelve R_2 and ten R_4 sorghum lines derived from Hegari were field tested for acid soil tolerance at Griffin, Georgia in 1989. These lines survived and produced seed under acid soil stress conditions in 1988. Among the 22 lines tested, 9 lines had no <u>in vitro</u> stress, 7 lines were from $AlCl_3$ <u>in vitro</u> stress, 3 lines were from chlorate <u>in vitro</u> stress, 2 lines were from hydroxy-proline <u>in vitro</u> stress, and 1 line was from $NaCl_3$

in vitro stress. A randomized complete block design with 3 replications was used due to limited seed. Non-regenerated Hegari and SC283 were used as checks and randomized within each block. Plots were 3.0 m long with 0.75 m spacing between rows.

Three site visits were conducted by TCCP personnel: 1) at 16 days after planting to evaluate stand conditions, 2) at 44 days after planting to rate for vigor and to record the number of surviving plants, and 3) at 108 days after planting to rate for vigor, to record the number of surviving plants, and to record and bag heads capable of producing seed. Vigor ratings were based on 1 to 5 scale with: 1 = healthy plants, 2 = slight nutritional deficiencies, 3 = plants stunted with some nutritional deficiencies, 4 = plants barely alive with severe nutritional deficiencies, and 5 = dead.

Statistical analysis was conducted with SAS (Statistical Analysis System), by using analysis of variance and Dunnett's t-test procedure. Seed was harvested from each line which produced harvestable seed. These lines were also planted at Griffin on a normal pH soil for seed increase (Expt. #17, Workplan 5).

Research Output

Significant differences ($P<.01$) between lines were found for all variables (vigor at 44 days, number of plants at 44 days, vigor at 108 days, number of plants at 108 days, number of heads at 108 days, seed weight, and number heads harvested). These differences can be attributed to mortality of some lines and variability between lines which survived these acid soil stress conditions.

Among the 22 lines tested, 5 lines survived and produced seed. Two of these lines were visually different from the Hegari phenotype and

appeared as if they had been crossed with SC283 in the preceding generation or were SC283 plants which were out-of-place due to "dribbling" during planting. These lines will not be evaluated in future generations. The other 3 lines which survived and produced seed are shown in Table 31. Among the 3 surviving lines, 2 lines (2030604-42 and 02301050-42) were stressed *in vitro* on $AlCl_3$.

According to Dunnett's t-test procedure, the 3 lines shown in Table 31 performed better than the non-regenerated control for all variables, however these differences were not significant.

Seed harvested from these lines will be increased in the TCCP greenhouse and planted with 12 replications in the low pH field at Griffin, Georgia during the summer of 1990.

Table 31. *In vitro* background and weight of seed harvested for 3 Hegari lines which survived 2 seasons of acid soil stress at Griffin, Georgia.

Line	Total Passages	Stress Level	Stressing Agent	Seed Harvested in 1989 (grams)
862125-4-6-16	6	0	-	9.6
2030604-42	8	300 ppm	$AlCl_3$	5.5
02301050-42	13	100 ppm	$AlCl_3$	3.3

EVALUATION OF ACID SOIL TOLERANCE IN R_5 SORGHUM LINES
(Expt. #16, Workplan 5)

Summary

Four R_5 lines which survived two previous seasons of acid soil stress were re-tested at Griffin, GA under low pH conditions in 1989. Results indicated that each R_5 line differed significantly from their non-regenerated control for vigor at 44 days, number of plants at 44 days, vigor at 108 days, number of plants at 108 days, and number of heads at 108 days. Seed harvested from these lines showed that each line had the ability to produce seed and complete its life cycle under these conditions. Phenotypic differences were observed within R_5 lines. Selections were made by Dr. Duncan and sent to a winter nursery in Puerto Rico for seed increase. In 1990, seed from Puerto Rico will be strip-planted on the Griffin low pH field. Distinct phenotypes will be determined at this time. Germplasm release will be dependent on the 1990 results.

Objectives

1) To confirm Hegari and RTx430 R_5 sorghum lines which survived 2 previous seasons of acid soil field testing for acid soil tolerance.

2) To compare these lines to their respective non-regenerated controls and to the acid-tolerant check (SC283).

Collaborating Scientists

Dr. Ronny Duncan, Sorghum Breeder, University of Georgia, Georgia Experiment Station, Griffin, Georgia.

Dr. Mark Brick, Legume Breeder, Colorado State University, Department of Agronomy, Ft. Collins, Colorado.

Dr. Phil Chapman, Statistician, Colorado State University, Department of Statistics, Ft. Collins, Colorado.

Research Approach

Two R_5 lines derived from Hegari (H2021 and H2539) and two R_5 lines derived from RTx430 (T75-44 and T75-46) were field tested for acid soil tolerance at Griffin, GA in 1989. These lines survived 2 previous seasons of acid soil testing. A randomized complete block design with 12 replications was used. Each block contained 1 plot of each R_5 line, 2 plots of RTx430 control (acid soil susceptible), 2 plots of Hegari control (acid soil susceptible), and 2 plots of SC283 control (acid soil tolerant). Plots were 3.0 m long with 0.75 m spacing between rows.

Three site visits were conducted by TCCP personnel: 1) at 16 days after planting to evaluate stand conditions, 2) at 44 days after planting to rate for vigor and to record the number of surviving plants, and 3) at 108 days after planting to rate for vigor, to record the number of surviving plants, and to record and bag heads capable of producing seed. Vigor ratings were based on a 1 to 5 scale with: 1 = healthy plants, 2 = slight nutritional deficiencies, 3 = plants stunted with some nutritional deficiencies, 4 = plants barely alive with severe nutritional deficiencies, and 5 = dead.

Soil samples from each plot and composites from each block were collected on the second site visit. Block samples were analyzed for soil pH at TCCP, Colorado State University Soil Testing Lab, and at the Georgia Experiment Station at Griffin. Plot samples were analyzed for soil pH at

TCCP. pH values for each plot were used as covariates in the analysis of variance.

Statistical analysis was conducted with SAS (Statistical Analysis System), by using analysis of variance, analysis of covariance, least-square mean significance probability, and regression analysis. Square-root transformations were conducted on variables with high levels of heterogeneity of error variance. Least-square means were adjusted using soil pH as a covariate for variables with significance by analysis of covariance.

Seed was harvested on a per plot basis and the number of harvested heads per plot was recorded. Yield means were computed to show that these lines were capable of producing seed, not as a yield test.

The four R_5 lines included in this experiment were also planted in a crossing nursery at Blairsville, Georgia (Experiment #18, Workplan 5). These lines were crossed to several male steriles to evaluate for general combining ability. F_1 seed from these crosses was planted in a winter nursery in Puerto Rico for phenotypic evaluation and seed increase. These lines were also planted at Griffin on a normal pH soil for seed increase (Experiment #17, Workplan 5).

Research Output

Soil pH values between blocks ranged from 4.39 to 4.65 with a mean of 4.31. These values are slightly higher than values from 1988.

Lines differed significantly for stand counts ($p < .01$), due to differences in germination and mortality of germinating seedlings before emergence. The relationship between stand and three dependent variables (number of plants at 44 days, number of plants at 108 days, and number of heads at 108 days) was analyzed by regression procedures. R-squared

values were .27, .21, and .21 respectively. These values show that differences in stand had little relationship to plants which survived acid soil stress.

Lines and reps differed significantly ($p < .01$) for vigor at 44 days, number of plants at 44 days, vigor at 108 days, number of plants at 108 days, and number of heads at 108 days. Means for these five variables are shown in Table 32. The covariate soil pH was significant for all variables except number of plants at 44 days. Means were adjusted using the covariate for the other variables. Means of SC283 check for number of plants at 44 days, number of plants at 108 days, and number of heads at 108 days are not shown in Table 32 since comparisons to SC283 were inappropriate due to SC283's high level of acid soil tolerance and ability to germinate and emerge under acid soil conditions.

Table 32. Means of four R_5 lines and three non-regenerated controls for vigor (44 days), number of plants (44 days), vigor (108 days), number of plants (108 days), and number of heads (108 days) at Griffin, GA 1989.

	44 days		108 days		
Entry	Vigor+ -score-	Plants -no./row-	Vigor+ -score-	Plants -no./row-	Heads -no. row-
H2021	2.66	16.31	2.07	12.56	7.37
H2539	3.10	20.83	2.95	12.73	6.39
T75-44	3.07	11.54	2.98	7.39	2.08
T75-46	3.33	12.77	2.94	8.45	4.60
Hegari check	4.51	0.67	4.52	0.29	0.30
RTx430 check	4.85	0.36	4.88	0.30	0.16
SC283 check	2.64	—	2.54	—	—

+Vigor ratings based on a 1 to 5 scale with 1= healthy and 5 = dead. Means adjusted using soil pH as a covariate.

Comparisons between regenerated lines and non-regenerated controls are shown in Table 33. Square-root transformations were conducted on

number of plants at 44 days, number of plants at 108 days, and number of heads at 108 days before significance of differences was determined. These transformations were necessary since few Hegari and RTx430 checks survived and consequently their error variance was extremely low. P-values of least square means show significant differences for each regenerated line compared to its non-regenerated check for all five variables. Comparisons of regenerated lines to the SC283 check are shown for vigor ratings only, due to the inappropriateness of comparison for variables related to plant number as previously mentioned. P-values > .05 for the comparisons between the regenerated lines and SC283 check indicate that the regenerated lines were not significantly different than SC283 for vigor ratings. However, the comparison between T75-46 and SC283 check for vigor at 44 days shows that T75-46 was significantly worse than SC283 check.

Table 33. Significance of probability (P-values)* of four R_5 lines compared to non-regenerated controls for vigor (44 days), number of plants (44 days), vigor (108 days), number of plants (108 days), and number of heads (108 days) at Griffin, GA 1989.

Comparison			44 days		108 days		
line	vs.	check	vigor	#plants+	vigor	#plants+	#heads+
H2021	vs.	Hegari	.0008*	.0004*	.0008*	.0004*	.0004*
H2539	vs.	Hegari	.0008*	.0004*	.0008*	.0004*	.0004*
T75-44	vs.	RTx430	.0008*	.0004*	.0008*	.0004*	.0076*
T75-46	vs.	RTx430	.0008*	.0004*	.0008*	.0004*	.0004*
H2021	vs.	SC283	1.0000	—	1.0000	—	—
H2539	vs.	SC283	.3920	—	1.0000	—	—
T75-44	vs.	SC283	.5352	—	1.0000	—	—
T75-46	vs.	SC283	.0376*	—	1.0000	—	—

*P-values multiplied by the number of comparisons in each column (4 or 8), so that probability of at least one erroneous significance in each column is <0.05 (by Bonferroni inequality). Comparisons having values <0.05 are significant.

+Significance of difference was determined after square-root transformation, which reduced but did not eliminate the heterogeneity of error variance.

Seed yield data is shown in Table 34. Among the two Hegari derived lines (H2021 and H2539), H2021 yielded the best. Both lines produced more seed than the Hegari check. Among the two RTx430 lines (T75-44 and T75-46), T75-44 yielded the best. Again, both lines produced more seed than the RTx430 check. Although the SC283 check produced the most seed (total seed harvested), it did not produce the most seed per head.

Table 34. Total seed harvested, seed weight per plot, total heads harvested, and seed weight per head for four R_5 lines and three non-regenerated controls at Griffin, GA 1989.

Line #	total seed harvested	seed wt/plot	Heads harvested	seed wt/head
	—g—	—g—	—no.—	—g—
H2021	330	27.50	69	4.78
H2539	92	7.67	26	3.54
T75-44	251	20.92	26	9.65
T75-46	58	4.83	34	1.70
Hegari check	5	.21	2	2.50
RTx430 check	0	0	0	0
SC283 check	488	40.66	176	2.78

Phenotypic differences were observed within plots of all four R_5 lines. Line H2021 had 69 heads harvested from 10 of 12 plots. Two plots had two different selections due to variability within the plot. Line H2539 had 26 heads harvested from 8 of 12 plots. Two plots of H2539 had two different selections due to variability within the plot. This genotype had a tendency for longer heads than the normal donor parent. Line T75-44 had 26 heads harvested from 4 of 12 plots. Four different phenotypes were found: 1) open-headed, short, 2) normal (resembles donor parent), 3) open-headed, tall, and 4) normal panicle, tall, and late maturity. Line T75-46 had 34 heads harvested from 9 of 12 plots. Four distinct phenotypes were found: 1) yellow endosperm, normal panicle, 2) white-seeded, 3) late maturity, normal panicle, white-seeded, and 4) white-seeded, normal panicle. This genotype had poor seed set in the panicle under stress.

