The International Plant Biotechnology Network

Tissue Culture for Crops Project

Newsletter #4 June 1985

TCCP Announces Formation of the International Plant Biotechnology Network

The International Plant Biotechnology Network (IPBNET) is being formed for the purpose of establishing working partnerships among researchers worldwide with interest in using plant biotechnology to accelerate the development of stress-tolerant crops.

The Network will emphasize the participation and concerns of researchers in developing countries (DC's) and will consist of basic and applied research groups in DC labs, U.S. labs, and plant breeding programs worldwide with interests in stress tolerance. It will carry out multi-national and multi-institutional collaborative research and serve as a vehicle for exchange of information and germplasm, institution building, and technology transfer.

Participation by DC's in the Network will be of two categories: equal-partner collaborators will be selected from existing tissue culture labs and from breeding programs currently active in stress tolerance and plant regeneration research. General partners will include DC institutions with interests in plant tissue culture and/or stress-tolerance, but which may or may not have programs currently active in these areas.

Equal-partner collaborators will work on specific agriculture-related problems and crops of local interest. They will be linked directly to the TCCP and its other collaborators (including International Agricultural Research Centers, several U.S. universities, and other DC laboratories) and will have priority for Network services. Support services available to equal-partner collaborators will include a training course for staff and technicians, a technical assistance program, technical workshops to include crop and stress-specific working groups, yearly conferences (at various locations) of Network members to promote information exchange, a bi-annual newsletter to include research results of Network members, a computerized filing system of titles to appropriate technical literature, and a limited number of direct grants from the TCCP to IPBNET collaborators.

General members will also receive Network information and have access to TCCP training facilities. They will be invited to yearly Network conferences and may also apply for grant monies to help create or strengthen existing plant biotechnology programs. Grant recipients will be targeted partly on their desire to become equal-partner collaborators.

Developing country members will be selected for participation in IPBNET according to 1) need for stress-tolerant crops, 2) organization and technological readiness to participate, 3) ability and willingness to contribute financially to training and networking on a partial matching basis, and 4) geographical location.

Network members will be responsible for free and rapid information exchange with other Network members and for obtaining partial funding for participation in the Network. Equal-partner members will serve as regional centers of excellence in plant biotechnology and will be required to attend yearly conferences. For participation in training, members will be requested to contribute transportation costs, and normal stipends or salaries paid in the country of origin. The Network will supply the remainder of the stipend required during training.

An informal, tri-lateral network has already been established among the TCCP, the International Rice Research Institute (IRRI) in Los Baños, Philippines, and Chulalongkorn University in Bangkok, Thailand. If you are interested in participating in IPBNET, or would like more information concerning IPBNET functions, please contact: Mr. Thomas A. Dykes, Network Director, TCCP/Botany Dept., Colorado State University, Fort Collins, CO 80523.

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The Tissue Culture for Crops Project

Project staff at CSU.

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Department of Botany
College of Natural Sciences
Colorado State University
Fort Collins, Colorado 80523, USA

Julie L. Fischer, Editor, Operations Director
Murray W. Nabors, Principal Investigator, Project Director
Kirby J. DeMott, Research Director
Thomas A. Dykes, Network Director

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International Rice Research Institute, Los Baños, Philippines
International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India
George T. Cox and Associates, Management Consultants, Seattle, Washington, USA
Colorado State University, Agricultural Experiment Station, Fort Collins, Colorado, USA
Department of Botany, Chulalongkorn University, Bangkok, Thailand
Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, Louisiana, USA

Participating Technical Staff:

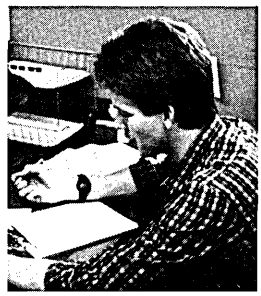
Murray Nabors, Carolyn Angleton, Barbara Ashton, Gwendolyn Bane, Linda Behrns, Christine Boris, Jeffrey Canfield, Leslie Conrad, Julie Cotton, Robert DeCino, Theresa DeMersseman, Kirby DeMott, Thomas Dykes, Fariha Faizi, Julie Fischer, Robert Fjellstrom, Tammie Fjellstrom, Jennifer Flannery, Touba Ghazi, Glenys Gunderson, Ruth Hendrickson, David Hewitt, Glen Hildreth, Koji Kawamura, Deborah Kelly, Raymond Ketchum, Terri King, Suzi Klein, Larry Klima, Christy MacKinnon, Marcus Murray, Debbie Neubaum, Lee Olton, Laurie Osborne, Kelly Pickering, Scott Price, Gaith Sasi, Lisa Schell, Peter Schuerman, Erin Scott, Denise See, Robin Spires, Nitschka ter Kuile, Callie Vanderbilt, Rebecca Ward, Mary Waters, Thomas Williams, Kerri Wright.

TCCP Appoints Three New Directors

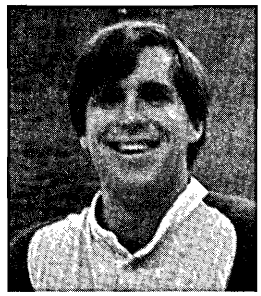
Kirby J. DeMott, Thomas A. Dykes, and Julie L. Fischer have been recently named Research Director, Network Director, and Operations Director of the TCCP, respectively. Mr. DeMott, who recently completed his Master of Science in Botany and Plant Pathology, is now overseeing all aspects of the Project's research, including coordination of various research groups and goals. Mr. DeMott is the TCCP's former Rice Project Manager.

Mr. Dykes, who holds a Master of Science in Botany and Plant Pathology, is now in charge of the International Plant Biotechnology Network (IPB-NET). Mr. Dykes is doing extensive traveling throughout the world to identify Network collaborators and is responsible for implementation of all Network activities. Mr. Dykes is the Project's former Research Operations Manager.

A recent Master of Business Administration graduate, Ms. Fischer is in charge of the Project's five-year budget. She is also responsible for Project human resource planning and evaluation, advertising/publications, and coordination of Project administrative activities.



Kirby J. DeMott



Thomas A. Dykes

Julie L. Fischer

Focus of Research is Expanded

In September, 1984, the TCCP received a second round of funding from the Agency for International Development (AID) to expand current research and begin research in other areas. Work has already moved into regeneration from cereal protoplasts and development of regeneration systems for legumes. In the near future, we will be expanding our efforts to include tissue culture of corn and sorghum. As mentioned in another article, we are also establishing a cooperative agreement with CIMMYT to work on the tissue culture of hybrid plants created by crossing wheat with other alien genera (wide crosses).

The TCCP has already developed methods for selecting rice cell lines tolerant to high levels of aluminum. The Project will also be developing methods for selecting nitrogen-efficient cell lines of rice. Preliminary experiments on nitrate efficiency, including the use of potassium chlorate or very low levels of nitrate, are underway. In addition, other methods of stress-selection through tissue culture will be explored in our expanded research effort.

The TCCP will also be studying the presence of conditioning factors in tissue culture media. Experiments conducted by Dr. N.V. Raghava Ram, former post doc for the Project, have noted the presence of conditioning phenomenon in tissue culture medium as a result of previous cell culture (Newsletter No. 3). We will be expanding our efforts in this area in an attempt to identify and quantify changes in the medium.

TCCP Project Director

Dr. Murray W. Nabors, TCCP Project Director, at his office in Fort Collins.

TCCP Begins Experiments on Legumes

We have recently begun investigations into regeneration systems of soybeans and will soon begin experiments on cowpea and great northern bean *(Phaseolus vulgaris)* as well. "Prize" cultivar has been used exclusively in our soybean experiments since it is a readily available explant source. Our callus cultures are obtained from immature embryos of plants grown under greenhouse conditions at the TCCP.

Extensive studies on the effect of initiation and maintenance media composition on obtaining embryogenic callus have been studied. Various concentrations of auxins and cytokinins have been tested. Nitrogen source and proline requirements have also been investigated.

Visual observation, calli weights, and transfer to fresh media were performed every four weeks for all treatments. Plantlets with trifoliate leaves (Fig. 1) were produced from hypocotyl segments and derived callus obtained from cultured pods. The best experimental results, in terms of callus quality, were obtained from hypocotyl segments isolated from larger (5-6 cm) pods.

Figure 1 — Soybean plantlets with trifoliate leaves.

TCCP Selects for Salt-Tolerant Oat Lines

In order to evaluate the effectiveness of *in vitro* selection of plant cells tolerant to salinity, the TCCP is developing hydroponic systems to test the response of regenerated plants, or their progeny, to increasing levels of salt. To date, extensive testing of oat lines selected for salt tolerance *in vitro* has shown that these lines are superior in seedling and mature plant growth (Table 1). In addition, salt-tolerant oat lines have increased seed yield as compared with non-tolerant oat lines when both are subjected to salt stress (Table 1).

Similar testing performed *in vitro* with salt-selected wheat lines has helped eliminate many salt-intolerant types so that the Project can concentrate on a few choice sources of material. The Project is presently conducting such tests with rice to determine the effects of salinity stress on the cultivars used and to compare salt-selected and non-salt-selected plants.

Upon completion of hydroponic testing, we plan to streamline the *in vitro* selection process by identifying the optimal salinity levels used in selection and duration of selection (time in culture).

Table 1. Comparison of growth in regenerated plants from *in vitro* salt-selected and non-selected oat callus in 6 g/l NaCl.

Growth parameter	Salt-selected	Non-selected
seedling vigor[1,2]	5.00	2.40
number of seedling leaves	5.30	3.30
number of early initiated tillers	.48	.03
seedling height (mm)	235.00	147.00
seedling dry weight (mg)	213.00	87.00
mature weight (mg)	2,292.00	499.00
number of flowering inflourescence	2.55	0.50
inflourescence weight (mg)	1,058.00	250.00
number of spikelets	27.70	5.50
number of seeds	29.70	8.50[3]
seed yield	543.00	141.00[3]

[1]Seedling is defined as a four-week-old plant.
[2]Vigor is rated on scale of 0-9; 0=dead, 9=no loss of vigor as compared to a non-stressed plant.
[3]Non-selected value is significantly less than the salt-selected value at the 10% level. All other values differ at the 5% level.

*Oat work funded totally by CSU Experiment Station.

Project Personnel Visit Thailand, Philippines

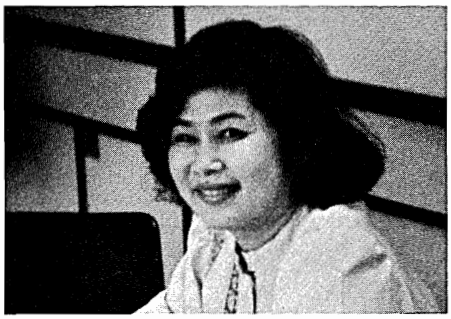
The TCCP is currently in the process of identifying other tissue culture laboratories to participate in The International Plant Biotechnology Network (IPBNET), and to field test plants regenerated from stress-selected cell cultures. In the past, the Project has collaborated, on an informal basis, with several other laboratories, but now seeks to expand and solidify these relationships. To this end, Dr. Murray W. Nabors and Kirby J. DeMott traveled in January to the Philippines and Thailand, where they conferred with several plant researchers.

During their visit to Los Baños, Philippines, TCCP personnel established an agreement with the International Rice Research Institute (IRRI) to begin testing promising lines of rice under various local conditions and to provide germplasm to the Project for continuing tissue culture experiments. They also met with Dr. Ramon Valmayor and Dr. Dely Gapasin of the Philippine Council for Agricultural and Resource Research Development (PCARRD), who helped to identify several tissue culture laboratories for possible participation in IPBNET. Several laboratories at the University of the Philippines in Los Baños were visited, as well as Dr. Nguyen Thi Thanh-Tuyen's laboratory at the Visayas State College of Agriculture (VISCA) on the island of Leyte.

In Bangkok, Thailand, Project personnel visited Mrs. Montakan Vajrabhaya's tissue culture laboratory. Mrs. Montakan is currently working to develop regeneration techniques for Thai varieties of rice, as well as sodium chloride- and acid-tolerant lines. Dr. Thavorn Vajrabhaya has been invited to participate in IPBNET's Management Review Group (MRG).

Dr. Tuyen (far left) and her staff at VISCA.

Dr. Zapata (third from right) and his staff at IRRI.



Mrs. Montakan Vajrabhava at Chulalongkorn University.

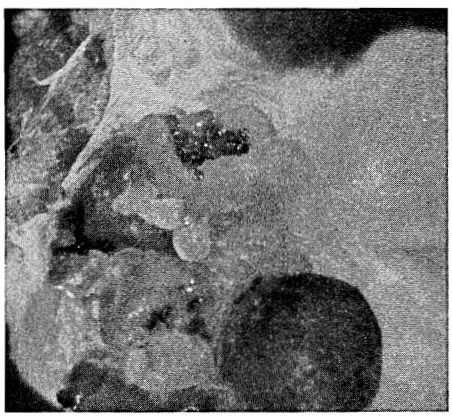
Large Scale Regeneration of Wheat Embryogenic Callus

The TCCP is the first laboratory to have established reliable, consistent methods for obtaining embryoids from callus initiated from mature kernels (or mature embryos) of Glennson, Pavon, and Chinese Spring cultivars of wheat. The reliable production and maintenance of somatic embryos (embryoids) is prerequisite for long-term, high-frequency regeneration of cereal callus cultures.