Representative seed from each selection for each R_5 line was selected from the low pH field by Dr. Duncan and sent to the winter nursery in Puerto Rico. Additionally, selections from the increase field (normal pH) at Griffin, selections from Blairsville, and the F_1 hybrids from

Blairsville were sent. Dr. Duncan plans to strip-plant each selection on the low pH block at Griffin and at Blairsville (high pH) in 1990. Bulked seed from the 1989 low pH field at Griffin will be planted beside these rows for comparison. Additionally, the F_1's will also be planted at both Griffin and Blairsville in 1990.

TECHNOLOGY DEVELOPMENT FOR TISSUE CULTURE, SELECTION AND REGENERATION

The principal objective of the pathbreaking research continues to develop reliable procedures for efficient plant regeneration within the designated crops. The procedures are then extended to include the necessary techniques for plant regeneration after long-term culture, then applied to *in vitro* selection for variants with increased tolerance to salts, aluminum and/or drought. Plants derived from selected, resistant cells are then field tested.

In the past year, the emphasis has been on the application of *in vitro* screening and improving culture reliability and efficiency with spring wheat and sorghum. The other important objectives include rice haploid research, the development of protoplast technologies, widecross research and molecular biology for uses in genetic approaches to stress tolerance.

RICE TISSUE CULTURE

In vitro Selection

Abstract. Rice tissue culture has been nearly phased out. The remainder is an experiment started during 1988. A comparison is being made between the original IR36 line and two R_2 lines which had been selected on NaCl media and found to have increased tolerance in greenhouse hydroponic testing.

Experimental. Embryogenic callus was produced under the usual conditions from two R_2 lines and seeds of the IR36 line. The R_2 lines had been selected from a NaCl tolerance screening in the greenhouse. The intent was to determine if the culture effectiveness and regeneration efficiency of the R_2 was improved, and if the increase in NaCl tolerance was reflected in the cultured cells.

Results and Discussion. The data to date indicate that the growth in the presence of NaCl is almost identical in the selected lines as it is in the original IR36 line. The study will continue to complete an evaluation of the plant regeneration efficiency.

Rice Anther Culture

Abstract. Anther-derived calli were maintained in culture for more than one year, and these calli have not lost the ability to differentiate into plants. The percentage of albino plantlet formation and haploids is low. The calli, on being cultured in media containing 100mM and 150mM NaCl, respectively, were found to take up Na^+ ions and growth was considerably reduced. The calli that were maintained in NaCl media had fewer starch granules in the cells. An ultrastructural analysis of an albino plant showed that the plastids failed to develop normal grana. The progeny of the regenerants are being taken to the next stage of the experiment, namely greenhouse testing for NaCl tolerance.

Materials and Methods. Anther calli obtained from rice cultivar "Yamahoushi" were used for all the experiments listed below. B5 medium with 1ppm indole acetic acid (IAA) and 0.5ppm benzyladenine (BA) was used for regenerating plants from androgenic calli. The regenerants were transferred to liquid medium with 0.1% LS salts until they developed a sufficient amount of roots. These were then placed in the greenhouse nursery for 2 weeks before being grown in tanks in the greenhouse. The plants were grouped based upon their morphology and seed set. Roots from randomly selected plants within these groups were harvested to investigate their ploidy level, as it was too time consuming to check the ploidy level of the 212 plants regenerated. Root tips were stained with 2 drops of 1% acetocarmine for 10 minutes. A coverslip was placed above it and the tip

smashed with a matchstick. Thumb pressure was applied to disperse the cells and then was viewed through a phase contrast microscope.

Callus that belonged to a single cell line was cultured in media without NaCl as well as with 100mM NaCl and 150mM NaCl. After a period of one week in culture these calli were freeze-dried at -196x C and fractured. The freeze fractured calli were mounted on a scanning electron microscope stud and an X-ray chemical microanalysis performed. The major elements that were analyzed were sodium, potassium, calcium, and chloride.

The calli that were cultured with and without NaCl were also fixed and embedded in plastic. These were then sectioned and stained for observation under a transmission electron microscope. The leaf from an albino regenerant was fixed, embedded in plastic, sectioned and stained for analysis under a transmission electron microscope, just as in calli analysis.

Results. Of the 212 plants that were regenerated from androgenic calli, 4 were albino, and 15 were found to be haploids. The presence of haploid and diploid regenerants in a single cell line demonstrates that the diploids were actually di-haploids, with the duplication of the chromosome number occurring early in cell line growth. This is not unknown in rice anther culture, but what is unique is that the percentage of haploid regenerants was very low, a fact which may be attributed to the induction of embryogenic cell lines. The same reasoning could be used to account for the insignificant level of albino plantlet regeneration. This means that only cells that are diploid and normal tend to become embryogenic while the other cells — haploids and defective ones — fail to become embryogenic and may be lost amidst the more rapidly-dividing embryogenic cells.

A major criticism laid against using solid medium for cell selection process is that only the cells that are in contact with the medium are subject to the selection process, while the cells above them may escape the toxicity of the selective agent. Later plants may be regenerated from these "escapees" which may account for the fewer tolerant lines at the whole plant level. In order to see whether this criticism is valid an experiment was conducted. A cell line was cultured in media containing NaCl and without it. Cells from the apex of the culture were harvested after one week of culture and an X-ray chemical microanalysis was performed. The microanalysis failed to detect any Na^+ outside the cell, but showed the presence of sodium ions inside the cells that were cultured in media containing different levels of NaCl. As the level of NaCl increased in the medium, the amount of sodium inside the cells also increased. The presence of sodium inside the cells affected the levels of potassium and calcium.

An ultrastructural analysis of the cells showed that cells cultured in medium without NaCl accumulated plenty of starch granules, while the amount of starch granules decreased as the NaCl level increased in the culture medium. This suggests that the cells utilized all the energy to counter the negative effects of Na^+, while the cells that are not exposed to NaCl had excess energy which they stored as starch granules.

As for the origin of excessive numbers of albino plants from anther culture of cereal grains, it was explained earlier in the literature that the microspores contain very few proplastids (precursors of chloroplasts), hence the albinos. There was also a report stating that inversions that occur in the plastid genome resulted in the production of albinos. An ultrastructural analysis of albino regenerants showed that these leaves do contain plastids that are similar to chloroplasts, except that the grana

in them were not properly developed. This could be due to a developmental gene mutation.

Conditioning Factor

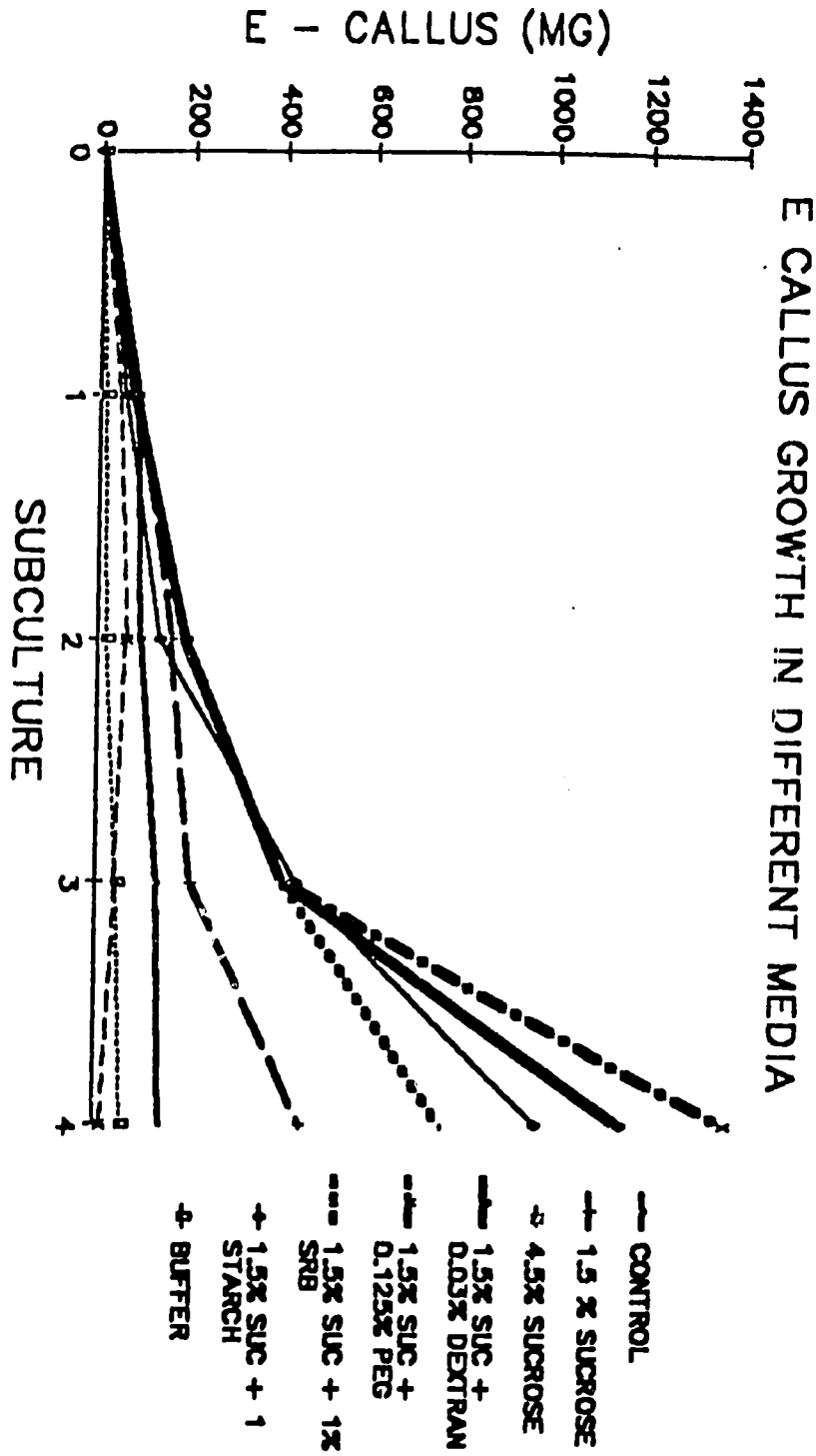
Abstract. In the previous annual report, based on callus initiation results, it was shown that there seemed to be an optimum pH that was desired by the tissues growing in in vitro conditions. Studies were aimed at determining the kinetics and quality of cell growth in different media including in those that have low levels of osmotic agents.

Materials and methods. Mature seeds of IR36 were dehusked, surface sterilized with 70% commercial Clorox for 30 min, rinsed three times with sterile de-ionized water, and plated singly on 10 ml of solidified L&S medium in vials. The cultures were incubated under a light regime at 23±2x C. After 28 days, callus was harvested from each vial, separated into E and NE callus, and weighed individually. The pH of the medium in each vial was also observed. Embryogenic callus was subcultured again on callus maintenance medium at 5.5 mg/ml. The medium was supplemented with one of the following compounds: 0.03% dextran (mol wt. 81,500); 0.125% PEG (mol wt. 20,000); 1% sorbitol; 1% rice starch; different levels of sugar; and buffers. A randomized block design was used throughout the experiments. Each experiment consisted of at least 30 vials and was replicated unless stated otherwise in the results.

Results. It was found that the cells turned non-embryogenic at a greater frequency as the sugar level was increased. Cells that were cultured in media containing dextran/PEG produced more E callus than the ones that contained sorbitol or starch. The results of data accumulated over 4 passages are presented in Figure 2. When the cultures were plated for plant differentiation, cultures in starch produced more plants than

others; however, the regeneration trials were not repeated and it is not known whether this is true even if the cultures were to be differentiated at earlier stages in their culture period.

Figure 1. The effect of different compounds on E callus growth in rice.



RICE PROTOPLAST ISOLATION, FUSION, CULTURE

Abstract

A simplified, successful, and broadly applicable methodology has been established for the isolation, culture, and fusion of protoplasts from suspension cell cultures of a wide variety of monocots and dicots. The method is identical for three cultivars of rice (Oryza sativa), two japonica and one indica, two salt marsh grasses (Distichlis spicata, and Spartina pectinata), and carrot (Daucus carota cv. Nantes). Protoplasts isolated from these suspensions readily form cell walls and divide to form callus. Protoplasts from Basmati rice have been fused with protoplasts of the salt marsh grasses, using a modified polyethylene glycol (PEG) technique. Protoplasts that have been through the fusion protocol form callus.

Experimental

Non-embryogenic suspensions of two japonica rice cultivars, Taipei 309 and Yamahoushi, one indica rice cultivar, Basmati, the two salt marsh grasses, Distichlis spicata and Spartina pectinata, and an embryogenic suspension of carrot, have been maintained for 1-2 years in a modified amino acid medium (2AA2).

Protoplasts were rapidly and easily obtained from these suspensions by incubating 3 or 4-day-old cells in mDSP1 medium containing 10% glucose as the osmoticum. Rice and carrot protoplasts were incubated in this medium with 1% Onozuka 'RS' cellulase, and 0.125% Sigma pectolyase. Cells of the salt marsh grasses were incubated in the same medium with 3% Onozuka 'RS' cellulase, 3% Sigma hemicellulase, 0.375% Sigma pectolyase. A 10 ml aliquot of suspension was transferred to a centrifuge tube. The cells were allowed to settle, or were pelleted, the supernatant removed

and replaced with 7 ml of filter-sterilized enzyme containing mDSP1. Cells were incubated in the dark at 28xC, 50 rpm, for 4 hours.

Isolated protoplasts were purified by filtering through a 20-30 um nylon mesh, and washing several times in mDSP1 medium. Protoplasts were plated at a density of 250,000 protoplasts/ml in 2 ml thin layers of mDSP1+10% glucose, in 60x15 mm petri dishes, and incubated in the dark at 28xC. After 5-10 days, fresh mDSP1 medium containing no glucose was added to the cells. Four weeks after protoplast isolation, cell colonies were washed from the petri dishes with 2AA2 medium, and transferred to petri plates containing 2AA2 medium solidified with 1% agar. These colonies were allowed to grow until large enough to transfer back into suspension or into jars.

Protoplast fusion has involved the fusion of Basmati rice protoplasts with protoplasts of the halophytes Distichlis spicata and Spartina pectinata. The fusion protocol was a PEG, high pH, high calcium chemical technique, highly modified from that of Kao, 1986. All manipulations during the fusion protocol were in a 40 ml 'Oak Ridge' centrifuge tube. Fusions were monitored by a stain/counter-stain technique using the fluorescent vital stains rhodamine isothiocyanate (RITC) and tetrachlorofluorescein (Cl4F).

Fused protoplasts were washed several times, and then plated using the same procedure as unfused protoplasts.

Results

Cell suspensions were best maintained in 2AA2 medium, an amino acid medium containing twice the concentration of amino acids previously reported (Muller and Grafe, 1978). Increasing the amino acids was necessary to prevent exhaustion of the medium, presumably the nitrogen

source, by the rapidly dividing salt marsh grass suspensions. Faster growth was obtained in 2AA2 than in a medium containing either the B5, LS, L6, or N6 major salts. Protoplasts were easily isolated from the suspension cultures, and rapidly formed colonies when plated in 2 ml thin layers in sealed 60x15 mm petri dishes.

Protoplasts were chemically fused with PEG 8000, modified from the procedure of Kao, 1986. Fusion did not occur when using PEG with a molecular weight below 8000; however, PEG 8000 and PEG 20,000 both promoted fusion.

A rapid and large scale fusion technique has been developed. Fusion was done at the interface of a PEG/protoplast suspension layer in a 40 ml centrifuge tube. All washes were done in the centrifuge tube. The advantage was that no protoplasts were lost in transferring from petri dish to centrifuge for washing. It was also possible to use much larger volumes of reagents and protoplasts than can be done on a cover slip in a petri dish (Kao, 1986). The disadvantage, of course, was that the protoplasts could not be monitored during the actual fusion process.

A vital staining procedure has been developed to allow the monitoring of protoplasts during fusion. Typically, investigators have used a combination of rhodamine isothiocyanate (RITC) and fluorescein isothicyanate (FITC) to stain the protoplasts prior to fusion, fuse them, and observe the fusion products by UV microscopy. One species appears reddish-orange (RITC), and the other species appears yellowish-green (FITC). It was found that while RITC stained protoplasts appear to stain brightly, FITC protoplasts stained comparatively dimly. Thus, a different stain, tetrachlorofluorescein (Cl4F), was found to give excellent results when used as a counter stain with RITC. Vital staining of the protoplasts

shows that fusion does occur between rice and salt marsh grasses.

The PEG fusion procedure did not appear to affect cell colony and callus formation. Colonies formed routinely, and reproducibly, from protoplasts that had been exposed to the PEG and high pH solutions during the fusion process.

Verification of hybrids is being done by isozyme analysis of putative hybrids and comparing the isozyme patterns of the hybrids to those of the parent cell lines. A hybrid cell selection procedure is being developed.

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SORGHUM TISSUE CULTURE

Abstract

Sorghum tissue culture has focused primarily on improving E callus growth and determining selection levels of aluminum. Several studies were completed in the past year. An evaluation of tissue culture performance of genotypes has demonstrated a substantial difference in efficiency of plant regeneration. The inclusion of 1% sorbitol in the culture medium improved growth of E callus. Selection level experiments of $AlCl_3$ are being tested in combination with the addition of sorbitol and increased sucrose concentration to the medium. Critical experiments were conducted for aluminum and drought tolerance. Attempts to get regeneration from the callus cultures were successful. Regenerated plants were grown in the greenhouse for seed increase and further field testing. Experiments in progress are designed to compare aluminum selection on pH normal for tissue culture and more acidic conditions which are typical for acidic soils.

Experimental

Initiation. Screening for callus initiation and E callus production on basal medium was successfully achieved on medium containing 2 mg/l 2,4-D and 0.5 mg/l kinetin (KIN).

Sucrose and Sorbitol. Experiments were conducted to determine the optimum levels of sucrose and sorbitol. The objective of the studies was to determine if varying the sucrose or sorbitol concentration would enhance E callus formation and growth. To test whether or not E callus could be successfully grown on different concentrations of sucrose and sorbitol, two experiments were conducted. In the first experiment, callus production of three cultivars of sorghum (RTx430, Hegari and M35-1) were

initiated and maintained for seven passages on medium containing 2%, 4%, 6%, 8%, 10% and 12% sucrose, respectively. After the initial period of culture, E callus was placed on regeneration medium containing 1 mg/l IAA and 0.5 mg/l BA for one passage. After one passage on regeneration medium, the produced plantlets were placed on 3 mg/l IBA medium for rooting. In a second experiment, calli of RTx430, Hegari, and M35-1 were initiated and maintained on 0%, 1%, and 2% sorbitol, respectively, for seven passages. A third experiment was conducted to test the interaction of sucrose and sorbitol on E callus initiation and growth. Callus of M35-1 was initiated on medium with 2% and 4% sucrose, 2% sucrose + 1% sorbitol, and 4% sucrose + 1% sorbitol. After four passages on these media, all E calli were weighed and transferred to 2% sucrose, 4% sucrose, 2% sucrose plus 100 mg/l $AlCl_3$, 2% sucrose plus 1% sorbitol, and 2% sucrose plus 1% sorbitol plus 100 mg/l $AlCl_3$. This experiment is in progress.

$AlCl_3$. Experiments were performed to produce plants after *in vitro* screening on several $AlCl_3$ stress levels. A total of ten sorghum varieties — Hegari, 2024-7-19 (Hegari R2 line), M35-1, SC283, SC599, RTAM428, RTx2536, SC214, RTx430, SC689, and SC574 — were grown on nonstressed media. After three to four months on maintenance media, the calli were moved to medium with or without $AlCl_3$. Embryogenic calli from the first two cultivars, Hegari and 2024-7-19, were placed on media containing 0, 100, and 200 mg/l $AlCl_3$. After three passages on stress medium, cultures were placed on regeneration medium. Plants were regenerated from *in vitro* selected cultures.

Embryogenic calli from M35-1 were moved onto media containing 0, 50, 100, and 150 mg/l $AlCl_3$ and maintained for three passages. Plant

regeneration was achieved after three passages on stress. The E calli from nine cultivars were transferred to media containing 100 mg/l Al. After four passages on stress, the calli were placed on regeneration media containing 1 mg/l IAA and 0.5 mg/l BA for plant regeneration.

Additional experiments were set-up to confirm earlier data. In the first experiment, M35-1 calli was initiated from mature embryos on media containing 0, 25, 50, 75 and 100 mg/l $AlCl_3$. Embryogenic calli obtained after in vitro selection were placed on regeneration medium after six passages on stress. In the second experiment, E calli of SC214 produced on nonstressed medium were moved to media containing 0, 75, and 150 mg/l $AlCl_3$. Currently, the calli are on the second passage of stress.

Experiments have been set-up to determine the effect of the pH of the medium on $AlCl_3$ toxicity. The aluminum tolerance is being tested at the normal pH of 5.5 - 5.7 and at the pH of 4.2 - 4.4 which usually prevail in acidic soils.

Result and Discussion

Effect of Genotype. The minimum requirement for a tissue culture system is plant regeneration. To have an efficient system for in vitro selection, embryogenic callus must be identified and maintained for the duration of the selection and plants recovered after a lengthy period in culture. The initial problem is the identification of E callus. Over the past two years, a large number of embryos from four cultivars have been plated. The cultivars Sc283 and M35 produced the most E callus, followed by Hegari, and RTx430 which produced the least E callus (Table 35).

Table 35. Effect of genotype on the percent of embryos producing E callus.

Cultivar	N	Percent*
RTx430	320	1.30 ± 0.83
Hegari	776	5.49 ± 1.48
Sc283	860	20.66 ± 5.50
M-35-1	282	32.30 ± 12.15

* Mean ± standard error

Sucrose and Sorbitol. Over six passages, E callus growth on 6%, 8%, 10%, and 12% sucrose declined, but on 2% and 4% sucrose the growth was the same as the control. On seventh passage, the E callus from RTx430 on 4% sucrose fell to almost nil. Embryogenic callus from M35-1 and Hegari on 2% and 4% sucrose treatments were performing well. The 0.1 grams of Hegari and M35-1 E callus placed on regeneration medium at the end of seventh passage produced 10 plants from 4% sucrose, 20 plants from 2% sucrose, and 27 plants from 4% sucrose, respectively. The conclusion is that the addition of sorbitol can increase E callus growth (Table 36). The E callus of M35-1 on 1% sorbitol grew faster, was of good quality, and produced more plants than callus on 0 or 2% sorbitol. Hegari E callus from 0% and 1% sorbitol was of poor quality and did not regenerate, while callus on 2% sorbitol regenerated plants. RTx430 E callus at the end of third passage on 0%, 1%, and 2% sorbitol died and no plants were obtained from this cultivar.

Table 36. Regenerated sorghum plant data from callus maintained up to nine months.

Cultivar	Passage	E Callus Wt.	Treatment	# Plants	P/g Callus
M35-1	9	2.6631	2% sucrose	20	7.51
		6.0344	4% sucrose	27	4.47
Hegari	9	0.3199	4% sucrose	10	31.26
RTx430	9	0	none	0	0
M35-1	9	0.2637	0% sorbitol	8	30.34
		3.1560	1% sorbitol	24	7.60
		6.8303	2% sorbitol	45	6.59
Hegari	9	5.0136	2% sorbitol	23	4.59
RTx430	9	0	none	0	0

<u>$AlCl_3$ Selection</u>. Growth of E callus on $AlCl_3$ levels was improved over the original experiments conducted. $AlCl_3$-tolerant cell lines of Hegari and R_2 line from Hegari selection (2024-7-19) were cultured on 0, 100 and 200 mg/l $AlCl_3$. At 200 mg/l $AlCl_3$, the Hegari E callus died after second passage; E callus decreased at the 100 mg/l and control treatments as well. The R_2 line showed the same response as Hegari. In Hegari, 2 plants were produced from the control, 3 plants from 100 and no plants from 200 mg/l $AlCl_3$. In 2024-7-19, no plants were obtained from the control due to contamination, 12 plants from 100 mg/l and 8 plants from 200 mg/l $AlCl_3$ were produced. Regenerated plants are shown in Table 37.