Wheat and corn are similar in that the ability of the scutellum to produce embryogenic callus decreases as the scutellum enlarges and matures. In all cereals, the use of seeds to produce regenerable callus greatly facilitates plant regeneration or mutant selection since flowering plants do not need to be continuously maintained.

In wheat, embryoids were derived from a smooth, dense (smooth embryogenic, Type I) callus which originated from the shoot region of the embryo. The majority of embryoids were formed from this smooth callus during the second passage of culture (7-12 weeks after callus initiation), although some embryoids can develop through the 17th week of culture. These embryoids can be easily maintained on medium containing 2-5 mg/l of 2,4 dichlorphenoxyacetic acid (2,4-D). Selective transfer of embryogenic callus (and minimum transfer of other calli types) is a key factor in successful maintenance. Regeneration medium can be of varied composition, but it is critical that 2,4-D be absent. Approximately 300 plants were regenerated from embryoids in six months. Regenerates are well-formed, and after a cold treatment, perform vigorously in the greenhouse. It has been estimated that, at ninth passage, an average of 75 plants per gram of embryogenic callus could be obtained. Regeneration of these embryoids has now occurred through the 13th passage (about one and one quarter years in culture).

The establishment of seed-derived, embryogenic wheat callus with long-term, high-frequency regeneration has greatly facilitated experiments on salt-drought, and aluminum tolerance.



Somatic embryos (embryoids) derived from callus of a mature wheat embryo.

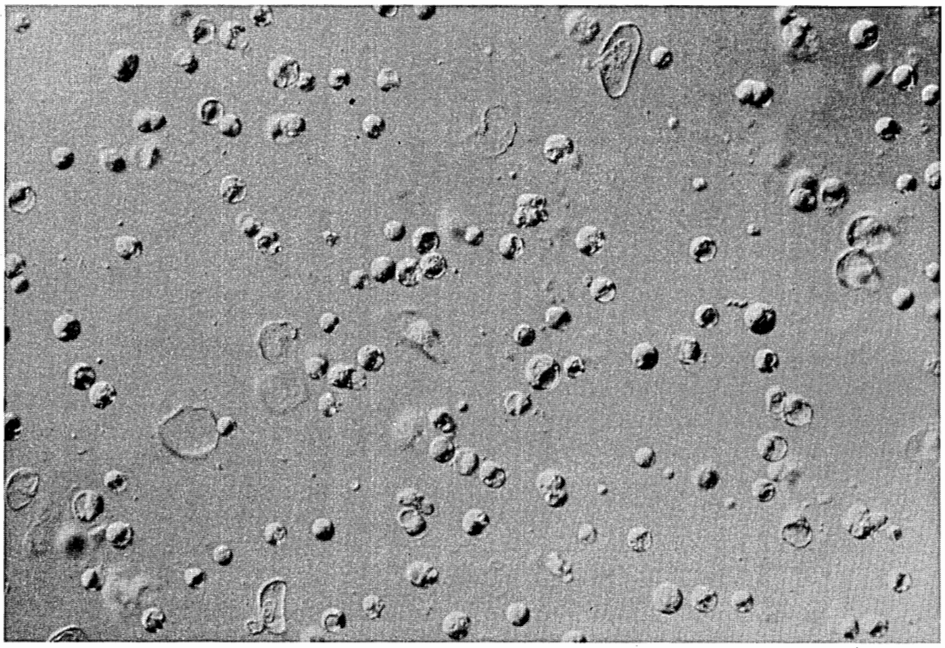
Vernalization (cold treatment) of regenerated wheat plants.

TCCP Works to Develop Methods of Plant Regeneration from Protoplasts

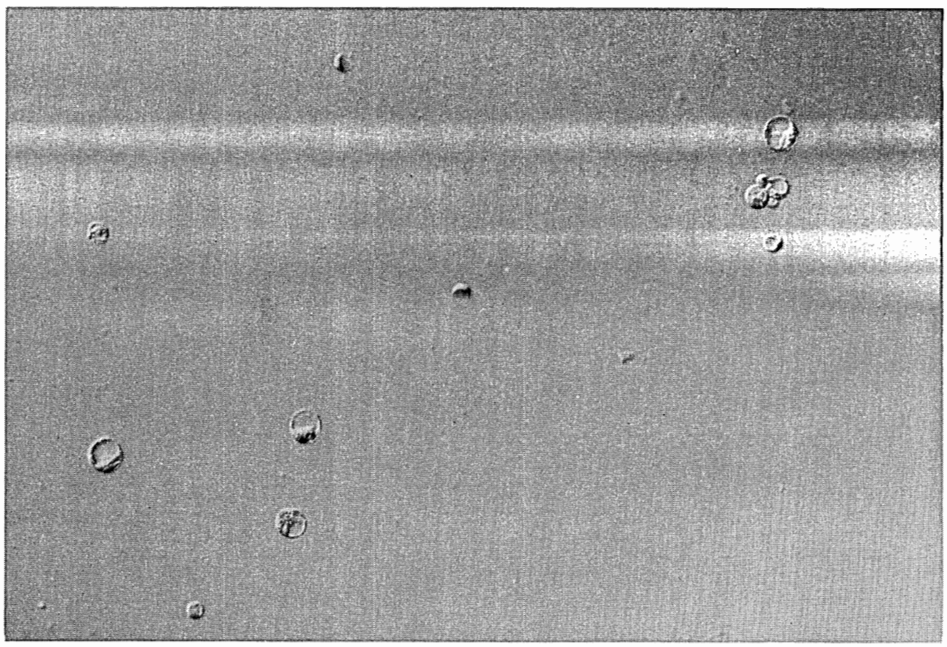
The TCCP has recently begun work on protoplast isolation, culture, and plant regeneration for future work on genetic manipulation of cereal crops. The goal of the Project is to develop a system of protoplast regeneration that will be applicable to a wide variety of monocots with minimal variation in procedures. Exhaustive research is being completed on enzyme combinations, concentrations, source and purity. In addition, extensive studies are being conducted on temperature and time of incubation, agitation speed, lighting conditions, osmotica, purification techniques, culture nutrients, hormones, culture density, culture conditions, and optimum time to add fresh media.

Work to date has concentrated on the isolation and purification of protoplasts from rice, wheat, proso millet, and corn. Isolations and purifications have also been successfully completed on a wide variety of dicots and less agronomically important monocots to test the efficacy of the procedures developed. Protoplasts are usually isolated from embryogenic callus cultures or suspension cultures of these cells.

The underlying philosophy of the protoplast studies is to maximize the number of protoplasts obtained from enzymatic digestion of embryogenic cells, while minimizing the severity of the isolation treatments (in terms of enzyme concentration time, and temperature of incubation), and eliminating contamination of the cultures of undigested cells. As of this writing, we have perfected digestion and purification techniques in both rice and wheat protoplast isolations, and have obtained cell wall regeneration in rice cultivar IR-36. Subsequent cell divisions and callus formation should be soon forthcoming for this cultivar, as well as for rice cultivar G-159, and wheat cultivars Pavon and Glennson.



Protoplasts isolated from wheat cv. Pavon prior to purification. Note presence of large number of cells. X200.



Protoplasts isolated from wheat cv. Pavon following purification. X200.

TCCP Continues Experiments on Rice Regeneration

Long-term, high-frequency regeneration has been obtained for Giza-159, IR-36, Mashuri, and Pokkali cultivars of rice. Regeneration from Mashuri callus is still being obtained after three years in culture. Using rice as a model system, we are developing stress selection procedures for salt, aluminum, drought, and nitrate efficiency. The Project will also begin to develop regeneration procedures for several cultivars of rice from the International Rice Research Institute (IRRI), a relatively simple task involving manipulation of hormones in culture media.

In an effort to assist the plant breeders at IRRI, we are using our tissue culture technology to "clone" rice plants. Using a limited amount of hybrid seed, we can increase the number of rice plants produced, per seed, over traditional breeding methods, and greatly reduce the time required to increase the hybrid germplasm.

Training Program/Facilities Established at TCCP

Construction site at CSU.

As part of an increased focus on technology transfer, the TCCP will be offering a training program for scientists and technicians in developing countries (DC's). The training program will be available to DC personnel to enable them to initiate or upgrade tissue culture programs tied to the agricultural needs of their own countries.

Training will emphasize, but not be limited to, the use of tissue culture as a method of obtaining stress-tolerant cereals and legumes. Training modules will include: media preparation and sterile technique, sterilization of explant material, initiation of callus cultures, plant regeneration, tissue culture as a means of mutation selection, propagation via meristem culture, protoplast isolation, literature utilization, and laboratory set-up and organization. Part of the training will focus on crops of individual interest.

Criteria for accepting trainees are: 1) a match between DC and broad Project goals, 2) a demonstrated need for training in reference to the potential use of tissue culture to solve local (DC) agricultural problems, and 3) financial commitment for transportation and partial stipend. Preference will be given to applicants with a background in Botany or Plant Physiology and a working proficiency in the English language.

The TCCP training center is currently under construction and will be completed in July, 1985. The first training session is targeted to begin in September, 1985. For more information, contact: Training Coordinator, The Plant Biotechnology Training Center, Botany Department, Colorado State University, Fort Collins, CO 80523.

Recent Visitors to the TCCP Laboratory

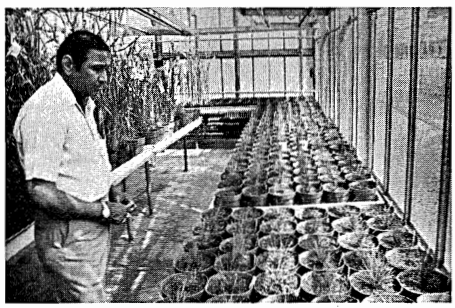
Dr. Anan Attachoo	Chulalongkorn University, Bangkok, Thailand
Dr. Marco Badino	Istituto Sperimentale per la Cerealicoltura, Italy
Mr. George Beggs	Ergo Group, Inc., Boulder, Colorado, USA
Dr. Supradit Bunnag	Chulalongkorn University, Bangkok, Thailand
Dr. Glen Burton	USDA, USA
Mr. Douglas Clark	USAID, Manila, Philippines
Mr. George Cox	Seattle, Washington, USA
Mr. Michael DeWitt	Menu, Fort Collins, Colorado, USA
Mr. Alan Forrest	Ergo Group, Inc., Boulder, Colorado, USA
Dr. David Hoisington	University of Missouri, Columbia, Missouri, USA
Dr. Karen Hughes	University of Tennessee, Knoxville, Tennessee, USA
Ms. Katherine Hutman	Ergo Group, Inc., Boulder, Colorado, USA
Dr. Torao Ishida	Asahi Chemical Company, Tokyo, Japan
Mr. Raymond Kitchell	Alexandria, Virginia, USA
Mr. David Lund	Enginetics, Inc., Arvada, Colorado, USA
Dr. N.V. Raghava Ram	Sungene Technologies, Palo Alto, California, USA
Mr. Al Roth	Enginetics, Inc., Arvada, Colorado, USA
Dr. Samart Samphantharak	Chulalongkorn University, Bangkok, Thailand
Dr. Dwight Tomes	Pioneer Hi-Bred International, Inc., Johnston, Iowa, USA
Dr. James Walker	USAID, Washington, D.C., USA

TCCP Personnel Plan Collaborative Research With CIMMYT

Frequently, wild grasses have traits such as disease resistance, salt tolerance and/or drought tolerance that would be extremely useful in cultivated species. Dr. A. Mujeeb-Kazi, of the Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT), is the head of a program that makes crosses between wheat, and wild grasses with useful traits. Crosses such as these are commonly referred to as wide crosses. By making such crosses, chromosomes of wheat and alien grass genera are placed in the same cell in hopes that random translocations will move the useful wild grass genus onto wheat chromosomes. Since there is considerable evidence that tissue culture increases the rate of chromosomal translocations in cells, the use of tissue culture in wide cross breeding may be highly beneficial.

In March, 1985, Thomas A. Dykes and Nitschka S. ter Kuile of the TCCP went to Mexico to plan cooperative research with the CIMMYT wide cross program. The first half of the trip was spent in the Ciudad Obregon locale of the Yaqui valley in the state of Sonora, Mexico. This valley produces the bulk of Mexican wheat, and is home to a major CIMMYT field station where much of the material generated through Dr. Kazi's wide cross program is being tested. The TCCP personnel also traveled to CIMMYT's headquarters in El Batán, near Mexico City, to visit Dr. Kazi's laboratory and greenhouse facilities.

As a result of this trip, several areas were identified in which research being done by the TCCP might complement research being done through CIMMYT's wide cross program. An agreement between CIMMYT and the TCCP to do collaborative research is currently being established.



Dr. A. Mujeeb-Kazi inspects intergeneric hybrid wheat plants at his greenhouse in El Batán.