Table 37. Number of plants which regenerated from nine passages on $AlCl_3$.

Cultivar	Passage	Stress Level	# Plants	Plants/Gram of Callus
Hegari	9	control	2	6.7
		100 ppm $AlCl_3$	3	30
		200 ppm $AlCl_3$	0	0
2024-7-19	9	control	0	0
		100 ppm $AlCl_3$	12	20
		200 ppm $AlCl_3$	8	26.7

In another experiment, M35-1 cultures were used to determine critical levels for $AlCl_3$ stress for E callus formation and plant regeneration. Embryogenic callus cell lines declined in weight over three passages on the stress and control. Embryogenic callus from 150 mg/l $AlCl_3$ failed to survive after third passage. At low levels of 50 and 100 mg/l, there was a significant effect on growth of E callus compared with the control. In this study, 150 mg/l $AlCl_3$ was considered the critical level for selection. Although no plants were regenerated from the control or 150 mg/l $AlCl_3$, 4 plants from 50 mg/l and 3 plants from 100 mg/l were produced and grown to maturity (Table 38).

Table 38. Number of sorghum plants regenerated from $AlCl_3$ stress experiment which matured and set seed.

Cultivar	Passage	Stress Levels	# Plants	Plants/Gram of Callus
M35-1	12 (6 stress)	control	0	0
		50 ppm $AlCl_3$	4	40
		100 ppm $AlCl_3$	3	15
		150 ppm $AlCl_3$	0	0

Leaf Culture. Experiments were performed on plant regeneration from leaf tissue culture. The purpose of this study was to identify the optimum age of tissue for somatic embryo formation. Seeds of Hegari, M35-1

and Sc283 were sterilized and germinated on LS medium without 2,4-D or kinetin. Seedlings were removed from the jars and selected at random five, seven and ten days after the seeds had been placed on germination medium. After each seedling had been measured, four sections of 1-2 mm, starting from the bottom of the seedling, were cut off and placed on 2D/.5K medium. Data were collected when there was significant callus formation. Embryogenic callus was transferred to 2D/.5K and regeneration medium every passage and maintained for five passages. Plants were obtained.

WHEAT TISSUE CULTURE

Abstract

Aluminum chloride tolerance studies for spring wheat have progressed to the germplasm screening stage. The selection levels had been determined for $AlCl_3$. The objective was to produce more germplasm from selected stress levels. Another study was to investigate the effect of sorbitol on increasing E callus growth. The experiments in progress are designed to select for aluminum tolerance at pH levels found in acidic soils.

Experimental

$AlCl_3$. In two experiments, mature embryos of Pavon and Glennson were initiated on medium containing 0, 100, and 200 ppm $AlCl_3$. Stepwise and long-term selections were used. In the stepwise approach, the cultures were transferred to increasing concentrations every third passage and then regenerated after cultures were grown on a constant level of $AlCl_3$ for nine passages. Regenerated plants were grown to maturity in the greenhouse. A second experiment was set-up to generate more germplasm from $AlCl_3$-tolerant cells. This experiment was similar to the previous experiments. The cultures on 0, 100, and 200 ppm $AlCl_3$ treatments are on ninth passage. After ninth passage in culture the calli will be transferred to regeneration medium to produce whole plants. An experiment now in progress is designed to determine the effect of pH of the medium on $AlCl_3$ toxicity. In this study, $AlCl_3$ tolerance is being tested at normal pH of 5.5 for tissue culture and acidic pH of 4.2 which prevails in acidic soils.

Sorbitol. An experiment was conducted to determine the effect of sorbitol on E callus growth. Mature embryos of Pavon and Glennson were

callused on medium containing 0%, 1%, and 2% sorbitol. Calli were maintained on their respective media for four passages. Data and E calli weight were recorded at the end of third and fourth passages. Plant regeneration was started on fifth passage.

Result and Discussion

$AlCl_3$. The results of the experiments demonstrated that constant level and stepwise selection can be used and that subsequent plant regeneration is quite successful (Table 39). Growth of E callus on various $AlCl_3$ levels was improved. The total number of shoots produced per treatment from each cultivar is shown in Table 39. Stepwise and constant long-term culture of Glennson experienced a few hours of 130° F heat due to a malfunction in the culture room. For this reason, no plants were obtained from regeneration after ninth passage on stress.

Table 39. Regenerated spring wheat plants from stepwise and long-term $AlCl_3$ stressed cultures.

CULTIVAR	PASSAGE	TREATMENT	MEDIUM	# PLANTS	PLANTS/g EC
Pavon	6	long term	2D (control)	74	148
Pavon	6	long term	2D + 100 ppm Al	70	140
Pavon	6	long term	2D + 200 ppm Al	54	108
Glennson	6	long term	2D (control)	70	140
Glennson	6	long term	2D + 100 ppm Al	64	128
Glennson	6	long term	2D + 200 ppm Al	76	108.6
Pavon	9	long term	2D (control)	157	104.7
Pavon	9	long term	2D + 100 ppm Al	466	194
Pavon	9	long term	2D + 200 ppm Al	55	183
Pavon	9	step wise	2D,2D+100,2D+200	158	92.9

Sorbitol. The inclusion of sorbitol did not enhance callus growth in spring wheat. At 2% sorbitol there was a decrease in growth (Table 40).

Table 40. Plants regenerated from various sorbitol levels.

CULTIVAR	PASSAGE	TREATMENT	# PLANTS	PLANTS/ GRAM OF CALLUS
Pavon	5	2D + 0% sorbitol	149	107.8
Pavon	5	2D + 1% sorbitol	125	96.2
Pavon	5	2D + 2% sorbitol	82	82
Glennson	5	2D + 0% sorbitol	752	250.6
Glennson	5	2D + 1% sorbitol	813	290.4
Glennson	5	2D + 2% sorbitol	436	114.7

These results are somewhat unexpected. In other cereals such as rice and sorghum, the presence of about 1% sorbitol has an enhancing effect on E callus production and plant regeneration efficiency.

MOLECULAR BIOLOGY

Summary

The molecular biology part of the project has been reduced to an activity which focuses on using RFLP markers to identify somaclonal variants. The research is being undertaken by a graduate student.

The research on the molecular biology of salt tolerance was discontinued at the end of 1988, when the designated funding period ended. The activity was a collaboration with Dr. J. Colbert of the Biology Department at CSU. Much of the laboratory work was performed by a graduate student, Mr. Isaac John, from Pakistan, who was supported by USAID Pakistan. Dr. Colbert and Mr. John are now at Iowa State University. The research is reported in the Annual Report of 1988 and in the paper by Gamborg et. al. to the International Plant Biotechnology Conference in Nairobi. (See list of references). The research is not sufficiently advanced to submit the data for publication in a refereed journal.

HALOPHYTIC KALLAR GRASS

The goal of the molecular biology program was to obtain the genes which could be used to confer salt tolerance to crop plants. The rationale was to investigate species which tolerate and complete their life cycle growing in high levels of salt and attempt to isolate the genes responsible for the salt tolerance trait. Since one of the principal objectives was to transfer salt tolerance to cereals, such as rice and wheat, the salt-tolerant species used was the halophyte, kallar grass (Leptochloa fusca). The experimental materials being used were suspension cultures. Results have shown that, when the cells are cultured in media containing 20 g/l NaCl, the growth is reduced to about 50%. Such cells

are considered to be stressed.

Initial experiments were performed to determine the presence of stress-induced proteins in cells of the kallar grass. That was investigated by isolation of total RNA from which the Poly(A)RNA was obtained. After in vitro translation of the poly(A)RNA, the polypeptides were analyzed by SDS polyacrylamide gel electrophoresis. There were no detectable stress-induced polypeptides observed from cultures stressed with up to 3% NaCl for up to 96 hours; however, this analysis would only detect changes in relatively abundant poly(A)RNA species. Dramatic changes in the levels of relatively rare poly(A)RNA species would be undetectable.

Cascade (Subtraction) Hybridization

The method of subtraction hybridization (Sargent 1987) was employed as an approach to detect subtle changes in gene expression. The method makes it possible to obtain an enrichment of low level mRNA's as the complementary DNA clones, which can be used as probes. In the procedure, poly(A)RNA from cells which had not been salt-stressed were hybridized with single-stranded cDNA derived from salt-stressed cells. The hybridization mixture was then fractionated on a hydroxyapatite column. The cDNA species corresponding to mRNA species present in the non-stressed cells form RNA-DNA duplexes. The resulting double-stranded molecules bind to the column under the conditions used. The cDNA species that corresponded to the mRNA species, which were present at the elevated levels in the poly(A)RNA isolated from salt-stressed cells, remained single-stranded and did not bind to the hydroxyapatite.

The results of initial experiments indicated that approximately 17% of the radioactivity in the cDNA population from salt-stressed cells was

increased in abundance relative to the mRNA population in the non-stressed cells. These results show that it may be possible to enrich the population of cDNA's from salt-stressed cells of kallar grass for messages which are induced by salt. The procedure is being used to prepare salt stress-enriched cDNA probes for use in the screening of the cDNA library. Such a library has been produced from poly(A)RNA isolated from salt-stressed cells of kallar grass. The library consists of about one half million clones with an average size of 1000 BP.

SALT STRESS-REGULATED mRNA INDUCTION IN RICE

Most crop species are sensitive to salt stress to varying degrees (Maas 1986). During salt stress, the plants of a particular species may respond by producing specific proteins. The research rationale in the TCCP program is to investigate plants of particular crops in which cells or regenerated plants have been selected for increased salt tolerance by *in vitro* screening. The investigation to date has focused on studies on rice cells to determine their response to salt stress. The response was measured by the production of polypeptides by *in vitro* translation using poly(A)RNA from salt-stressed and non-stressed cells.

Experimental and Results

A cell suspension culture (RB 387) of Basmati rice (B-370) was used for the experiments. The cells were cultured in L6 medium (Kumar *et. al.* 1988) and were used six days after subculture as inoculum. In the initial experiments, the cells were tested at 0 to 30 g/l NaCl for time periods of up to 48 hours. After treatment, the cells were harvested, frozen in liquid nitrogen, and stored until RNA isolation. Cells of the same age were also used in experiments to test the effect of other salts ($NaSO_4$,

KCl, $CaCl_2$ and $AlCl_3$, $MgCl_2$) at similar concentrations. Tests with PEG at 300 mg/l and ABA at 10 mg/l were also included. In these experiments the cells were harvested after 24 hours. The effect of salt stress was studied with seedlings grown in hydroponics in nutrient solutions which contained up to 30 gm/l NaCl. Total RNA was isolated from each sample. The poly(A)RNA was then isolated by affinity chromatography on a poly(U) Sephadex column (Colbert et. al. 1985). The in vitro translation was performed with the poly(A)RNA in a nuclease-treated, rabbit reticulocyte lysate. The polypeptides were analyzed by electrophoresis using 18% SDS polyacrylamide gel. The gels were stained with Coomassie blue, dried and exposed to Kodak X-ARS film. The results to date have shown that several polypeptides are produced in response to stress by NaCl, $AlCl_3$, PEG as well as ABA (Table 41).

Table 41. Stress Induced Proteins Produced by Rice Cell Cultures and Seedling Tissues in Response to Stressing Agents.

CELL CULTURES: Stressing agent	Proteins (KD)
Sodium chloride	16.3, 20, 24
Aluminum chloride	26, 39.8
Abscisic acid	20, 21.4, 24, 30, 39.8
SEEDLING/SHOOTS:	
Sodium chloride	17.6, 20, 40, 47, 51.5
SEEDLING/ROOTS:	
Sodium chloride	31.6, 51.5, 53.4

In rice shoots, five different mRNA's were induced that encoded proteins of 17.6 to 51.5 kDa within twelve hours of salt stress, while the synthesis of three other proteins of 53.4, 51.5, and 31.6 kDA were enhanced in seedling roots in response to stress. The mRNA which encoded 51.5 kDa protein was induced both in shoots and roots exposed to NaCl for twelve hours or more.

In cell cultures exposed to NaCl, the proteins of low molecular weight, ranging from 16.3 to 24 kDa, were overproduced. Most of the mRNA's encoding these proteins were induced within twelve hours in cells exposed to $\geq$ 0.75% NaCl. These proteins were also accumulated in response to $CaCl_2$, Na_2SO_4 and KCl, but not with $AlCl_3$ or $MgCl_2$. The cells exposed to ABA for 24 hours triggered the transcription of mRNA's found in both salinity and non-osmotic metallic ion-exposed cells. These findings suggest that tissue specific transcriptional mechanisms regulate gene expression in rice when exposed to stress factors.