Recent Publications Available from TCCP

T.A. Dykes, J.J. Wahler, and M.W. Nabors. Embryogenesis and plant regeneration in tissue cultures derived from mature and immature somatic tissues of rice *(Oryza sativa)* and wheat *(Triticum aestivum)*. Submitted for publication.

J.W. Heyser, M.W. Nabors, C. MacKinnon, T.A. Dykes, K.J. DeMott, D.C. Kautzman and A. Mujeeb-Kazi. Long-term, high-frequency plant regeneration and the induction of somatic embryogenesis in callus cultures of wheat *(Triticum aestivum L.)*. Z. Pflanzenzuch. In Press (1984).

M.W. Nabors and T.A. Dykes. Obtaining cereal cultures with increased tolerance to salt, drought, and acid stressed soils through tissue culture. Inter-Center Seminar on IARCs and Biotechnology, IRRI, Los Baños, Laguna, Philippines (1984).

N.V. Raghava Ram and M.W. Nabors. Plant regeneration from tissue cultures of Pokkali rice is promoted by optimizing callus to medium volume ratio and by a medium conditioning factor produced by embryogenic callus. Plant Cell, Tissue and Organ Culture. In Press (1985).

N.V. Raghava Ram and M.W. Nabors. Salinity Tolerance. In: *Biotechnology*. Eds. P.N. Cheremisinoff and R.P. Ouellette. Technomic Publishing Co., Inc., Lancaster (1985).

R.A. Simons, M.W. Nabors, and C.W. Lee. A model of mutant selection in plant suspension cultures. J. Plant Physiol. 116:95-102 (1984).

Tissue Culture for Crops: Stress Resistant Plants from Cell Culture. Newsletter No. 3. Eds. T.A. Dykes and K.J. DeMott. Department of Botany and Plant Pathology, Colorado State University (1984).

Positions Available at the TCCP

Research Associate in Plant Tissue Culture. The TCCP is seeking a TRAINING COORDINATOR to develop and administer a training program directed at scientists/technicians from developing countries. An M.S. in Botany or related field and two years experience in tissue culture, crop breeding, and/or teaching Botany/Biology is required. Position is full-time, 40 hours per week. Salary, $20,000 plus benefits. Application by resume. Apply at nearest Job Service Center, referring to J.O. # CO 2593819, on or before July 15, 1985.

Research Associate in Plant Tissue Culture. Two positions available at the TCCP requiring a Ph.D., or a Master's with at least two years postgraduate experience in plant tissue culture.

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TCCP to Hold Plant Biotechnology Conference

The First Annual Conference of the International Plant Biotechnology Network (IPBNET) will be held by the TCCP at the University Park Holiday Inn (adjacent to CSU) in Fort Collins, Colorado, October 21-25, 1985. The conference will focus on tissue culture propagation of cereal crops and will include workshops on somatic embryogenesis and plant regeneration, design and management of a tissue culture laboratory, isolation and manipulation of plant protoplasts, and selection of stress resistance in plant tissue cultures. The Keynote Speaker will be Dr. Trevor Thorpe of University of Calgary, Alberta, Canada. In addition, Dr. E.C. Cocking, of University of Nottingham, England, will be speaking on protoplast regeneration. Other speakers will include experts in the science of tissue culture, and Network collaborators.

Plan on flying a commercial airline to Denver's Stapleton Airport. Transportation from Denver to Fort Collins will be arranged by the TCCP.

Please enclose check or money order for $75.00 (payable to TCCP) to cover the cost of registration, all luncheons, breaks, and the Monday Dinner Banquet. A block of rooms has been reserved at the University Park Holiday Inn, at a cost of $49.50/person/day, single occupancy, or $27.75/person/day, double occupancy. *Registration for the conference must be received no later than September 30, 1985.*

For registration or more information, contact Ms. Julie L. Fischer, Operations Director, TCCP/Botany Dept., Colorado State University, Fort Collins, CO 80523, (303)491-1813.

CONFERENCE REGISTRATION FORM

Name: _____

Affiliation/Organization: _____

Address: _____

Arrival Date: Saturday, Oct. 19 _____ Sunday, Oct. 20 _____ Time: _____

Departure Date: _____ Time: _____

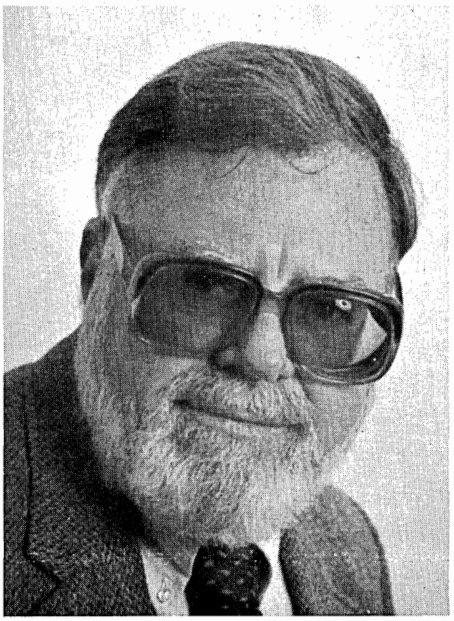
I would like a Single Room _____ Double Room _____ Roommate Name: _____

Special Dietary Requirements: _____

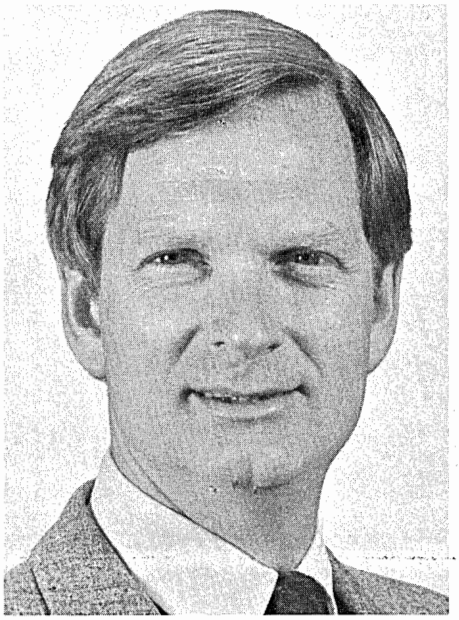
Management Consultants Assist TCCP

Mr. Raymond E. Kitchell, management consultant for USAID, Washington, D.C., recently spent a week at the TCCP designing a five-year project work plan. The work plan is a detailed outline of research to be accomplished through 1989.

In addition to Mr. Kitchell's work, Mr. George T. Cox, a management consultant for the TCCP, has spent considerable time developing a five-year implementation plan (as a follow-up to the work plan) for the Project. During his most recent visit, Mr. Cox developed a detailed time line for implementation of various Project outputs, including publications, conference and training activities, and plant regeneration and stress selection systems. Mr. Cox has been involved with the TCCP since 1980, when he completed the Project's first management audit.



Raymond E. Kitchell



George T. Cox

Positions Available . . .

(continued from page 7)

GROUP LEADER - LEGUMES, is a position which involves development of techniques for callus culture of legumes, emphasizing regeneration systems. GROUP LEADER - NEW EXPERIMENTS, is a position which involves the study of efficient stress selection systems for cereal tissue cultures and media conditioning by plant tissue culture. Application deadline: July 1, 1985. Position available immediately. Salary commensurate with qualifications. Send curriculum vitae and 3 letters of recommendation to:

Dr. Murray W. Nabors, Director
Tissue Culture for Crops Project
Department of Botany
Colorado State University
Fort Collins, CO 80523 USA

Tissue Culture for Crops Project
Department of Botany
Colorado State University
Fort Collins, Colorado 80523 U.S.A.

The International Plant Biotechnology Network

Tissue Culture for Crops Project

Newsletter #5 January 1986

TCCP Holds First Annual IPBNet Conference

On October 21-25, 1985, the First Annual Conference of the International Plant Biotechnology Network was held in Fort Collins, Colorado. Ninety-four persons from 21 different countries attended.

The focus of the Conference was exchange of information. Several collaborators and participants presented papers, posters, and/or slides of their current research, with topics ranging from genetic engineering and protoplast fusion to techniques for producing salt-, drought-, disease-, and temperature-tolerant plants through tissue culture.

The keynote address, entitled the "Application of Tissue Culture Technology to Agriculture and Forestry", was given by Dr. Trevor A. Thorpe, University of Calgary, Canada. Additionally, Dr. E.C. Cocking, University of Nottingham, U.K., spoke on protoplast and cell fusion technologies, and Dr. Karen Hughes, University of Tennessee, Knoxville, presented a seminar on mutation selection and transformation.

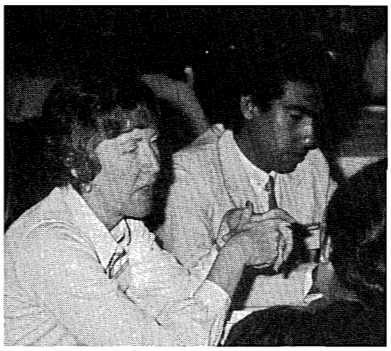
The Conference was concluded with Planning Workshops in which participants responded to questions about the structure, purpose, and objectives of IPBNet. This information will be used by the TCCP to plan future conferences and activities.

Proceedings of the Conference are being compiled and will be published in the next IPBNet Newsletter.

The next annual IPBNet Conference, to be held in Bangkok, Thailand in January 1987, will be hosted by Dr. Thavorn and Mrs. Montakan Vajrabhaya of Chulalongkorn University. Preliminary bulletins will be mailed in February 1986.

Dr. Trevor A. Thorpe (left)

Dr. E.C. Cocking



Dr. Karen Hughes (left)

Dr. James Meiman (CSU) and Dr. Tejpal Gill (USAID) (right)

Dr. Thavorn and Mrs. Montakan Vajrabhaya

Prepared under the support of the United States Agency for International Development, Cooperative Agreement No. DAN-4137-A-00-4053-00.

All reported opinions, conclusions, and recommendations are those of the authors (contractors) and not those of the funding agency or the United States government.

Project Staff is Expanded

In order to fulfill its increased objectives, the TCCP recently hired three new Directors/Coordinators.

Ms. Susan J. Ferguson, newly appointed Network Coordinator, came aboard in May 1985. Ms. Ferguson has a B.A. in Political Science and extensive overseas experience, particularly in Latin America. She replaces Mr. Thomas A. Dykes, who left the Project in August 1985.

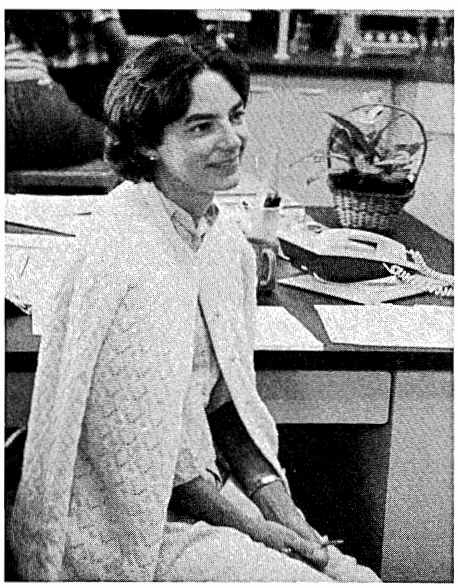
Dr. Oluf L. Gamborg, well-known and pioneering tissue culture and protoplast scientist, was appointed Associate Director in August 1985. Dr. Gamborg has been travelling extensively in connection with IPBNet, and provides technical assistance to the IPBNet Training Program.

Appointed Co-Research Coordinator in August 1985, Dr. Christy MacKinnon recently received her Ph.D. in Botany from Colorado State University. Dr. MacKinnon, along with Co-Research Coordinator Mr. Kirby DeMott, is managing the research efforts of the TCCP.

In addition to these employees, the TCCP also offers a warm welcome to six new graduate students: Mr. Douglas McMurray (USA), Mr. Akbar Mohmand (Pakistan), Ms. Poungpet Poonsapaya (Thailand), Mr. Sathish Puthigae (India), Ms. Sunitha Siriwardana (Sri Lanka), and Ms. Supaporn Tang (Thailand).

Ms. Susan J. Ferguson

Dr. Oluf L. Gamborg



Dr. Christy MacKinnon

The Tissue Culture for Crops Project

Published by the Tissue Culture for Crops Project (TCCP)
Department of Botany
College of Natural Sciences
Colorado State University
Fort Collins, Colorado 80523, USA

Julie L. Fischer, Editor, Operations Director
Murray W. Nabors, Principal Investigator, Project Director
Oluf L. Gamborg, Associate Director
Kirby J. DeMott, Christy MacKinnon, Research Coordinators
Susan J. Ferguson, Network Coordinator

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Cooperating Agencies include:

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U.S. Agency for International Development (AID), Washington, D.C., USA
George T. Cox and Associates, Management Consultants, Seattle, Washington, USA
Colorado State University, Agricultural Experiment Station, Fort Collins, Colorado, USA

Participating Technical Staff:

Murray Nabors, Barbara Ashton, Gwendolyn Bane, Linda Behrns, Julie Cotton, Theresa DeMersseman, Kirby DeMott, Nancy Ekren, Fariha Faizi, Susan Ferguson, Julie Fischer, Robert Fjellstrom, Tammie Fjellstrom, Lori Frederickson, Oluf Gamborg, Touba Ghazi, Paula Glasgow, Glenys Gunderson, David Hewitt, Glen Hildreth, Jeannette Hut, Koji Kawamura, Deborah Kelly, Raymond Ketchum, Larry Klima, Eric Leftwich, Bryan Lobmeyer, Christy MacKinnon, Douglas McMurray, Akbar Mohmand, Marcus Murray, Kim Nabors, Deborah Neubaum, Lee Olton, Laurie Osborne, Robert Oswald, Poungpet Poonsapaya, Gregory Pott, Scott Price, Gaith Sasi, Sathish Puthigae, Stephanie Schaal, Peter Schuerman, Erin Scott, Debra Singleton, Sunitha Siriwardana, Supaporn Tang, Nitschka ter Kuile, David Timm, Susan Todd, Callie Vanderbilt, Mary Waters, Kerri Wright.