USING RFLP MARKER TO IDENTIFY SOMACLONAL VARIANTS

Experimental Design

DNA from ten plants in each of six categories will be hybridized with ten different putative single copy gene probes for RFLP analysis. Category A is a control to check on possible RFLPs among IR36 seed lots, with one plant from each of 10 seed lots of non-tissue-cultured material. Category B is chemical mutagen (ethyl methane sulphonate) treated seed. Category C contains somaclonal variants based on either morphological differences, a high rate of sterile seeds, or screening test performance significantly different from the controls (Table 42). Three categories (D-F) are defined by number of passages in culture before regeneration. Low passage number was defined as 1-7 passages, medium passage number as 8-14, and high passage number as 15 or more. The specific regenerant lines used are listed in Table 43, along with number of passages in culture, passages on NaCl stress, and stress level. Where more than 10 lines are listed within a category, the ten lines with the best DNA extracted will be used.

Results and Discussion

The DNA labeling system, ChemiProbe (FMC BioProducts), which was used during much of the past year, proved to be inadequate for resolution of single copy genes. This labeling and DNA detection system involves sulfonation of the cytosine moiety of the probe DNA. After restriction endonuclease digestion, gel electrophoresis, Southern blotting and hybridization, a monoclonal antibody and enzyme-anti-immunoglobulin conjugate binds to the modified DNA and converts a chromogenic substrate into an insoluble dye which should show up as a band at the appropriate location on the hybridization membrane. The bands observed were extremely faint, even when the protocol for maximum detection sensitivity was used. Increasing the DNA concentration, chromogenic substrate concentration and incubation times resulted in an unacceptable level of background. After numerous attempts to optimize the system, the decision was made to switch to a different non-radioactive labeling system.

The Genius DNA labeling and detection kit (Boehringer Mannheim Biochemicals) is now being used. Switching to this system involves re-doing all the probe preparations and labeling. The fractionation of probe plasmid DNAs on gels to isolate the inserts is now in progress. The insert DNA will be labeled with a nucleotide analogue (digoxigenin-11-dUTP) by the random primed labeling technique. The detection procedure is similar to that used for sulfonated probes.

Isolation of genomic DNA from greenhouse grown plants in the defined categories continued throughout the year. Transformation, amplification, and isolation of plasmid DNA was completed for all ten probes.

Table 42. Somaclonal variant lines.

line #	R#	Tot. pass.	Strs. pass.	NaCl g/l	variation
300045	4	13	0	-	partial sterility
300050	4	14	0	-	partial sterility
300092	4	13	0	-	partial sterility
303026-46	3	4	4	3	salt screen performance
303030-46	3	4	4	3	salt screen performance
303044-45	3	2	2	3	salt screen performance
303049-47	3	2	2	3	salt screen performance
303075-45	3	3	3	3	salt screen performance
303143-45	3	5	5	3	salt screen performance
303036					dwarf turf type

Table 43. Regenerant lines.

cat.	line #	R#	Tot. pass.	Strs. pass.	NaCl g/l
low	300002	1	7	0	-
low	303030	1	4	4	3
low	303033	1	4	4	3
low	303035	1	4	4	3
low	303049	1	2	2	3
low	303079	1	3	3	3
low	303112	1	4	4	8
low	303138	1	5	5	3
low	303146	1	5	5	3
low	303152	1	5	5	3
low	303174	1	5	5	3
low	306003	1	5	5	6
low	306005	1	5	5	6
low	306026	1	4	4	6
med	300035	1	13	0	-
med	300042	1	9	0	-
med	300044	1	8	0	-
med	300048	1	13	0	-
med	303101	1	11	11	3
med	303102	1	11	3	3
med	306051	1	12	12	6
med	3001026	1	9	0	-
med	3001078	1	7	0	-
med	3090011	1	9	9	9
med	3090012	1	9	9	9
high	300037	1	15	0	-
high	3001012	1	22	0	-
high	3001015	1	20	0	-
high	3001020	1	20	0	-
high	3001038	1	19	0	-
high	3001043	1	22	0	-
high	3060070	1	18	6	-

+ 3 more lines to be added

COLLABORATIVE RESEARCH AND NON-USAID FUNDED ACTIVITIES

A significant component of the TCCP is the graduate students, visiting research scientists, and the technical as well as professional training of research workers from developing countries. An essential condition for having a strong training program is to have an active and progressive research component.

The research at TCCP attracts a large number of scientists and research workers from all parts of the world. After having been at TCCP they sometimes become part of the network of collaborators.

In some of the projects described below, the funding for essential expenses has been obtained from outside sources. All of the projects are related directly to the purposes of TCCP for research, training, and network collaboration with developing country scientists, US institutions and IARC organizations.

TISSUE CULTURE OF WINTER WHEAT

Background and Abstract

The project on tissue culture of winter wheat is supported by the Agricultural Experiment Station at CSU. The objectives and goals are to establish the tissue culture methods for Colorado winter wheat and utilize *in vitro* screening with Polyethylene Glycol (PEG) for selection of lines with increased field drought tolerance.

The tissue culture procedure has been developed for the efficient production of E callus and *in vitro* screening on PEG. The methods are well established for the cultivars Hawk and Bennett.

A total of 199 lines from regenerated plants of Hawk and Bennett have been produced. A seed increase was performed during 1988-1989. A total of 191 lines are now being field tested in Colorado. Tissue culture

experiments are in progress to produce additional regenerated plant lines after selection on PEG.

Experimental and Results

The tissue culture selection and plant regeneration was performed by the procedures developed for winter wheat. Both Hawk and Bennett cultivars are performing well in culture. Selection is in progress on PEG at 6% and 9% PEG 8000, respectively. After regeneration and plantlet establishment, they are vernalized prior to transplanting to pots and growing to maturity in the greenhouse

Results and Discussion

More than 200 lines of tissue culture-regenerated plants have been produced, including 42 lines of PEG-stress-selected and 149 non-stress-selected lines. The seed increase during the 1988-89 season yielded R_2 seeds. A total of 191 R_1 lines have been seeded to produce R_2 seeds for use in field screening and evaluation. A sampling of R_2 lines from Bennett and Hawk are being field tested at Akron, Colorado under typical eastern Colorado drought conditions during the 1989-90 season. Data collected will include yield tests on six row plots with four replications in a RCB design.

The seed increase and field testing is a collaboration with Dr. J. Quick, a Wheat Breeder in the Agronomy Department at CSU. Dr. Quick has also been the advisor throughout the project.

TISSUE CULTURE OF SPRING WHEAT

Background

The project was designed to extend collaboration through a sub-agreement from TCCP to Dr. A. B. Zamora, Institute of Plant Breeding, UPLB, Laguna, Philippines. The goal of the research was to transfer technology and utilize tissue culture for the production of wheat germplasm with increased tolerance to higher temperatures as experienced in the tropics.

The TCCP financial support for the project was discontinued at the end of 1988 because of lack of funds.

Summary from Report by Dr. Zamora (See list of Publications)

A total of 30 plants from tissue culture were grown to maturity in the greenhouse. The R_1 and R_2 plants, respectively, were grown in the field and evaluated. Some differences were observed between the regenerated and the non-regenerated (control) plants. Generally, the R_1 and the R_2 plants had a higher number of spikelets per spike, and a higher number of tillers than the parental line.

The principal accomplishment was the establishment of the tissue culture facility, trained personnel, and the demonstration of success in the use of the technology in plants that have reached the field testing stage. The heat tolerance screening research is incomplete.

TISSUE CULTURE OF BEAN

Background and Abstract

During 1988, a member of the staff of the Biotechnology Research Unit at CIAT, Mr. Paul Chavarriaga-Aguierre, was at TCCP for six months. At that time Mr. Chavarriaga acquired the plant regeneration technology

which had been developed by Dr. S. Kumar while he was at TCCP. The method has worked for Tepary bean (Phaseolus acutifolius), but the procedure has not been successful for plant regeneration in dry bean (P. vulgaris) at TCCP or in any other laboratory.

Research on a minor but effective scale is being continued in collaboration with CIAT. The objective is to define the conditions for plant regeneration. The research concerns three components in particular: 1) the genetic sources of the plant materials, 2) the explant tissue source, and 3) media components. We have regular exchange of results and planning with CIAT. Paul Chavarriaga was at TCCP September 16-24, 1989 for a discussion and planning session. The goal and interest is to develop a procedure which could be employed to produce transgenic plants.

Experimental and Results - Dry Bean

At TCCP, several explant sources from a number of genetic lines of both tepary bean and dry bean have been tested. One line of tepary bean and eight lines of dry bean are being used. The explant tissues were from lines recommended by CIAT and others from the Agronomy Department at CSU. The dry beans included lines grown in Central and South America. The explant tissue sources have included meristems, axillary buds and leaf tissues taken at different times of seedling development.

Meristem tissues give rise to multiple shoots or to callus depending on the hormones being used. Each of the shoots can be transferred to another medium in which rooting occurs. The number of shoots from a meristem varies from three to five. This method was used on a tepary bean line and could be used for the multiplication of a desirable line by micropropagation, but a method needs to be tested with dry bean. While the meristem method could have potential, for most purposes a method is

needed for plant regeneration de novo from somatic cells.

One of the desirable and potential explant tissue sources is leaf tissue. Experiments are now concentrating on using immature leaf tissues from a variety of genetic lines. The experiments are designed to determine the media combinations and hormones which can be used for the induction of plant regeneration by somatic embryogenesis. In line with what has been observed in pea (Pisum sativum) and the major cereals, it is anticipated that several subcultures over a two to four month period will be necessary to acheive the desired results.

The results of our experiments with bean have shown that 2,4-D, NAA, and Picloram are effective auxins for callus formation. At relatively high concentrations, IAA and IBA also induce callus formation and might be better because a callus differentiation appears to occur. Benzyladenine and zeatin are effective cytokinins for callus production from the bean leaf tissue. The MS appears to be a better basic medium than L6. The latter was developed for grain legume plant regeneration from suspension cultures at TCCP. The media now being used are for initiation. A second class of media are being developed, and are expected to support the development of somatic embryogenesis and subsequent plant regeneration.

At CIAT the research focuses on alternate activities which complement those at TCCP. They include the use of immature embryos as explant sources. The use of the cell suspension culture-filtration method developed at TCCP is also being investigated at CIAT.

Experiments and Results - Tepary Bean

The research with tepary bean has been carried out by Dr. Ahmed Abou-Salha, who is at TCCP on a Peace Fellowship from Egypt. Using a new approach in media combinations, it has been possible to achieve plant

regeneration from leaf tissue of one of the tepary bean lines. The shoot induction was achieved from leaf tissues in two separate media.

At CIAT, Paul Chavarriaga has obtained a large number of tepary bean plants by using a grafting procedure. The shoots were produced from a cell culture, which was established at TCCP in 1986 and monitored for more than one year at CIAT. The shoots were grafted on to root stocks and plants produced.

Plant regeneration continues to be very difficult in any of the Phaseolus species. The recent results in the collaborative research between CIAT and TCCP is promising and the data are expected to be prepared for publication shortly.

METHODS FOR GENE TRANSFER AND EXPRESSION IN COLORADO POTATOES

Background and Abstract

The project is supported by the Agricultural Experiment Station at CSU. The objectives are to establish an efficient procedure for plant regeneration in Colorado potato cultivars and define the conditions for the introduction and expression of genes in potatoes grown in Colorado. The research involves collaborators at CSU and the San Luis Valley Research Center, Colorado.

Experimental and Results

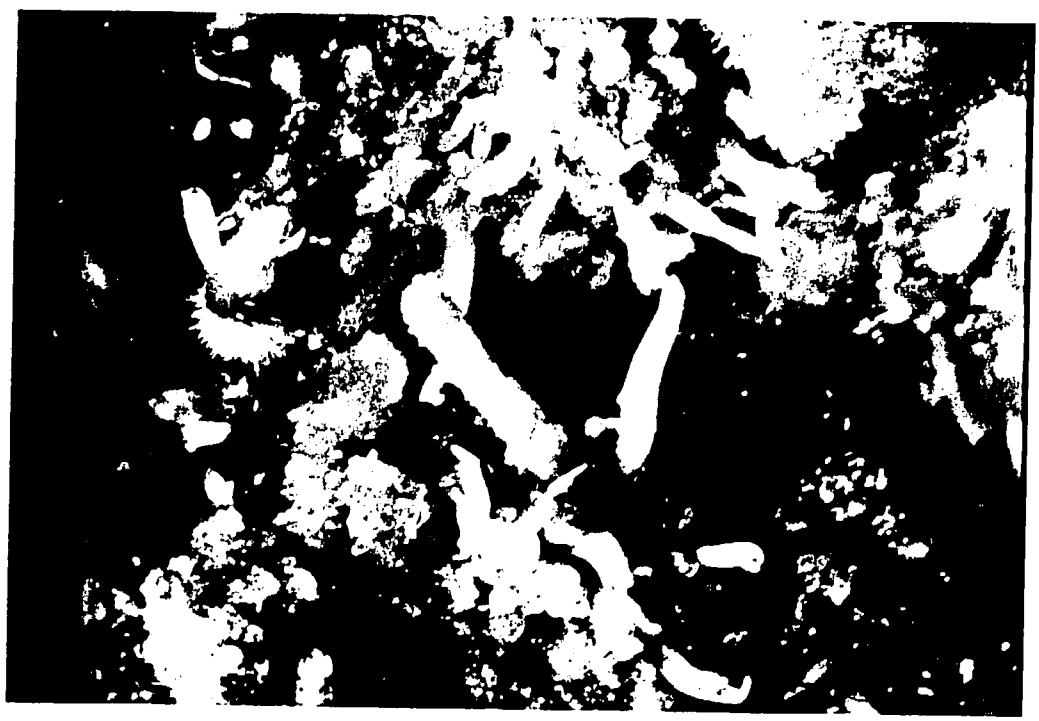
The first task is to define the conditions and procedures for rapid and efficient plant regeneration from a tissue in cultivars of importance to Colorado. In the research to date, several of the procedures used for potato varieties not grown in Colorado have been tested. Most of the methods were not successful with cv Centennial, which is the most important variety in Colorado.

The explants are obtained from shoots propagated aseptically. In the early stages of the experimentation, the shoots were very spindly and the leaves very small. It was discovered that aeration of the gas phase is critical. The propagation is now being done in Erlenmeyer flasks which are stoppered by cotton plugs. The shoots are vigorous and the leaves are of normal size.

Using stem and leaf explants from the in vitro-produced shoots and culturing on an MS medium supplemented with B5 vitamins, callus production occurred within one month and masses of small shoots were observed after about two months (Figure 3). The hormone composition was zeatin, IAA, and GA3. The callus was produced on both stem and leaf tissues. The small shootlets can then be placed on the same medium as used for micropropagation, on which rooting will occur. Although the observations were made on three parallel experiments, the procedure has been repeated. Moreover, the results have shown that it is essential to use the stem of the first internode. Callus with masses of shoots are produced within one month. The research on introduction and expression of genes is now being persued.

The TCCP lab has been approved by Federal, State and University Biosafety authorities for the use of Agrobacterium tumefaciens, which will be employed in gene transfer experiments. The expression vectors are supplied by a collaborator. The research will not go beyond the greenhouse stage.

Figure 3. Plant regeneration from potato callus.



SORGHUM ANTHER CULTURE

Background and Abstract

The research on anther culture in sorghum is aimed at defining the conditions for establishing the anther culture methods for use in sorghum and to apply the procedure in tissue culture selection and plant breeding. The technology will be developed and applied to the identification of aluminum tolerance among haploid cell lines created by the anther culture method. The F_1 sorghum hybrids were developed and are made available by Dr. Gourley at Mississippi State University. The objective is to isolate recombinant lines that combine the aluminum tolerance trait with other traits from the commercial cultivar. The experimentation has recently begun.

Experimental

The protocol consists of culturing anthers or microspores in media which support the growth of callus for subsequent somatic embryogenesis and plant regeneration. After a procedure has been developed, the method will be applied in attempts to select for aluminum tolerance and identify the genetics of aluminum tolerance.

The funding for this project is from the Office of the International Programs at CSU which provides funds for student assistance to USAID Projects.

TISSUE CULTURE OF CORN

Dr. Nguyen T. Thanh-Tuyen, Visayas State College of Agriculture, Visca, Philippines. From Progress Report by Dr. Thanh-Tuyen.

Experimental and Results

Maintenance of E callus derived from immature embryos of corn was attempted with the use of mannitol and abscisic acid (ABA) at 3 and 6% sucrose. The aim is to prolong the regeneration ability of the callus in

preparation for the *in vitro* selection work. A new commercial variety of white corn, i.e. SMARC 1283, was used in the study.

In terms of percent cultures producing E callus, at 3% sucrose, addition of mannitol at 1.5 to 5% to the initiation medium was not beneficial to callus production. At 6% sucrose, the presence of mannitol slightly improved embryogenic response. Incorporation of ABA in to the culture medium for 2 passages prior to regeneration medium retained high embryogenic response until the 5th passage; however, plant regeneration was considerably suppressed, even on medium containing KIN at 0.5 to 5.0 mg/l.

For verification of the possible action of ABA in retaining embryogenic response and in enhancing plant regeneration as reported for rice and corn inbreds, ABA was added at 0, 0.5, 1.0, and 5.0 mg/l in combination with either 5% mannitol or 3% sucrose in various passages. Results showed that addition of ABA in the first passage totally suppressed callus formation on immature embryos of both IPB var. 2 and SMARC 1283. In the subsequent passages, use of ABA did not improve E callus formation nor enhance plant regeneration.

In order to improve plant regeneration from E callus of corn, stress-inducing chemicals, particularly NaCl, were added to the culture medium before regeneration. Remarkable improvement of plant regeneration from NaCl-stressed cultures was observed. A two to three-fold increase in the frequency of plant regeneration was observed when NaCl was added to the medium during the second and third passage. Beyond the fourth passage, however, plant regeneration ability of the cultures declined in all treatments regardless of the addition of NaCl prior to regeneration. The loss of regeneration ability of the callus could be attributed to the aging of the cultures which no longer responded to any *in vitro* treatments.

CONSULTING ASSIGNMENTS

The following trips and assignments were performed by Dr. Oluf Gamborg with the financial support of the Sponsor (S) as listed under each assignment.

November 2 to 17, 1988

Bogota and Cali, Colombia, S.A. Consultant and Specialist in Biotechnology for Agriculture to The Government of Colombia (Instituto Colombiano Y Agropecuario) for The World Bank. Included visit to CIAT at Cali.

The objective was to provide an analysis of the current and proposed research activities of ICA in the area of plant biotechnology, and to make recommendations for strengthening future research and dissemination of results.

Consultancy Report: External Review of Plant Biotechnology Program. ICA, Government of Colombia. Bogota. 30pp.

Sponsor: Instituto Interamericano De Cooperacion Para Agricultura Oficina en Colombia, and the World Bank.

November 23 to December 3, 1988

New Delhi, India. Consultant to The Government of India, under assignment with Winrock International.

The objective was to review and provide scientific and technical advice in the establishment of the National Facility for Plant Tissue Culture Repository at the National Bureau of Plant Genetic Resources in New Delhi.

Consultancy Report and Recommendations to Winrock International, USAID and Government of India. 27pp.

Sponsor: Winrock International/ USAID.

May 8 to May 28, 1989

Sana`a, Yemen Arab Republic. Invited to present a paper at the First National Symposium on Horticulture -- Tissue Culture Technologies: Opportunities for Developing Countries -- and to evaluate potentials of tissue culture technologies in crop production and improvement in YAR. The final report contained detailed recommendations for establishing a plant tissue culture facility.

Sponsor: ICARD Yemen Core II Project, USAID and the Government of Yemen Arabic Republic.

October 3 to October 29, 1989

Bogor, Indonesia. Consultancy for the Applied Agricultural Research Project - Phase II, and the AARD, Ministry of Agriculture, Indonesia. The objective was to review proposals and plans for setting-up research programs and centers of excellence in biotechnology in crops, livestock, and fisheries as well as to make recommendations for designing and conducting research, with technical and professional training in biotechnology.

Final report to AARP II and USAID.

Sponsor: USAID through Winrock International.

TRAINING

The primary goal of the TCCP Training Program has been achieved consistently in the past year, despite the persistent difficulty in obtaining sponsors for the selected candidates. The technology transfer has been affected as intended with a total of eight participants being trained during three separate short courses held in the 1988-1989 period (Table 44). The six-month courses were not held this year due to lack of funding sources for the nominees, although continued interest is evident by the applications received (Table 45).

Table 44. Candidate information of the short courses held in 1988-1989

Name	Country	Topic	Period
M. Ahmad	Pakistan	*In vitro* & greenhouse selection strategies for salt tolerance	Dec 88- Feb 89
B.H Niazi	Pakistan	"	
N. Ahmad	Pakistan	"	
S. Ismail	Pakistan	"	Sep 89- Oct 89
P.B. Odhano	Pakistan	"	
R.H. Qureshi	Pakistan	"	
S.H. Zaheer	Pakistan	"	
Al Hamidi	Saudi Arabia	*In vitro* selection for stress tolerance and micropropagation of fruit crops	Oct 89-Dec 89

Table 45. Applications received for 1988 and 1989 training courses.

Name	Country	Interest	Date, or reason for not attending
Fall 1988			
Akiew	Philippines	Oat	No Funding (NF)
Binh	Vietnam	Rice	NF
Khanna	India	Triticale	NF
Mohan	India	Oil seeds	NF
Mukhri	Indonesia	Soybean	Attending Spring 1990
Soliman	Egypt	Wheat	NF
Spring 1989			
Abou-Salha	Egypt	Beans	Visiting scholar, 10/89 - 3/90
Alloufa	Brazil	Beans	NF
Belarmino	Philippines	Sweet Potato	NF
Bora	India	Barley	NF
Joshi	India	Brassica	NF
Kibuchi	Kenya	Cassava	NF
Maifang	China	Rice	NF
Okundaye	Nigeria	Wheat	NF
Silverio	Philippines	?	NF

The eight participants of the short courses received training on the basic and applied concepts of plant tissue culture, adaptable for crop plant improvement. The two groups from Pakistan conducted their research on *in vitro* and greenhouse screening for salt tolerance in cereals. The Saudi Arabian trainee gained skills in micropropagation of a variety of horticultural crops as well as the basic concepts of *in vitro* selection strategies for obtaining stress-tolerant plants.

Two candidates for the spring 1990 courses have confirmed funding while three others await confirmation (Table 46).

Table 46. Applications received for 1990 training courses.

Name	Country	Interest
Spring 1990		
Al-Ani	Iraq	Tomato, wheat, Citrus
Mohamed	Sudan	Potato, Tomato, Date
Mukhri	Indonesia	Soybean
Sundaram	Norway	?
Fall 1990		
Mohamed	Ghana	Corn, Sorghum, Rice
Short courses 1990		
Orense	Philippines	Coconut

REGIONAL TRAINING

The third regional course held at CATIE, Costa Rica proved to be a continued success of technology transfer to the Latin American scientists. The TCCP Training Coordinator traveled to CATIE for three weeks in September 1989 to assist as an instructor in this program. The main tasks accomplished this year included familiarizing the group with the basic concepts of establishing plant tissue cultures and assisting the students in planning and implementing their research projects on various tropical crops. Fourteen participants representing six countries graduated from the 10-week 1989 course (Table 47). The CATIE instructing staff represented diverse disciplines of tropical crops research and their expertise was used efficiently in training the participants.

Table 47. Participant Information on third regional training course at CATIE, Costa Rica, Sept. 1 - Nov. 11, 1989.