Trainees:

Francisco Gutierrez, Iteu Hidayat, Flordeliza Javier, Rachmat Kartapradja, Ben Musyimi.

Recent Publications from the TCCP

Gamborg, O.L. and M.W. Nabors. Protoplasts and somatic hybridization - Constraints and prospects. Proceedings of UNIDO Workshop on Biotechnology in Agriculture. Delhi, India. In Press (1985).

Gamborg, O.L., R.E.B. Ketchum and M.W. Nabors. Tissue culture and cell biotechnology for increased salt tolerance in crop plants. Proceedings of Biosaline Research Workshop, Karachi, Pakistan. In Press (1985).

Heyser, J.W., M.W. Nabors, C. MacKinnon, T.A. Dykes, K.J. DeMott, D.C. Kautzman, and A. Mujeeb-Kazi. Long-term, high-frequency plant regeneration and the induction of somatic embryogenesis in callus cultures of wheat *(Triticum aestivum L.)*. Z. Pflanzenzuchtg. 94:218-23 (1985).

The International Plant Biotechnology Network; Tissue Culture for Crops. Newsletter No. 4. J.L. Fischer (Ed.). Department of Botany, Colorado State University (1985).

Raghava Ram, N.V. and M.W. Nabors. Plant regeneration from tissue cultures of Pokkali rice is promoted by optimizing callus to medium volume ratio and by a medium-conditioning factor produced by embryogenic callus. Plant Cell Tissue Organ Culture 4:241-248 (1985).

Tissue Culture Selection Obtains Salt Tolerance Not Readily Found in Field-Grown Seeds

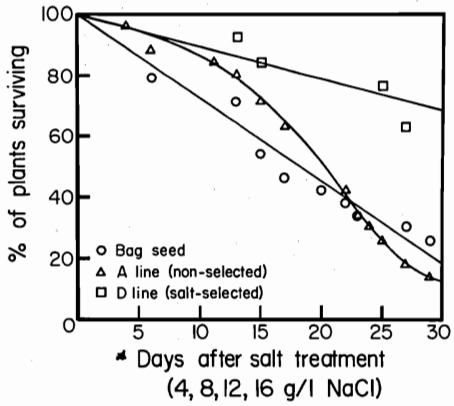


Figure 1

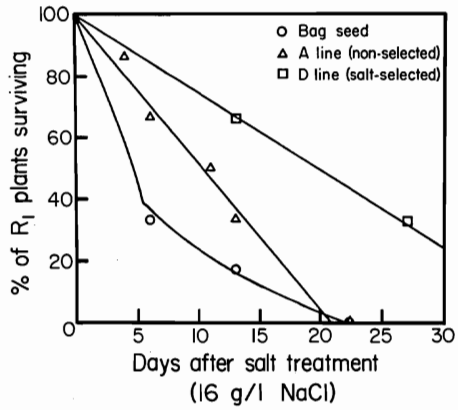


Figure 2

In experiments supported by the CSU Experiment Station, TCCP graduate student Robert Fjellstrom has obtained preliminary results which help to answer an important question often posed to tissue culture workers by plant breeders. Since most commonly-used cultivars of crop plants have not been selected for tolerance to particular stresses, it is possible that considerable heterozygosity for tolerance exists in natural populations. Therefore, it is also possible that, when tissue culturists obtain resistance to, for example, NaCl, they have simply selected a seed or other starting explant which already had naturally-occurring tolerance.

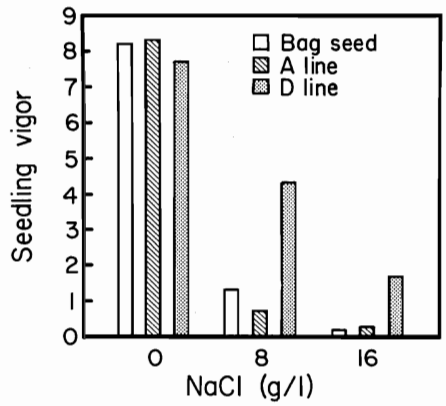


Figure 3

Using the hydroponic testing methods discussed in this newsletter, oat seedlings were placed in solutions containing 0.0, 4.0, 8.0, 12.0 or 16.0 g/1 NaCl. Seedlings were from three sources: 1) field-grown seeds, 2) R_1 plants derived from cell cultures selected for salt tolerance, and 3) R_1 plants derived from cell cultures not selected for tolerance.

Figures 1, 2, and 3 clearly show that, at high salt levels, only plants from selected cultures are surviving. Field-grown seeds clearly do not commonly possess increased levels of tolerance. R_1 plants derived from non-selected cell cultures possess more tolerance than plants from field-grown seed, probably because cell culture increases mutation rates, leading to the appearance of somaclonal variants with increased salinity tolerance.

Areas of Research at the TCCP

CROP	TECHNIQUE	SELECTION
corn	regeneration from callus	heat tolerance
rice	regeneration from suspension	salt tolerance
wheat	regeneration from protoplasts	drought tolerance
millet	promotion of gene introgression in widecrosses	aluminum tolerance
sorghum	greenhouse testing	nitrate reductase efficiency
tropical legumes	field testing	

Several combinations of the above crops, techniques, and selections are currently being investigated by the TCCP.

Research Initiated on Somatic Embryogenesis and Plant Regeneration in Soybean

Using "Prize" cultivar of soybean, the induction of somatic embryogenesis and the influence of growth regulators on subsequent development, including plant regeneration, has been studied.

Callus cultures were obtained from four sources: Immature embryos, cotyledons, seed hypocotyls, and hypocotyls from germinated seedlings.

Two types of embryoids derived from callus cultures. One type was termed "rough embryoid", because of its dimpled appearance. Rough embryoids arose from friable callus initiated on 2,4-D, and from compact-green callus initiated on IAA. Rough embryoids in both callus types appeared as compacted masses, and were not directly regenerable.

The second embryoid type was called "shiny" or "smooth", and was produced from cotyledonary segments on media containing 10 mg/L, 2,4-D and 10 mg/L 2,4-D in combination with 0.5 to 1 μM ABA (Fig. 4a).

Smooth, shiny embryoids, after one to two weeks incubation in the dark, produced four different shapes of shoots and a few shoots with roots (Fig. 4b). Shoots initiated from shiny embryoids were then transferred to B5 medium, containing 0.5 μM IBA, for rooting.

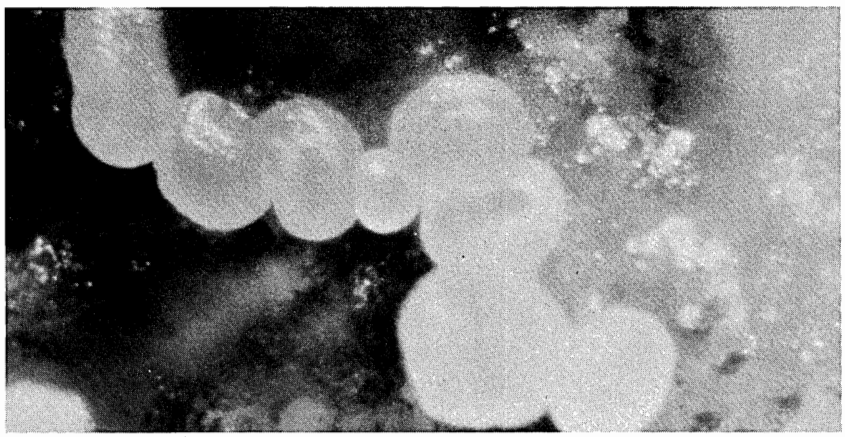


Figure 4a

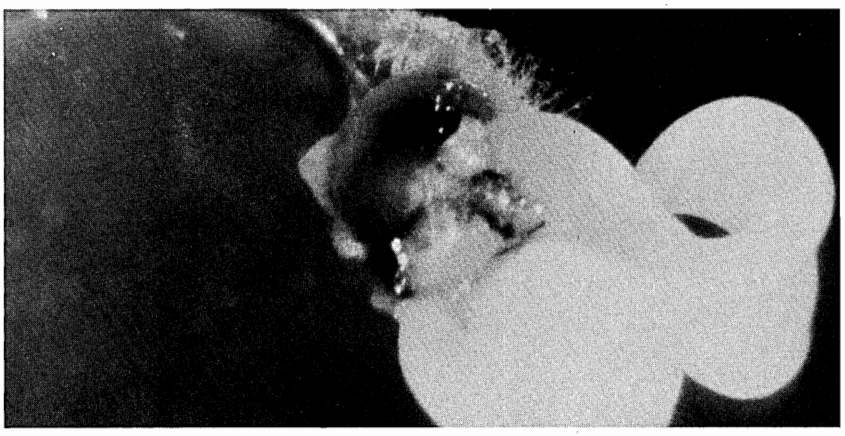


Figure 4b

Simple, Uniform Hydroponic Testing Method Now Available

Greenhouse and field testing of plants regenerated from salt-, drought-, or acid-tolerant cell cultures is an important goal of TCCP research. In such testing, it is usually not possible to use R_0 plants, (those regenerated directly from tissue culture), because such plants need special care during the early stages of growth in the greenhouse, and it is, in some cases, difficult to obtain a sufficient number of plants the same age. Most TCCP greenhouse testing is carried out on R_1 (plants from seeds of selfed R_0) or later generation plants. It is therefore important to develop testing methods which (1) allow for testing throughout the complete life cycle of the plant, and (2) allow for uniform stressing and nutrition of the plants.

Scientists at the TCCP have sought to develop simple hydroponic methods which meet the two above criteria. These methods are now available for all major cereals.

Small plastic tubs (24×30×13 cm) are filled with nutrient solution which is changed every seven days. A piece of styrofoam, in which 12, 2 cm holes are bored for plants, is then placed over the tub. Control plants (from field-grown seed) are included in each tub along with the plants to be tested.

Seeds are germinated in vermiculite-filled 12.5 cm wide pots. The pots are placed in trays with 5 cm of standing water. No nutrients are supplied. Enough seeds are planted to obtain eight seedlings per pot. At 14 days, when the seedlings have two leaves, they are transplanted to the tubs. The pH is adjusted to 5.5 in tubs containing wheat, sorghum, corn, or oats. Except for rice, all tubs are supplied with bubbled air from a small aquarium pump. Tap water can be used to mix the nutrient solutions for the tubs if an ionic analysis is available so that nutrient concentrations can be corrected.

The next step is to transplant seedlings from pots to tubs. The pots are submerged in a container of water to easily remove all vermiculite from the roots. The seedlings are then moved to the tubs, held in place in the holes in the styrofoam with non-absorbent glass wool. Salt damage can be compounded by excessive sunlight, heat or wind, therefore, experiments should be properly randomized to eliminate potential problems with these variables within a greenhouse.

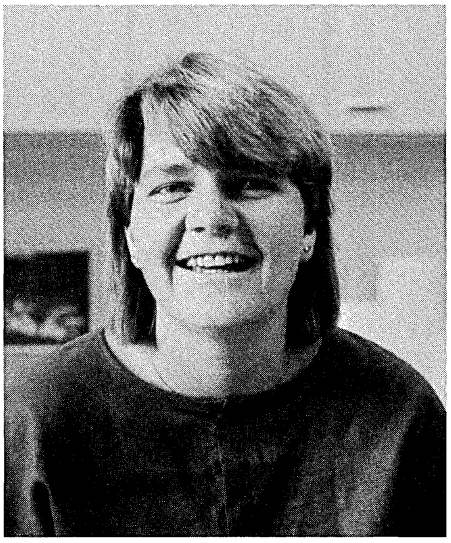
Currently, TCCP scientists are investigating several variables in the hydroponic culture system, including (1) NaCl to $CaCl_2$ ratios in testing for salt tolerance, (2) best chelators for iron nutrition, (3) use of peat pots, and (4) best nutrient solutions for sorghum and Napier grass.

Recipes of nutrient solutions currently used for each cereal, as well as more information, are available from the TCCP.

Widecross Research Program Initiated Between CIMMYT and TCCP

A Memorandum of Agreement between the TCCP and CIMMYT (Centro Internacional de Mejoramiento de Maiz y Trigo) was signed in May 1985. Collaborative research arrangements include the development of tissue culture techniques for application to the CIMMYT widecross program and production/field-testing of progeny from plants regenerated from tissue cultures.