Name	Country	Funding	Project
A.T. Valerin	Costa Rica	CATIE/TCCP	Micropropagation of gerbera
S. Nunez Mata	Costa Rica	" "	Virus elimination in plantain
R.E. Puente	El Salvador	CATIE	Micropropagation of musa
R.A. Merino	Mexico	PROMECAFE	Tissue culture of coffee
R. Salazar A.	Costa Rica	CATIE/TCCP	Micropropagation of *Frantzia tacaco*
L.Guevara	Guatemala	USAID/Guatem.	Micropropagation of potato
L.A. Rodriguez	Nicaragua	PROMECAFE	Tissue culture of coffee
F.Tschen C.	Guatemala	USAID/Guatem.	Micropropagation of sweet potato
S. T. Vallejo	Ecuador	IDEA	Micropropagation of orchid
R. V. Chacon	Venezuela	CAF	Morphogenesis of potato
L. Yepez G.	Venezuela	CAF	Micropropagation of *Aloe vera* L.
J. Gonzalez	Ecuador	Personal	Morphogenetic studies in banana
H. Sancho C.	Costa Rica	CATIE/TCCP	Micropropagation and virus elimination in *Xanthosoma saggitifolium*
A. Bogado	Argentina	Personal	*In vitro* selection for drought-tolerant cotton

The first IAV Hassan II regional training course was held in Agadir, Morocco, May 15-June 23, 1989. Nine participants from four African Francophone countries were trained (Table 48). The TCCP Project Director assisted in the course instruction. Plans for a second course, to be held in May 1990, are underway.

Table 48. Participant information for the first IAV Hassan II regional training course held in Agadir, Morocco, May 15-June 23, 1989

Name	Country	Funding	Interest
Loukili Fatiha	Morocco	Private	potato, ornamentals
Lyamani Abderrahmane	Morocco	USAID(MIAC)	cereals
Belaadel Mohamed	Morocco	Personal	citrus, strawberry
Nsnsarellah Nasserlehq	Morocco	USAID/(MIAC)	cereals
Ndamage Georges	Rwanda	FAO	potato, banana
Antonio Nassallu	Guinea (Bissau)	FAO(PNUD)	potato, sweet potato, banana
Morel Gilbert	Guinea (Conakry)	USAID (African Bureau)	citrus, banana

There is growing interest in short courses and several participants have effectively undergone specialized training on specific techniques. Long-term training, however, is also requested specifically by applicants. Through a cafeteria system of training offered throughout the year, we have been of greater assistance in serving the varied needs of scientists to implement plant biotechnology in the developing nations.

NETWORK

The biggest event of 1989 was the Third IPBNet Conference held in Nairobi, Kenya January 9-12, 1989. Conference participants numbered 92 from 30 countries. In addition, 113 inquiries were received.

Conference program topics included plant breeding, tissue culture, and novel genetic biotechnologies. Nine invited speakers presented research in their respective areas of expertise. In addition to oral and poster presentations, four roundtable discussions were held covering the areas of National Policies, Applications of Biotechnologies, Networking, and Financial Resources. These discussions served as a base for future planning in plant biotechnology collaborative programs and IPBNet's role in assisting scientists internationally.

As a direct result of discussions during the Conference, APBNet, the African Plant Biotechnology Network, was formed. The APBNet, coordinated by S.K. Hahn at IITA, is holding its first conference in July 1990.

It was agreed that the Fourth IPBNet Conference would be held in San Jose, Costa Rica, and plans will commence in January 1990 for a 1991 Conference. Dr. Victor Villalobos and CATIE have agreed to carry through with the Conference in the event that funding for the TCCP is not committed by AID for 1991. The TCCP will make preliminary arrangements and announcements during the 1990 fiscal year. Top priority will be to garner financial support for speakers, travel, and participation by local (Latin American) scientists.

In June, Kerri Wright attended the CTA/FAO symposium on "Plant Biotechnologies for Developing Countries" in Luxembourg. The symposium was to evaluate the relevance of new plant biotechnologies and to adopt a long-term policy and related action program which FAO would instigate.

Current needs to be included in the action plan:

1) the current status of plant biotechnology with specific reference to needs of developing countries.

2) an appraisal of the socio-economic impact of biotechnology developments.

3) identification of activities to be promoted in the future.

It should be noted that, in reference to item #3, there was overwhelming support for IPBNet from various African scientists who participated in the Third IPBNet Conference, and from various IARC affiliates.

The symposium combined keynote and plenary presentations with regional presentations on the state of biotech in Africa, Asia, Latin America, and the Caribbean. Five concurrent workshops followed on 1) Plant Protection, 2) Cereals, 3) Roots, Tubers, Plantains and Bananas, 4) Trees, Palms, Coffee, Tea, and Cocoa, and 5) Legumes, Vegetables and Oilseed crops. An overview of these discussions well comprise the major part of the symposium paper.

In what was to be the last grant year of the TCCP, Collaborative agreements were not expanded, except in the area of field testing. Table 49 shows TCCP collaborations for FY 89.

Table 49. Current collaborative agreements and sub-agreements.

Institution and Country	Activity	IPBNet Collaborator
CATIE, Costa Rica	Regional Training Course taught in Spanish emphasizing crops of regional importance.	Victor Villalobos
CIAT, Colombia	Field testing for aluminum tolerant rice.	Robert Ziegler
	Short-term training of personnel from CIAT Biotechnology Unit.	William Roca
CIMMYT, Mexico	Widecross and field evaluation of wheat. Personnel exchange	A. Mujeeb-Kazi
IAV Hassan II, Morocco	Regional Training Course taught in French emphasizing crops of regional importance.	Mohamed Aaouine M'Hamed Sedrati
ICRISAT, India	Field testing for salt-tolerant spring wheat	Choukr-Allah
	Sorghum drought testing through SADCC/ICRISAT program in Zimbabwe	Lee House Ian Robertson
	Exchange of chickpea germplasm and personnel from ICRISAT in a collaborative program to transfer biotechnology. Field testing of pigeonpea.	H. van Rheenen
NIAB, Pakistan	Research into producing rice/kallar grass hybrids. Field testing of salt-tolerant rice.	S.H.M. Naqvi Kauser Malik Yusuf Zafar
PCARRD, Philippines	Facilitation of sub-grant awards, coordination of collaborative research in the Philippines.	Crisanto Escaño
UPLB, Philippines (sub-agreement)	Field testing of rice germplasm for salt tolerance. (Completed summer 1989)	Jose Hernandez
ViSCA, Philippines (sub-agreement)	Research on regeneration and stress selection techniques for corn and rice.	Nguyen Thanh-Tuyen

Univ. of Arizona, Yuma Experiment Station	Field evaluation of sorghum for salt tolerance and millet seed increase.	Robert Voigt Bill Blackledge
Univ. of Georgia, Griffin Experiment Station	Field evaluation for aluminum-tolerant sorghum germplasm.	Ronny Duncan
Univ. of Georgia, Tifton Experiment Station	Field evaluation of FAW resistance.	David Isenhour
Louisiana State University, Crawley Experiment Station	Rice germplasm increase and evaluation.	M. C. Rush
Colorado State Univ., Ft. Collins & Cortez Experiment Station	Field Evaluation of tepary, mothbean, pigeonpea.	Mark Brick

In 1989, the TCCP mailing list swelled to 1,430 persons, in part because of the Third IPBNet Conference. The list includes: 785 from Developing countries, 118 from Developed Countries, 259 from the USA, 152 from USAID, and 116 Conference participants/inquiries.

In June 1989, IPBNet Newsletter No. 10 and the 1989 edition of the IPBNet Directory were distributed to everyone on the mailing list. Two newsletters are planned for fiscal 1990. Unfortunately, however, a 1990 edition of the IPBNet Directory will not be published due to AID budget cuts and redirection of funds into the whole plant testing program. The 1989 Directory cost $6,668 for 2,000 copies. The 1990 budget is inadequate to cover a Directory in addition to newsletters, refereed publications, and copying costs.

The strength of IPBNet programs in 1990 and beyond will largely depend on AID's financial commitment. Alternatively, IPBNet could begin charging membership fees to cover operating and publishing costs. This

option should be carefully considered over the next several months. The IPBNet has become an integral part of the worldwide scientific community, filling a vital gap in information exchange in and between developing and developed countries.

FINANCIAL HIGHLIGHTS

Total expenses for Grant Year Five were $832,234. This amount includes $11,000 for the Winter Wheat Project which was supported by the Colorado State Experiment Station. Of the remaining $821,234, $773,052 was AID money. The amount of AID dollars spent was $3,745 less than budgeted in the Grant Year 5 Work Plan (a .5% difference), and 26% less than the amount spent in Grant Year Four. Most of the expense reduction was achieved through personnel lay-offs and attrition; AID personnel expenses were nearly 20% less than last year, a savings of $95,684. Personnel cuts were made primarily in tissue culture and molecular biology. In addition, the Lab Manager, Research Coordinator, and Network Coordinator all resigned over the course of the year. The personnel distribution changes were noted in last year's monthly budget reports.

A total of $73,968 in non-AID revenues was realized in 1988-89. About one-third of this was registration fees and donations for the Third IPBNet Conference. More than one-third was comprised of grants from various CSU departments. The remaining amount included training and consulting fees. All revenues, with the exception of the Experiment Station grant, were in support of AID Project activities.

As of September 30, 1989, a total of $4,544,000 had been committed by AID to support the TCCP through April 10, 1990. The $456,000 which remains of the original $5,000,000 projected will carry the Project through November 1990 at the present rate of spending. The additional eight months are needed to complete the 1990 field season, gather and analyze data, and to prepare a final report.

APPENDIX I

ICCP BUDGET DISTRIBUTION FOR FISCAL YEAR 1988-89

AID Account

	NETWORK	TRAINING	WHOLE PLANT	RICE	WHEAT	SORGHUM	LEGUMES	MILLET	CORN	WIDECROSS	PROTOPLAST	TOTAL
PERSONNEL	89667	61486	132540	30805	20548	14422	7880	748	3008	21199	15955	398258
TRAVEL	17479	2497	13097									33073
MATERIALS	5706	3898	8573	1994	1351	984	443	62	219	5683	1068	29987
OPERATING EXP	8317	11133	12794	2957	1930	1341	1015	151	337	1960	1738	43680
CONFERENCE	7475											7475
FIELD TESTING			4025									4025
PRINT/COPY	3452	211	411	89	60	38	1		10	66	27	4363
RENT										5700		5700
TUITION				6357						425	2063	8845
EQUIPMENT										2720		2720
SUBGRANTS			9443	8251	2344				4075			24113
INDIRECT COST	52541	31487	71925	14175	9497	5683	3726	384	1419	11500	7490	210827
TOTAL EXPENSES	184636	110713	252808	64628	35729	23468	13064	1346	9067	49253	28340	773052
REVENUES												773272

Training Account

PERSONNEL	2491	2491
TRAVEL	69	69
SUPPLIES/SERVICES	2835	2835
INDIRECT COST	796	796
TOTAL EXPENSES	6191	6191
REVENUES	11386	11386

Conference Account

TRAVEL	700	700
SUPPLIES/SERVIC	10723	10723
PRINTING	5675	5675
INDIRECT COST	2715	2715
TOTAL EXPENSES	20814	20814
REVENUES	21313	21313

TCCP BUDGET DISTRIBUTION, CONTINUED

Development Account

	NETWORK	TRAINING	WHOLE PLANT	RICE	WHEAT	SORGHUM	LEGUMES	MILLET	CORN	WIDECROSS	PROTOPLAST	TOTAL
CONF TRAVEL	4887											4887
HOSPITALITY	647											647
TOTAL EXPENSES	5534											5534
REVENUES	10546											10546

Other Expenses

GRAD STUDENTS				11654							3989	15643
WINTER WHEAT					11000							11000
TOTAL EXPENSES				11654	11000						3989	26643

Other Revenues

EM GRANT				2080								2080
INT'N'L PROG GRANT				2000								2000
BIOLOGY DEPT (GRADS)				11654							3989	15643
EXPT STAT (WINTER WHEAT)					11000							11000
TOTAL REVENUES				15734	11000						3989	30723

All Accounts

GRAND TOT EXP	210984	116904	252808	76282	46729	23468	13064	1346	9057	49253	32329	832234
GRAND TOT REV	31859	11386	0	15734	11000	0	0	0	0	0	3989	847240

APPENDIX II

PUBLICATIONS AND REPORTS FROM TCCP

Mohmand, A.S. and M.W. Nabors. 1990. Somaclonal variant plants of wheat derived from mature embryo explants of three genotypes. Plant Cell Reports. (In Press).

Gamborg, O.L. 1989. Consultancy Report. Report on Proposals for Establishing Agricultural Biotechnology Research Programs in Indonesia. For Agency for Agricultural Research and Development, Government of Indonesia. October 29, 1989. 36 pp.

Gamborg, O.L. 1989. Consultant Report: The Potentials of Tissue Culture Technologies in Crop Production and Improvement for Yemen Arab Republic. For Yemen Core II Project, USAID, and the Government of Yemen Arab Republic. Sana'a, Yemen Arab Republic. 29 pp.