In September 1985, Ms. Nitschka S. ter Kuile of the TCCP travelled to CIMMYT to conduct experiments in embryo and meristem culture under the direction of Dr. A. Mujeeb-Kazi. Ms. ter Kuile will return to CIMMYT in January 1986 to continue the research. Additional experiments are planned at the TCCP on *in vitro* induced chromosome alterations.



Ms. Nitschka S. ter Kuile

Cooperative Research Experiments Initiated Between Bangladesh and TCCP

Dr. Nilufer H. Karim

Dr. Nilufer Hye Karim from the Bangladesh Rice Research Institute (BRRI), visited the Project facilities from October to November, 1985. During her four-week visit, Dr. Karim attended the First Annual Conference of the International Plant Biotechnology Network. She also designed and initiated a cooperative experiment on salt tolerance of callus induction in japonica and indica rice varieties. Regeneration experiments on salt-stressed media were planned as well. In addition to her research, Dr. Karim lectured and demonstrated rice anther culture techniques to the scientists at the IPBNet Training Program.

Salt Tolerance in Rice Plants Derived from Salt-Stressed Cell Cultures

Preliminary findings from hydroponic greenhouse experiments with rice (cvs. Giza 159 and IR 36) indicate that the progeny of plants regenerated from cell cultures containing six or nine grams per liter salt (NaCl) had greater salt tolerance than those from cultures containing zero grams per liter salt. Whether this tolerance is greater, or genetically different, than that found within the natural variability of the parental rice varieties used is yet to be determined.

TCCP Initiates Small Grants Program

The TCCP seeks to accelerate the development of food crop varieties with high yields under conditions of environmental stress. To this end, the TCCP will provide limited financial assistance, in the form of small grants, to scientists in developing countries who conduct research compatible with these objectives.

The scope of small grant research must involve plant biotechnology, including tissue culture and other *in vitro* technologies such as meristem culture, anther culture, protoplasts in plant cell, and molecular genetic manipulation. The proposed research must involve a major food crop important to the country for which the research is intended, and should be directed toward the eventual production of crops resistant to environmental stresses such as salt, drought, aluminum, etc.

The research must be performed in a country or at an International Agricultural Research Center currently supported by programs of the United States Agency for International Development, or in a United States Research Institution with collaborative ties to research in developing countries. The principal investigator must be an experienced scientist with proven research capability.

All proposals must be submitted in English, and should be no more than six (6) double-spaced, typewritten pages in length. Supporting information such as curriculum vitae and list of publications of the principal investigator is to be attached and is not included in the six-page limit. A separate budget should also be attached. The level of funding for each grant will not exceed US $20,000 per year.

Proposals will be evaluated in terms of (1) technical merit, (2) influence of expected results on the development of stress-tolerant crops, (3) qualifications of the principal investigator, and (4) time and cost. Projects funded under this program will be reviewed annually, and funded on a year-to-year basis.

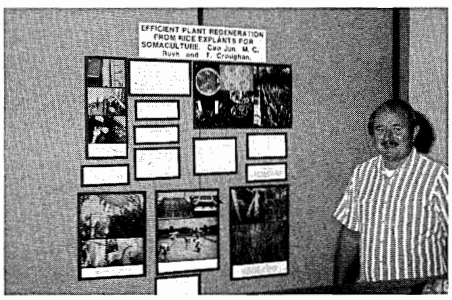
Applications for small grants will be reviewed in June and December. For application and list of current research priorities, write: Dr. Oluf L. Gamborg, Associate Director, Tissue Culture for Crops Project, Colorado State University, Fort Collins, CO 80523.

Small Grants Awarded

The first two sub-contracts in the TCCP's small grants program have been awarded to Dr. M.C. Rush, Louisiana State University Agriculture Center, USA, and Dr. Nguyen Thanh-Tuyen, Visayas State College of Agriculture, Philippines.

Dr. Rush's work will focus on development of drought- and acid-tolerant, regenerable lines of corn. Dr. Tuyen's research will seek to improve existing systems for generating rice plants through somaculture, to include development of useful variations such as disease resistance and soil-stress tolerance.

The Project extends its congratulations to these two researchers.



Dr. Milton C. Rush

Dr. Nguyen Thanh-Tuyen (left)

IPBNet Representatives Visit Potential Collaborators

Representatives of the International Plant Biotechnology Network (IPBNet) visited numerous countries in Asia, Latin America, Africa, and the Middle East during 1985. The purpose of the trips was to make contact with national programs and individual scientists and to explore the tissue culture facilities available in each country. Visits were also made to the USAID missions to discuss scientific collaboration.

Mr. Thomas Dykes, former Network Director, began an extensive journey through Asia in May 1985. At the International Rice Research Institute (IRRI) in the Philippines, Mr. Dykes presented a paper at the International Rice Genetics Symposium and discussed collaborative research with IRRI scientists. In Indonesia, he visited the Horticulture Research Institute in Lembang. Ms. Iteu Hidayat, a technician at the Institute, attended the first session of the IPBNet Training Program and will begin graduate studies at Colorado State University in the Spring of 1986. Mr. Dykes also met with scientists at ICRISAT (International Crops Research Institute for Semi-Arid Tropics) in India, and visited various research institutions in Bangladesh, Pakistan, and Sri Lanka.

Upon his return from Asia, Mr. Dykes began a trip to Latin America, where he travelled to Costa Rica, Panama, and Ecuador. In Costa Rica, he visited the Centro Agronomico Tropical de Investigacion y Ensenanza (CATIE) in Turrialba. Mr. Dykes also attended the Interciencia Symposium entitled, "Biotechnology in the Americas II: Applications to Tropical Agriculture." In Panama, Mr. Dykes visited IDIAP, the Panamanian Institute of Agriculture, and spoke with several scientists on the applications of plant biotechnology in Panama. While in Ecuador, Mr. Dykes visited the Central University in Quito, the Technical University in Ambato, and the Polytech school in Riobamba.

In September and October, Dr. James Walker of USAID/Washington and Ms. Susan Ferguson of the TCCP travelled to Africa, where they met with researchers in seven countries. In Morocco, Dr. Walker and Ms. Ferguson visited five of the six existing tissue culture laboratories in the country. They also toured tissue culture facilities in Liberia, Zimbabwe and Kenya.

While in Jordan, Dr. Walker and Ms. Ferguson visited the University of Jordan and spoke with the Ministry of Agriculture about their plans for a new tissue culture laboratory. Several researchers from the Ministry of Agriculture have interest in the IPBNet Training Program, and one candidate, Ms. Abeer Abu Hassan, will participate in the Spring session.

In Egypt, Ms. Ferguson met with several University of Alexandria faculty members who are currently involved in tissue culture research. These researchers have definite interest in the IPBNet Training Program, as well as the development of stress-tolerant cereal crops for Egypt.

Finally, Ms. Ferguson visited the International Center for Agricultural Research in the Dry Areas (ICARDA) in Syria, where she met with group leaders of various research programs to discuss possible areas of collaborative research with the Tissue Culture for Crops Project.

Gordon Conference Attended by TCCP Scientists

The Plant Cell and Tissue Culture session of the Gordon Conferences, held in Plymouth, New Hampshire the week of June 17-21, was attended by approximately 150 U.S. and foreign scientists. Lectures were given by invited speakers on such topics as protoplast technology, somaclonal variation, and haploid culture.

Dr. Murray W. Nabors and Dr. Christy MacKinnon represented the Project at the conference and presented three posters: 1) "Greenhouse testing of oats derived from salt-tolerant and salt-sensitive tissue cultures", by Robert Fjellstrom and M.W. Nabors, 2) "Preliminary Results: plant regeneration from soybean callus", by Touba Ghazi and M.W. Nabors, and 3) "Long-term, high-frequency, regenerable callus from developmentally mature embryo explants", by Christy MacKinnon, Glenys Gunderson, Thomas Dykes, and M.W. Nabors.

The International Plant Biotechnology Network - Update; January-December 1985

I. Outreach
a. Informal survey sent to 400 potential collaborators in 38 different countries
b. 150 survey responses received
c. IPBNet representatives personally contacted and visited research facilities in 20 countries
d. First Annual IPBNet Conference held in Fort Collins, Colorado
e. Tissue Culture for Crops Project facilities visited by 130 scientists, professors, and administrators.

II. Education
a. Ten graduate students from six countries pursuing degrees with the TCCP at Colorado State University
b. One student from Thai-U.S. graduate student exchange studying at Colorado State University
c. Five trainees from four countries enrolled in the Fall 1985 session of the IPBNet Training Program
d. Ten trainees accepted to the March 1986 session of the IPBNet Training Program

III. Information
a. First IPBNet Newsletter published and distributed in June 1985
b. Computerized literature files reviewed and updated with 10,000 entries

IV. Formalized Collaboration and partnerships
Centro Internacional de Mejoramiento de Maiz y Trigo (Mexico)
Chulalongkorn University (Thailand)
International Rice Research Institute (Philippines)
Louisiana State University (United States)
Philippine Council for Agriculture and Resources and Research Development (Philippines)
Visayas State College of Agriculture (Philippines)

V. Potential Collaboration and Partnerships
Agricultural Development Systems (Egypt)
Bangladesh Agricultural Research Council (Bangladesh)
Centro Agronomico Tropical de Investigation y Ensenanza (Costa Rica)
Centro Internacional de Agricultural Tropical (Columbia)
Centro Internacional de las Papas (Peru)
Indian Agricultural Research Institute (India)
Indian Council of Agricultural Research (India)
Institut Agronomique et Veterinaire (Morocco)
Institut Pertanian Bogor (Indonesia)
Instituto de Investigaciones Agropecuarias de Panama (Panama)
Instituto Nacional de Investigaciones Agropecvarias (Ecuador)
International Crops Research Institute for the Semi-Arid Tropics (India)
Internationalized Institute of Tropical Agriculture (Nigeria)
Kasetsart University (Thailand)
Kenya Agricultural Research Institute (Kenya)
Ministry of Agriculture (Jordan)
National Agricultural Research Centre (Pakistan)
Nuclear Institute for Agriculture and Biology (Pakistan)
Pakistan Agricultural Research Council (Pakistan)
Prince of Songkla University (Thailand)
University of the Philippines at Los Banõs (Philippines)
University of Tennessee (United States)
University of Calgary (Canada)
University of Zimbabwe (Zimbabwe)

TCCP Scientist Attends Workshops in India, Pakistan, and Thailand

Dr. Oluf L. Gamborg represented the Project at the UNIDO workshop on Biotechnology in Agriculture, the workshop on Biosaline Research, and the Salinity Monitoring Tour, held in September 1985.

The workshop on Biotechnology in Agriculture was held in Delhi, India, September 17-22, 1985 and was sponsored by the United Nations Industrial Development Organization and the Department of Science and Technology, Government of India. Dr. Gamborg presented a paper on protoplasts and reported on current research and collaborative efforts of the TCCP and IPBNet. He also served on a panel to prepare a research agenda for the proposed International Center for Genetic Engineering and Biotechnology (plants), to be located in Delhi, India.

The workshop on Biosaline Research, jointly organized by Pakistan and the USA, was held in Karachi, Pakistan, September 22-26, 1985. Foreign and Pakistani scientists presented papers on a wide variety of salt tolerance research topics, including biology, physiology, tissue culture, genetics, soil science, forestry, agriculture, and ecology. Dr. Gamborg presented a paper on salt tolerance of crop plants through tissue culture and reported on the research and collaborative work of the TCCP and IPBNet.

The Salinity Monitoring Tour was organized by Dr. M. Akbar of the International Rice Research Institute (IRRI), the Philippines, and included visits to salinity research centers in India, Pakistan and Thailand. The objectives of the tour were to observe first-hand the research, breeding, crop development, and testing performed at the Stations; to discuss germplasm collection, screening methods, generation of new breeding materials, collaborative research, breeding, testing and development of rice plants for different types of soil salinity conditions, waterlogging, and alkalinity.

Project Scientist Attends Plant Molecular Biology Congress

The First International Congress of Plant Molecular Biology was held in Savannah, Georgia, October 27 to November 2, 1985, and was attended by approximately 2000 persons from 35 countries, among them, Dr. Oluf L. Gamborg of the TCCP. Five plenary sessions, up-to-date, status reviews of selected topics, were held, covering the topics of plant molecular biology, cell genetic engineering, and plant cell culture. A brief synopsis of each session follows.

Session 1. Expression of Nuclear, Chloroplast, and Mitochondrial Genes.

A highlight was the research presented by Dr. R. Goldberg on gene expression during seed/embryo development in soybean and tobacco. Reportedly, seed protein genes are expressed only during seed development. When the seed protein genes are transferred by genetic transformation to tobacco cells, they are expressed only in the seeds. This type of organ specific expression also applies to other genes normally expressed in a particular organ.

Session 2. Mobile Genetic Elements.

Dr. Barbara McClintock, Nobel Laureate 1983, gave a presentation on her research in maize genetics performed in the 1940s. At that time, she established the existence of transposable elements. These elements, or transposons, have now been identified completely by molecular, recombinant DNA methods. They also occur in other plants, but were first identified in bacteria and yeast. Attempts are now being made to utilize them as vectors for gene transfer in plants. As vectors, they also have potential as a means of identifying loci for genes coding for agronomically important traits.

Session 3. Plant Microbe Interaction.

Progress is being made in the understanding of the symbiotic nitrogen fixation between Rhizobia spp (bacteria) and legume plants. The process of bacterial invasion, nodule formation and active nitrogen fixation has several control points which are being identified in the Rhizobia/alfalfa plant system.

Session 4. Applications to Economic Problems.

A. Genes that code for proteins/enzymes which metabolize herbicides have been cloned and transferred to crop plants and expressed. The plants now tolerate quantities of herbicides which kill the surrounding weeds.

B. A stress response protein has been identified. A plant exposed to wounding elicits a "chain" reaction which leads to the production of an inhibitor of proteinases. The components are low molecular weight proteins which may make up five to ten percent of soluble proteins. They combine irreversibly with the active site of proteinases. The genes for proteins are being cloned.

C. Somaclonal variants. Information is now available on the molecular basis of variants that are expressed as deletion of an enzyme (ADH), as nucleotide substitution in which a triplet was replaced (GLU replaced by VAL) or as changes in mobility of fractions of the gliadin proteins of wheat. There was also evidence that chromosome rearrangement had occurred. None of the examples were shown to relate directly to quantitative agronomic traits.

Session 5. Plant Transformation Systems.

Genes that have been cloned can now be transferred to plants and expressed. The transfer (transformation) can be done by using the Ti-plasmid vector, a viral vector (CaMV), or without a vector. Methods are available to inject the DNA into plant protoplasts by microinjection, electroporation, chemical (PEG) and heat treatment. It is clear that the need now is for identifying and cloning the genes that code for traits of importance in plant development and productivity.

Recent Visitors to the TCCP Facilities

Ms. Vivian Anderson	USAID, Washington, D.C., USA
Dr. Clifford Campbell	Uncle Ben's Rice, Houston, Texas, USA
Dr. Dely Gapasin	ViSCA, Leyte, Philippines
Dr. Oluf Gamborg	Belmont, California, USA
Dr. Tejpal Gill	USAID, Washington, D.C., USA
Dr. Samuel Go	ViSCA, Leyte, Philippines
Dr. C.L.L. Gowda	ICRISAT, Pantacheru, India
Dr. David Hsi	New Mexico State University, Los Lunas, New Mexico, USA
Dr. Fred Johnson	BIFAD, Washington, D.C., USA
Dr. Nilufer Karim	BRRI, Dacca, Bangladesh
Dr. Amris Makmur	Bogor University, Bogor, Indonesia
Dr. A. Mujeeb-Kazi	CIMMYT, El Batan, Mexico
Dr. Shao Qiquan	Institute of Genetics, Academia Sinica, Bejing, China
Dr. Bhal Somaroo	ICARDA, Aleppo, Syria
Dr. Dwight Tomes	Pioneer Hi-Bred International, Inc., Johnston, Iowa, USA
Dr. James Walker	USAID, Washington, D.C., USA
Dr. James Welsh	Montana State University, Billings, Montana, USA
Ms. Aziah Yusof	Forest Research Institute, Malaysia

Contributions to IPBNet Newsletter Solicited

The Tissue Culture for Crops Project invites IPBNet collaborators to submit lists of current publications (references only), and summaries of current research of importance to Network members, for publication in future IPBNet newsletters. Summaries of research should be no more than one, double-spaced, typewritten page in length.

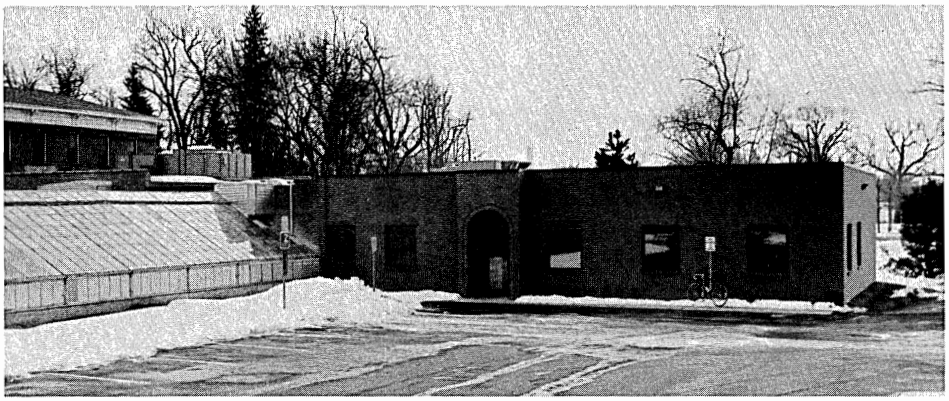
For publication in the next newsletter, copy should reach the TCCP no later than May 1, 1986. Send entries to: Ms. Julie L. Fischer, Editor, Tissue Culture for Crops Project, Colorado State University, Fort Collins, CO 80523.

New, Expanded Facility Houses TCCP

On August 12, 1985, the Tissue Culture for Crops Project moved to a new, specially-designed, 5300 sq. ft. laboratory and office facility on the edge of the Colorado State University campus. The new facility, which also houses the IPBNet Training Center, lies adjacent to the existing 8000 sq. ft. TCCP greenhouse.

Important features of the new facility include a separate equipment room with detached floor (to prevent vibration of delicate equipment) and fume hood, a temperature-controlled, 50% larger culture room, quiet study area, and conference room/library.

To inaugurate the new building, the Project held an Open House on September 10, 1985. Over sixty persons, including Dr. Judson Harper, CSU Vice President for Research, Dr. John Raich, Dean of the College of Natural Sciences, Dr. Robert Parke, Botany Department Chairman, and Dr. James Walker, then USAID Senior Program Manager, attended the gathering and toured the facility.



Tissue Culture for Crops Project laboratory, office, and greenhouse facility at CSU.

Upcoming Tissue Culture Courses

A two-week, intensive course consisting of lecture-discussions and laboratory exercises in most aspects of plant cell and tissue culture will be held at the University of Tennessee, Knoxville, August 18-29, 1986. This course is designed for persons with a degree in science or with some experience in plant tissue culture. The course will provide hands-on laboratory training in plant cell and tissue culture techniques and a thorough theoretical and practical understanding of tissue culture technology. The course will cover establishment, growth, nutrition and handling of single cells, callus cultures, suspension cultures and organ cultures. The status, methodology and prospects of protoplasts, cryogenic storage and production of drugs and chemicals by plant cell cultures will be covered. Mutant selection, cell cycle analysis and cytogenetics in cultures will be major topics.

The course will be limited to sixteen participants. Places will be reserved upon receipt of the $1100 per person fee.

For further information, contact Dr. Donald K. Dougall, Botany Department,

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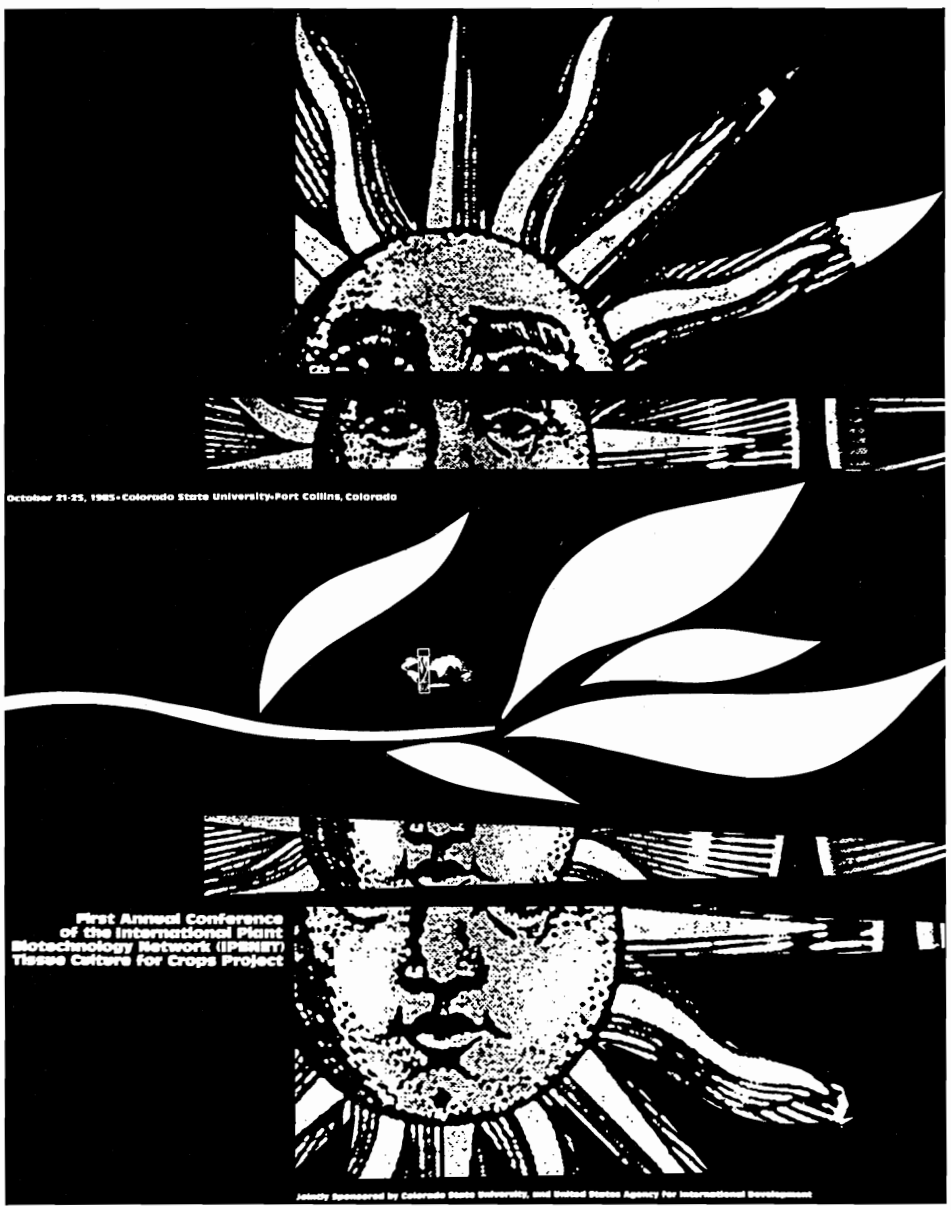
IPBNet Conference Posters Available



A limited supply of beautifully designed, commemorative conference posters is available from the TCCP. To receive a copy, write Ms. Julie L. Fischer, Conference Coordinator, Tissue Culture for Crops Project, Colorado State University, Fort Collins, CO 80523.

Upcoming Courses . . .

(continued from page 9)

The University of Tennessee, Knoxville, Tennessee 37996-1100, USA, (615)974-2256.

Plant Cell and Tissue Culture
Dates: July 7-11, 1986
Sponsored by: The Center for Advanced Training in Cell and Molecular Biology Catholic University of America
Cost: U.S. $650.00 per person
For more information, contact: Dr. Roland M. Nardone, Professor of Biology and Director, Center for Advanced Training in Cell and Molecular Biology, Catholic University of America, Washington, D.C. 20064, USA, (202)635-6161.

Plant Cell and Tissue Culture
Dates: varies
Sponsored by Flow Laboratories, Inc., and the Center for Advanced Training in Cell and Molecular Biology, Catholic University of America
Cost: varies
For more information and a schedule of topics, dates and places, contact: Flow Technical Services, 7655 Old Springhouse Road, McLean, VA 22102, USA, (800)368-3569.

Plant Cell and Tissue Culture
Dates: September 1, 1986 - February 20, 1987
Sponsored by: The Tissue Culture for Crops Project, International Plant Biotechnology Network Training Center
Cost: U.S. $8,000.00 plus transportation
Application deadline: June 1, 1986
For more information, contact: Training Coordinator, Tissue Culture for Crops Project, Colorado State University, Fort Collins, CO 80523, USA.

Plant Cell and Tissue Culture
Dates: To be announced
Sponsored by: Joint FAO/IAEA Division of Isotope and Radiation Applications of Atomic Energy for Food and Agricultural Development
For more information, contact: Dr. Bjorn Sigurbjornsson, Director, Joint FAO/IAEA Division, International Atomic Energy Agency, Wagramerstrasse 5, P.O. Box 100, A-1400 Vienna, AUSTRIA.

Information on other plant biotechnology training courses is solicited for inclusion in future newsletters.

Position Available at the TCCP

Research Associate. The Tissue Culture for Crops Project at Colorado State University is seeking a **Greenhouse Manager.** The successful candidate should have at least a B.S. (preferably an M.S.) in Horticulture or a related field and two years' greenhouse management experience. The candidate should be experienced in all phases of plant care (preferably cereal crops), greenhouse maintenance and design, and have the proven ability to lead a staff of ten, full- and part-time workers.

Application deadline March 1, 1986. Position open May 1, 1986. Salary commensurate with qualifications. Send resume and three letters of reference to:

Dr. Murray W. Nabors, Director
Tissue Culture for Crops Project
Colorado State University
Fort Collins, CO 80523.