Gamborg, O.L. 1989. Review of "Applications of Plant Cell and Tissue Culture," CIBA Foundation Symposium. In: Trends in Genetics. Elsevier Publications, Cambridge, England. Vol 5, p 352.

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Tissue Culture for Crops Project. 1989. IPBNet Directory. K.L. Wright and J.L.F. Ketchum (eds.), TCCP, CSU, Fort Collins, Colorado

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Wright, K. 1989. M.Sc. Thesis. Evaluation of a short-term tissue culture training course.

Gamborg, O.L. 1988. Consultant Report. Review of the National Facility and Program for Plant Tissue Culture Repository of the National Bureau of Plant Genetic Resources in New Delhi. For Indian Council of Agricultural Research, Government of India, New Delhi, India.

Gamborg, O.L. 1988. Consulting Report. Recommendations for Programs in Biotechnologies for Application in Research and Development for McCormick and Co. Inc., Hunt Valley, Maryland and Gilroy Foods Inc., Gilroy, California.

Gamborg, O.L. and C. L. Niblett. 1988. Consulting Report. External Review of Plant Biotechnology Program. Instituto Colombiano Agropecuario. Subgerencia de Investigacion y Transferencia. Government of Colombia, Bogota, Colombia, South America.

Hidayat, I.M. 1988. M.Sc. Thesis. Heat Screening and Leaf Protoplast Isolation from Three Diploid Solanum Species; and Plant Regeneration from Leaf Protoplast, Cell Suspension Culture, and Protoplast Isolation from cell suspension culture of diploid Gp. Tuberosum X Gp. Phureja (US-W-9310) and Gp. Phureja/Stenotonum (77-19).

PUBLICATIONS FROM COLLABORATORS

Mujeeb-Kazi, A., N. Ter-Kuile and M. Nabors. Callus culture and regeneration potential of Triticum aestivum, T. turgidum and of T. Aestivum x Aegilops variabilis hybrids. Plant Cell, Tissue and Organ Culture. In Preparation.

Mujeeb-Kazi, A., N. Ter-Kuile and M. Nabors. Callus culture mediated amphiploids of Triticum aestivum and T. turgidum x Aegilops variabilis. Journal of Heredity. In Preparation.

Mujeeb-Kazi, A., N. Ter-Kuile and M. Nabors. A structural chromosome 5B modification and enhanced meiotic pairing in regenerants from callus of Triticum aestivum x Aegilops variabilis hybrids. Theoretical and Applied Genetics. In Preparation.

Asiedu, R., A. Mujeeb-Kazi and N. Ter-Kuile. Marker-assisted introgression of alien chromatin into wheat. In: 2nd Int. Symposium on Genetic Manipulation in Crops, August 29-31, CIMMYT, El-Batan, Mexico. Ed. A. Mujeeb-Kazi, L.A. Sitch and G. Hettel (In Press).

Asiedu, R., N. Ter-Kuile and A. Mujeeb-Kazi. Diagnostic markers in wheat wide crosses. In: 2nd Int. Symposium on Genetic Manipulation in Crops, August 29-31, CIMMYT, El-Batan, Mexico. Ed. A. Mujeeb-Kazi, L.A. Sitch and G. Hettel (In Press).

Jahan, Q., N. Ter-Kuile, N. Hashmi, A. Vahidy and A. Mujeeb-Kazi. The status of 1B/1R translocation chromosome in some recent varietal releases and in the 1989 national uniform wheat yield trials (NUWYT) in Pakistan. Pakistan Journal of Botany (In Press).

Mujeeb-Kazi, A., S. Roldan, D.Y. Suh, N. Ter-Kuile and S. Farooq. Production and cytogenetics of Triticum aestivum L. hybrids with some rhizomatous Agropyron species. Theoretical and Applied Genetics (In Press).

Mujeeb-Kazi, A., L.A. Sitch, G. Fedak, S. Farooq and N. Ter-Kuile. The range of chromosomal variations in intergeneric hybrids involving some Triticeae. Cytologia (In Press).

Mujeeb-Kazi, A., N. Ter-Kuile, Q. Jahan, A. Vahidy and K.A. Siddiqui. N-banding polymorphisms in Triticum aestivum L. cultivars Jauhar-78, Sind-81 and Sarsabz. Pakistan Journal of Botany (In Press).

Thanh-Tuyen, N.T., M.N. Dionzon. 1989. Field performance of upland rice somaclones in acidic soil. (Manuscript in preparation).

Thanh-Tuyen, N.T., B.C. Paduano. 1989. Enhancement of plant regeneration from embryogenic callus of corn with stress-inducing chemicals. Manuscript in Preparation.

Thanh-Tuyen, N.T., B.C. Paduano. 1989. Genotype and medium effects on initiation of embryogenic callus and plant regeneration in Zea mays L. (Submitted for Publication).

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APPENDIX III

TCCP STAFF LIST

PERMANENT STAFF

Name	Title/Area	Comments
Murray Nabors	Project Director	
Oluf Gamborg	Associate Director	
Gary Hanning	Research Coordinator	Resigned 6/30/89
Julie Ketchum	Operations Director	
Jolanta Menert	Greenhouse Manager	
Lee Olton	Office Manager	
Sunitha Siriwardana	Training Coordinator	
Reagan Waskom	Research Coordinator/ Whole Plant	
Kerri Wright	Network Coordinator	Resigned 8/8/89
Daniel Miller	Research Associate /Whole Plant	
Carol Ostrom	Research Associate/ Tissue Culture	.375 Time
Fariha Sayied	Research Associate/ Tissue Culture	Re-hired 7/24/89
Sue Sidinger	Office Assistant	Resigned 4/11/89

GRADUATE STUDENTS

		Comments (Funding)
Iteu Hidayat	Protoplasts/Potato	Graduated 10/88
Ray Ketchum	Protoplasts	(Biology/TCCP)
Doug McMurray	Rice/RFLP	(TCCP)
Amani Mnayer	Tomato	(Biology)
Akbar Mohmand	Wheat/Drought	graduated 3/89
Anna Novero	Rice/NR	graduated 5/89
Poungpet Poonsapaya	Rice/Suspension	(Biology/TCCP)
Sathish Puthigae	Rice/Cond. Factor	(Biology/TCCP)

Gaith Sasi	Triticale	(Libyan Gov't)
Nanan Widiyanto	Rice/Blast	(World Bank)

STUDENT SUPPORT

(All students are 25% time unless otherwise noted)

Carol Brumley	Greenhouse	
Barbara Bucknam	Office	
Jennifer Clark	Greenhouse	
Laurie Clauss	Office	
Dawn Dow	Greenhouse	
Doree Dufresne	Office	
Jan Emming	Greenhouse	
Michelle Fink	Lab	
Hal Gibson	Greenhouse	
Lynn Herrmann	Whole Plant	
Julie Hicks	Greenhouse	
Priscilla Johnston	Lab	
Alanna Kulchak	Lab	
Heather Loomis	Office	
Robert McComb	Greenhouse	
Ron Monroe	Greenhouse	
Aparna Nageswaran	Office	
Debbie Neubaum	Lab	
Dana Nichols	Lab	
Nitschka ter Kuile	CIMMYT	100% Time
David Timm	Whole Plant	50% Time
Kristin Vanderbilt	Lab	
Julie Vanderkolk	Whole Plant	
Brian Wood	Greenhouse	

APPENDIX IV: VISITORS TO THE TCCP

Illo Adani	Econ Couns. of Nigeria
M.A. Adoum	Ambassador of Chad
Shozo Aoki	Japan
Richard S. Avo	Colorado State University, USA
S.A. Awan	Mjo Food, Agric. Coop., Islamabad
J.F. Baker	Aust. Farmers Tour, Australia
Tom Bartlett	Colorado State University, USA
Peter & Marj Beshly	Aust. Farmers Tour, Australia
Benjamin Bounkoulou	Ambassador of Congo
Bob Briggs	Colorado Greenhouse Growers Assoc., USA
Dr. Kekoura Camara	Ambassador of Guinea
Pamela Chatterjee	SPWO, New Delhi, India
Paul Chavarriaga	CIAT, Cali, Colombia
Alain Nicaise Coffie	Economic Counselor, Cote D'Ivoire
Lucienne Darlan	Economic Counselor, Central Africa
Mamoun Dawelbeit	Rahad Res. Sta., Sudan
Moumouni A. Djermakoye	Ambassador of Nigeria
Jerry Dodd	University of Wyoming, USA
Jerry L. Drew	V.P., African-American Institute
Chris Ells	Kitayama Bros., Colorado, USA
Peter & Les Elnor	Aust. Farmers Tour, Australia
Bonnie Ennis	Adams County Extension, Colorado, USA
Amedee Farga	Gabon
Lemays Favitson-Boulandi	Econ Couns. Emb. of Chad
Marco Flores	Colorado State University, USA
Jagadish C. Gautam	Min. Agric., Nepal
Charles Gomis	Ambassador of Cote D'Ivoire
Leo Hartman	Veldkamps Greenhouse, Colorado, USA
M. & J. Hatty	Aust. Farmers Tour, Australia
George Himi	Denver, Colorado, USA
K. Hinata	Tohoko Univ., Japan
Hibeki Hiramo	Japan
T. Honmura	Kagoshima Pref. College of Ag., Japan
Tom Iwater	Aurora, Colorado, USA
Jing-fen Jai	Lanzhou University, China
Harry & Lesley Johnson	Aust. Farmers Tour, Australia
Ibra Deguene Ka	Ambassador of Senegal
Paul Desire Kabore	Ambassador of Burkina Faso
Carl Kaspar	Pikes Peak Greenhouse, Colorado, USA
Poyohisa Katsu	Tif, Japan
Jeanette Katuuk	IKIP, Indonesia
A. Mujeeb Kazi	CIMMYT, Mexico
Yoichi Kumazawa	Tendo-shi, Japan
Austin Suzgo Kumwenda	Rice Research Sta., Malawi
Toshiyuki Matsukana	Wakayama Pref., Japan
T. Miyazaki	Japan
Dr. Sugiono Moeljopawiro	AARD, Indonesia
Takayuki Morishima	Tochigi Pref., Japan
Ichizo Nagamine	Aomori. Pref. Japan
Katsumi Nagomo	Miyazaki Pref., Japan
Fijio Nakai	Japan
Theophile Nata	Ambassador of Benin
Potter Ne	Taku Takai, Japan

Kikutiyo Okad	Toyota, Japan
Kazushige Omori	Japan Tobacco Inc., Japan
M.A.M. Pasha	Agric. Res. Inst., Pakistan
Dorothee Rabemananjara	FOFIFA, Madagascar
Gopel Rajbhandary	MARSC, Nepal
Francois Rasolo	FOFIFA, Madagascar
Pascal Ravohitrarivo	FOFIFA, Madagascar
E.B. Reshing	Aust. Farmers Tour, Australia
Mohamed Saleem	Agric. Res. Corp., Sudan
Farouk Salih	Shambat Res. Station, Sudan
Nouhoum Samassekou	Ambassador of Mali
T. Sasaki	Japan
Ellom-Kodjo Schuppius	Ambassador of Togo
Della E. Sedoh	Economic Counselor, Republic of Togo
John Shirley	Aust. Farmers Tour, Australia
Huluki Siker	Japan
Gueye Silcarneyni	Min. Couns., Emb. of Senegal
Renee Sohnonvi	Econ. Couns. Emb. of Benin
Dave Swift	NREL/Colorado State University, USA
R.J. Swuntz	Tagawa Greenhouses, Colorado, USA
Khadim Hussain Tahir	Atomic Energy Agric. Res. Center, Pakistan
Moshe Tal	Ben Gurion University, Israel
Michiyo Tanizaki	Wakayama Pref., Japan
Dr. Abdel-Rahim Tawfik	Virginia Polytechnical Institute, USA
Christian Lingama Toleque	Ambassador of Central African Republic
K. Tonomo	Japan
Ichiro Uchisa	Japan
Aloys Uwimana	Ambassador of Rwanda
Dr. Warid Warid	University of Cairo, Egypt
Ron Williams	Aust. Farmers Tour, Australia
H. Yamamoto	Japan
Tomako Yochida	Kanagawa, Japan
Shoei Yokokawa	Yamagata City, Japan