First IPBNet Training Session Held

In September 1985, the first training session in tissue culture was implemented at the IPBNet Training Center in Fort Collins, Colorado. Five persons from four different countries are enrolled in the course. The trainees have successfully completed several weeks of intensive, formal course work and have applied the tissue culture techniques they have learned to the improvement of a crop species of their choice.

Iteu Hidayat (Indonesia), Anton Ben Musyimi (Kenya), and Francisco Gutierrez (Mexico), have developed experimental portfolios on obtaining virus-free potato, cassava, and sweet potato plants, respectively, through tissue culture. Dr. Flordeliza Javier (Philippines) has focused her interest on regeneration techniques of woody perennial crops. Rachmat Kartapradja (Indonesia), is investigating procedures for stem and leaf explants of tomato.

The Spring session of the Training Program will commence in March 1986 and related applications are now being processed. For more information on the Training Program, contact: Training Coordinator, Tissue Culture for Crops Project, Colorado State University, Fort Collins, CO 80523.

(Left to right) Rachmat Kartapradja, Flordeliza Javier, Iteu Hidayat, Ben Musyimi, Francisco Gutierrez, and Sunitha Siriwardana.

For more information, contact: Tissue Culture for Crops Project
Colorado State University
Fort Collins, Colorado 80523, USA

Telex: 9109309011 CSU CID FTCN
ATTN Murray Nabors, Botany, 6996

Phone: (303) 491-6996

2052

The International Plant Biotechnology Network

Tissue Culture for Crops Project

Newsletter #6 July 1986

Research Update

The principal research goals of the Project are the application of several tissue culture methods to increase the production rate of crop plant cultivars with enhanced tolerance to environmental stress. The research has focused particularly on salt, drought, and aluminum ion tolerance.

The major and significant contributions of the research have resulted in the development of reliable, efficient procedures for regenerating plants from cultured cells of rice, wheat, sorghum, sweet sorghum, millet, and soybean. Another significant achievement is the capability to regenerate plants after cell lines have been in culture for extended periods. For example, large numbers of rice plants have been obtained from rice cultures maintained for up to four and one half years.

The TCCP has completed virtually all its regeneration experiments with rice, wheat, millet, and sorghum. For each plant, methods are now available for obtaining long-term, high-frequency plant regeneration from callus cultures initiated from mature embryos (seeds). Recently, the Project has determined optimal callus culture and plant regeneration procedures for IRRI cultivars IR-6, IR-54, IR-56, and IR-60. Work also continues, in collaboration with Dr. S.S. Virmani of the Plant Breeding Department at IRRI, on perfecting plant regeneration techniques from callus obtained from various rice hybrids.

In all cases, cereal tissue cultures are initiated on Murashige and Skoog's basic medium containing various concentrations of 2,4-D and kinetin. Regeneration media usually contain various concentrations of benzyladenine and IAA, although not all possible concentrations have been tried.

Plants regenerated from tissue cultures are selfed to obtain sufficient seed for greenhouse and field tests to identify stress tolerance and other useful agronomic traits. Specific, detailed methods for each crop will be published in an upcoming Progress Report.

Field Testing of Plants Derived from Tissue Cultures

Researchers: K. Petersen, M. Thompson

Seeds of plants regenerated and selected after tissue culture screening are being grown to maturity and tested at field locations. Rice is being tested at Louisiana State University in collaboration with Dr. Milton C. Rush. A total of 147 lines of the cultivars Calrose, Giza 159, IR-36, Mahsuri, and Pokkali are being tested. Another testing site is the USDA Station at Beaumont, Texas where Dr. Charles N. Bollich is the collaborator. At the Beaumont location, a total of 92 lines of five cultivars are being tested.

The testing includes screening for tolerance and evaluation for valuable agronomic traits such as disease and pest resistance and potential yield characteristics. Plans have been completed to test more than 100 lines of rice for salinity tolerance in the Philippines, in collaboration with the Philippine Council for Agriculture and Resources Research and Development (PCARRD) in Los Baños.

At Texas A & M College Station, field testing is in progress with grain sorghum. Two hundred seventy lines have been planted by collaborator Dr. Fred Miller. The plants are being screened and evaluated for useful, agronomic characteristics. Special precautions are being taken to ensure that self-pollination will occur. About 370 lines of grain sorghum are also being grown and field tested by Dr. Ronny Duncan at Experiment, Georgia. Some of the plants are being grown and screened for tolerance to acidic soil and aluminum toxicity while others are being screened for useful characteristics on normal soil.

With Dr. David J. Isenhour as collaborator, field testing with grain sorghum is being performed at the Coastal Plain Station, University of Georgia. Dr. Isenhour has a research

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- **Recent Publications, pg. 12**
- **Second Annual Conference, pg. 11**

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The Tissue Culture For Crops Project

Project staff at CSU

Published by the Tissue Culture for Crops Project (TCCP)
Department of Botany
College of Natural Sciences
Colorado State University
Fort Collins, Colorado 80523, USA

Julie L. Fischer, Editor, Operations Director
Murray W. Nabors, Project Director
Oluf L. Gamborg, Associate Director
Susan J. Ferguson, Kerri L. Wright, Regional Network Coordinators

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Cooperating Agencies include:
Colorado State University, Fort Collins, Colorado, USA
Colorado State University, Agricultural Experiment Station, Fort Collins, Colorado, USA
George T. Cox and Associates, Management Consultants, Woodinville, Washington, USA

Participating Technical Staff: Murray Nabors, Barbara Ashton, Leann Avery, Gwendolyn Bane, Julie Cotton, Theresa DeMersseman, Fariha Faizi, Susan Ferguson, Julie Fischer, Lori Frederickson, Oluf Gamborg, Touba Ghazi, Hal Gibson, David Hewitt, Glen Hildreth, Harrison Hughes, Jeannette Hut, Koji Kawamura, Deborah Kelly, Larry Klima, Suresh Kumar, Clark Livingston, Marcus Murray, Deborah Neubaum, Lee Olton, Laurie Osborne, Robert Oswald, Kathy Petersen, Gregory Pott, Scott Price, Monica Rusk, Stephanie Schaal, Peter Schuerman, Dennis Shippee, Debra Singleton, Kim Spitler-Nabors, Nitschka ter Kuile, David Timm, Michael Thompson, Susan Todd, Callie Vanderbilt, Kristin Vanderbilt, Reagan Waskom, Kerri Wright, Zong-xiu Sun.

Graduate Students:

Iteu Hidayat, Raymond Ketchum, Douglas McMurray, Amani Mnayer, Akbar Mohmand, Poungpet Poonsapaya, Sathish Puthigae, Gaith Sasi, Sunitha Siriwardana, Nanan Widiyanto.

Trainees:

Olivia Damasco, Merlina Dionzon, Abeer Hassan, Simon Ndirangu, Mussarat Raza, Takaniko Ruabete.

Acknowledgments:

Grateful acknowledgment is given to Dr. Oluf L. Gamborg for his assistance in preparing the research sections of this newsletter.

New Faces At The TCCP

The Project extends its congratulations to several newly-appointed/promoted employees: Dr. A.S. Kumar, Postdoc, Ms. Kerri Wright, Regional Network Coordinator; Mr. Reagan Waskom, Greenhouse Manager; Ms. Gwendolyn Bane, Assistant Greenhouse Manager; Mr. Glen Hildreth, Lab Manager; Ms. Kathy Petersen and Mr. Michael Thompson, Group Leaders — Whole Plant Testing and Production.

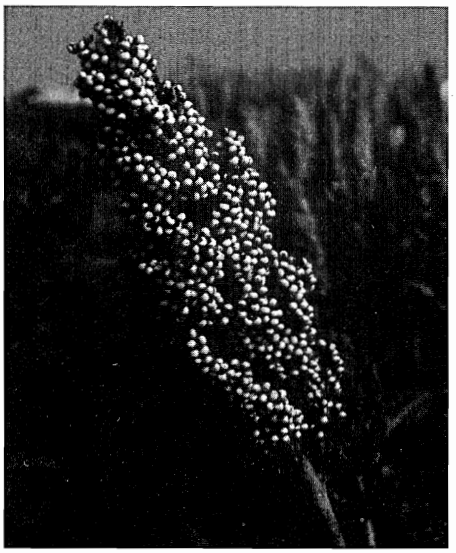
Research . . .

(continued from page 1)

program on insect pests and is screening sorghum lines for resistance to sorghum midge and fall army worms. The testing is being performed with two separate plantings to permit separate testings for each of the insects.

About 100 lines of spring wheat (Chinese Spring, Pavon, Glennson) are being grown in field plots for seed increase. Plans are being developed for field and greenhouse testing when the seeds become available.

Tissue culture regenerated sorghum in the field



Seed head of regenerated sorghum

In a collaborative program with Dr. Allan McHughen, Crop Development Center, University of Saskatchewan, Saskatoon, Canada, testing is in progress on over 100 lines of salt-tolerant oats derived from callus culture.* The plants are being grown in field plots on saline soils outside Saskatoon. The entries are composed of F_1, F_2, and F_3 progeny, which were selected in previous greenhouse screenings for salt tolerance.

In all of the testings, the seeds of valuable lines are being collected and will be used in more extensive testings at specific locations such as Thailand, India, Africa, Pakistan, and the Philippines.

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*Supported by CSU Agricultural Experiment Station, Fort Collins, Colorado.

Hydroponics and Greenhouse Testing
Researchers: K. Petersen, M. Thompson, R. Waskom

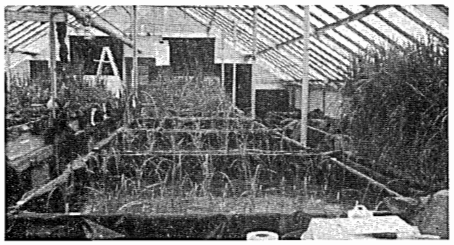
Hydroponic testing is currently underway on seeds of rice plants regenerated from callus after several months of culture in media containing 3, 5 and 7 g/l NaCl respectively. The plants are being grown in a hydroponic sand culture system which has been designed and used very successfully for rice. Greenhouse screening and seed increase is also in progress with grain sorghum on lines from plants regenerated after culture on 0.9 and 1.2% NaCl, respectively.

Plants from Suspension Cultures of Rice
Researchers: D. Kelly, P. Poonsapaya

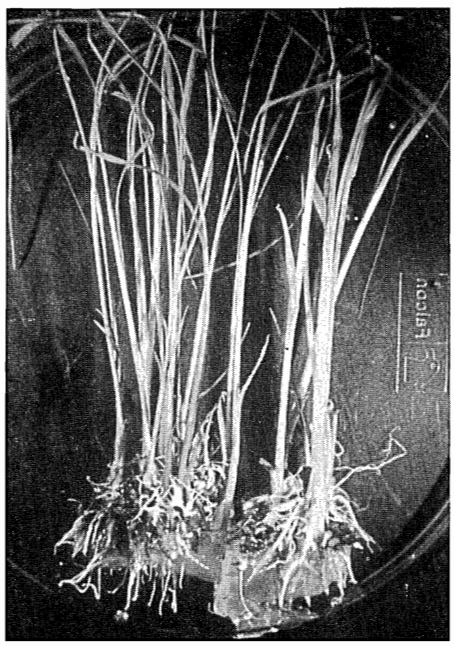
Plants have been regenerated from cells cultured in liquid suspensions for several months. This is a new and valuable accomplishment, because the liquid suspension culture system is an alternate and perhaps more effective culture approach for selection and screening for stress-tolerant variants. The current investigations focus on longer term culturing and higher plant regeneration efficiency.

Rice with Tolerance to Salt and Aluminum
Researchers: J. Cotton, K. Wright

Two cultures have been regenerated after continuous culturing on solid media with 6 and 9 g/l of NaCl, respectively, for up to two years. Approximately 100 plants from each experiment were obtained and grown to maturity for seed production. In other investigations, plants of cv. G-159 have been regenerated after the callus had been cultured for four months on agar



Tissue culture regenerated rice grown in hydroponic sand culture (above and below)



Tissue culture regenerated rice plants

Regenerated rice plants grown to maturity

media with 11 g/l of NaCl and others after two months at 11 g/l and then two months on 13 g/l.

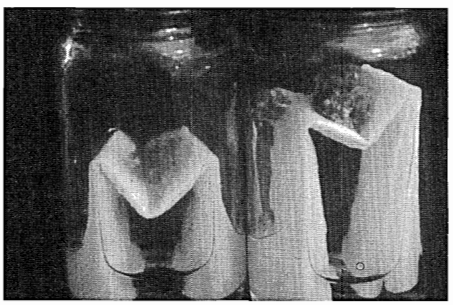
Embryogenic callus of cv. IR-36 has been cultured for four months on 9 g/l NaCl and plants regenerated. The liquid culture method has been employed in salt stress selection. Embryogenic callus of cv. G-159 was cultured for 56 days in liquid medium with 11 g/l NaCl and plants regenerated. The regenerated plants from these experiments are being grown to maturity. Callus culture and plant regeneration methods for IR-6, IR-54, IR-56, and IR-60 have also been developed.

Selection for Salt Tolerance in Wheat
Researchers: F. Faizi, S. Ferguson

Several strategies are being used for selection. Most often the embryogenic callus produced from an explant of mature embryos from seeds are placed on media with stepwise increasing concentrations of salt, after which plant regeneration is achieved. In two bread wheat cultivars, embryogenic callus has been grown on media with 6 g/l NaCl for six months. Plants were then regenerated, grown to maturity and seeds produced.

Selection for Drought Tolerance in Wheat
Researcher: A. Mohmand

Screening for drought tolerance generally involves the use of polyethylene glycol (PEG 4000) in the culture medium. Since this polymer interferes with solidification of agar media, the embryos of mature seeds are placed on a filter paper bridge. Using this technique, production of both total and embryogenic callus is nearly doubled compared to callus on solid medium to



Testing for drought tolerance using filter paper bridge system

which PEG is added just prior to solidification. Suitability of the paper bridge approach for drought tolerance screening for variants produced in culture and for plant regeneration efficiency has

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Research . . .

(continued from page 3)

not been fully evaluated, but the present observations indicate that the approach would result in plant regeneration by somatic embryogenesis.

Sorghum Regenerated from Long-Term Cultures on Salt Media
Researcher: K. Petersen

Methods for efficient plant regeneration by somatic embryogenesis have been demonstrated extensively on both grain sorghum and sweet sorghum*. Plants have been regenerated from cultures of grain and sweet sorghum after a growing period of up to three months in 1.2% salt and up to seven months in 0.9% salt, respectively. The plants have been grown to maturity for seed production.

*Supported by the Gas Research Institute, Chicago, Illinois.

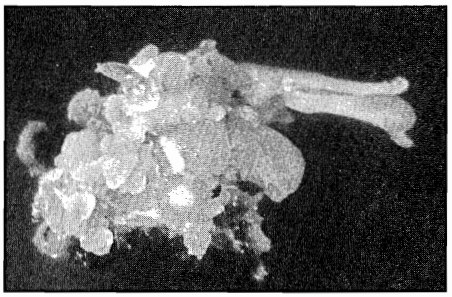
Salt-Tolerant Napier Grass *Pennisetum purpureum)**
Researcher: G. Hildreth

Plantlets regenerated by somatic embryogenesis *in vitro* have been grown for more than two months in the greenhouse in pots with saline soil. The levels of NaCl were gradually increased from 4 to 15 g/l.

Plants which survived these conditions are now being propagated. More than 200 entries of plants have been regenerated from embryogenic callus after extended culture on high levels of hydroxyproline. Current data suggest that Napier grass accumulates proline when the plants are grown under salt stress. Hydroxy-proline-tolerant variants might accumulate more proline.

Salt tolerance testing of regenerated Napier grass (plants growing in 15 g/l NaCl)

Mature sorghum from tissue culture regenerated plantlets



Sorghum regeneration after several months under salt stress

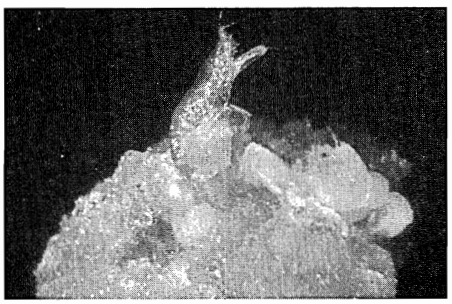
*Supported by the Gas Research Institute, Chicago, Illinois.

Pearl Millet Regeneration by Somatic Embryogenesis
Researcher: B. Ashton

Plant regeneration has been achieved after culturing callus from mature embryos for up to seven months. Plants have also been obtained from callus after culturing in media with 8 g/l of NaCl. The regenerated plants are being grown to maturity.

Regeneration in Cultivars and Inbred Lines of Corn
Researcher: S. Schaal

Plant regeneration capability has been demonstrated with several hybrid cultivars and inbred lines of corn. When immature embryos of corn are cultured, the tissue gives rise to two separate types of callus. Plant regeneration is consistently achieved by somatic embryogenesis from what is identified as Type II after a period of up to four months. The Type I callus is more abundant and grows at a more rapid rate, but plant regeneration is not successful beyond a two-month culture period.



Napier grass regenerated by embryogenesis (control)

Soybean Regeneration by Somatic Embryogenesis
Researchers: F. Faizi, T. Ghazi

A procedure has been developed for consistent plant regeneration from callus derived from immature embryos of soybean. The cotyledon tissue produces a particular callus which, upon further culture, gives rise to embryos which can grow to mature plants. The research is a collaborative project between the TCCP and Dr. Harvinder Cheema of Punjab University, Chandigarh, India. These observations were reported at the same time as the first report on plant regeneration in soybean by somatic embryogensis was published.

Wide Cross Breeding in Wheat
Researcher: N. ter Kuile

In continuing collaboration with Dr. A. Mujeeb-Kazi of CIMMYT, the TCCP is conducting experiments to acquire the tissue culture methods to facilitate the hybridization and chromosome transfer process of disease, salt, and drought tolerance within wheat species and between wheat and grasses. Plant regeneration has been successful in four bread wheat and three Durum wheat varieties.

Plants have been regenerated from embryogenic callus originating from immature embryos and cultured for several months. The embryo rescue methodology developed earlier has been used on 367 accessions of hybrids between *T. aestivum* and nine separate wild species of *Triticum.* More than 10,000 embryos have been cultured and are being grown to maturity.

Physiology of Stress Tolerance
Researcher: R. Ketchum

Since no crop plant species are halophytic, research into the fundamental basis for salt tolerance is being pursued with identified halophytic species.

The grass species *Distichlis spicata* (seashore saltgrass) is particularly valuable material. A rapidly growing, uniform cell suspension culture of this species was used to determine the correlations between proline accumulation and stress tolerance. There is a direct correlation between the concentrations of proline and the chemical/osmotic equivalent of salt in the medium. The data also show that the proline

accumulates in the cell cytosol and negligible amounts are present in the vacuoles. Investigations with metabolic inhibitors have shown that proline levels are normal when cycloheximide is present in salt-stressed media, whereas the presence of actinomycin D had no effect on high proline accumulation. Both inhibitor compounds were toxic at the concentrations used (10 μg/l) and stopped cell division.

Biotechnology for Identifying and Transferring Stress Tolerance Genes
Researchers: O. Gamborg, Z. Sun

The transfer of stress tolerance from alien species to crop plants is rarely feasible by conventional sexual crossing methods because of incompatibility barriers. Cell fusion hybridization procedures can be considered in making crosses between highly stress-tolerant species and crop cultivars. The hybrid could then be backcrossed to the crop cultivar.

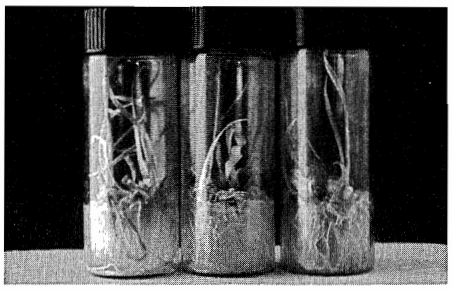
A collaborative program exploring the cell fusion approach has been initiated with the Nuclear Institute of Agriculture and Biology in Faisalabad, Pakistan. A highly salt-tolerant tropical forage grass, with high photosynthetic efficiency and associate nitrogen fixation, has potential and is being considered in a cell fusion hybridization with cereals.

Soybean grown to maturity from plantlet regenerated in tissue culture

Molecular Biology is being employed to detect gene products (proteins) and identification of genes for salt tolerance. The approach being taken is to use plant materials from highly-tolerant species (halophytes). The cell suspension culture of saltgrass *(D. spicata)* used for the physiological studies is being used as the experimental material. These cells grow rapidly in the absence and in the presence of salt. The approach is to identify stress-induced proteins, to clone the respective gene(s) and to determine their possible role(s) in helping the cells cope with salt stress.

Analyses of RNA and proteins are being performed on stressed and non-stressed halophytic cells.*

*The Molecular Biology program is a collaboration with Dr. James Colbert, Botany Department, CSU, Fort Collins, Colorado.



Tissue culture regenerated wheat plants

Due to the large number of methods for expressing salinity levels and water potentials presently being used in the literature, the following table is included for general reference.

Comparison Of Properties Of Aqueous Solutions Of NaCl At 20°

MOLARITY m • 1^{-1} H_2O	NaCl g • 1^{-1}	MOLALITY m • kg^{-1} H_2O	OSMOLALITY osm • kg^{-1} H_2O	WATER POTENTIAL MPa	WATER POTENTIAL -bars	ELECTRICAL CONDUCTIVITY mmho • cm^{-1}
0.050	2.922	0.050	0.092	0.230	2.30	5.0
0.100	5.844	0.100	0.185	0.454	4.54	9.4
0.150	8.766	0.151	0.278	0.676	6.76	13.7
0.200	11.689	0.201	0.371	0.900	9.00	17.9
0.250	14.610	0.251	0.464	1.123	11.23	22.1
0.300	17.533	0.302	0.557	1.344	13.44	26.2
0.350	20.455	0.353	0.651	1.569	15.69	30.3
0.400	23.377	0.404	0.744	1.791	17.91	34.3
0.450	26.299	0.454	0.838	2.016	20.16	38.2
0.500	29.221	0.505	0.932	2.241	22.41	42.2
0.550	32.144	0.556	1.026	2.464	24.64	46.1
0.600	35.066	0.608	1.120	2.694	26.94	50.0
0.650	37.988	0.659	1.215	2.926	29.26	53.9
0.700	40.910	0.710	1.309	3.151	31.51	57.8
0.750	43.832	0.762	1.404	3.388	33.88	60.8
0.800	46.754	0.813	1.499	3.612	36.12	64.3
0.850	49.676	0.865	1.594	3.850	38.50	67.7
0.900	52.598	0.916	1.689	4.079	40.79	71.1
0.950	55.521	0.968	1.785	4.311	43.11	74.5
1.000	58.4428	1.020	1.880	4.550	45.50	77.9

Compiled by R. Ketchum

References

Lang, A.R.G. 1967. Osmotic coefficients and water potentials of sodium chloride solutions from 0 to 40°C. Aust. J. Chem. 20: 2017-2023.

CRC Handbook of Chemistry and Physics. 1978-1979. ed. Weast R.C. 59th edition. CRC Press, Inc. Boca Raton.

IPBNet Conference - 1985 List of Participants

Name	Address
Dr. Mohammed Aaouine	I.A.V. Hassan II Morocco
Dr. Mohamed Ammati	Complexe, d'Algadir Morocco
Dr. Azis Azirin Asandhi	Lembang Horticulture Research Institute, Indonesia
Dr. Irvin Asher	Agency for International Development, U.S.A.
Ms. Barbara Ashton	Tissue for Culture Crops Project (TCCP), Colorado State University U.S.A.
Ms. Gwen Bane	TCCP, Colorado State University U.S.A.
Dr. Ramon Barba	Institute of Plant Breeding University of the Philippines at Los Baños, Philippines
Mr. David G. Beall	Enginetics, Inc. U.S.A.
Ms. Linda Behrns	TCCP, Colorado State University U.S.A.
Dr. D.S. Brar	Punjab Agricultural University India
Dr. John Carman	Utah State University U.S.A.
Dr. John Chandler	USDA-ARS, University of California, U.S.A.
Dr. Harvinder Cheema	Punjab University India
Dr. Hassane Chlyah	Universite Mohammed Morocco
Dr. V.L. Chopra	Biotechnology Centre Indian Agricultural Research Inst. India
Dr. E.C. Cocking	University of Nottingham United Kingdom
Ms. Julie Cotton	TCCP, Colorado State University U.S.A.
Mr. George Cox	U.S.A.
Mr. Kirby DeMott	TCCP, Colorado State University U.S.A.
Dr. Ati Srie Duriat	USDA-ARS U.S.A.
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Ms. Susan Ferguson	TCCP, Colorado State University U.S.A.
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Ms. Lori Frederickson	TCCP, Colorado State University U.S.A.
Dr. Oluf Gamborg	TCCP, Colorado State University U.S.A.
Ms. Touba Ghazi	TCCP, Colorado State University U.S.A.
Dr. Tejpal Gill	Agency for International Development, U.S.A.
Dr. Phillip Gordon	Yale University U.S.A.
Dr. C.L.L. Gowda	ICRISAT India
Dr. Glenys Gunderson	TCCP, Colorado State University U.S.A.
Mr. Francisco Lopez Gutierrez	Centro Investigacions Biologicas de Baja California Sur U.S.A.
Ms. Iteu Hidayat	Lembang Horticultural Research Institute Indonesia
Mr. Glen Hildreth	TCCP, Colorado State University U.S.A.
Dr. Harrison Hughes	Colorado State University U.S.A.
Dr. Karen Hughes	University of Tennessee U.S.A.
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Dr. Sinikka Kallio	University of Davis, KGMIRA Co., Espoo Research Centre Finland
Dr. Nilufer Hye Karim	BRRI Joydebpur Bangladesh
Mr. Rachmat Kartapradja	Lembang Research Institute for Horticultural Crops Indonesia
Dr. A. Mujeeb-Kazi	CIMMYT Mexico
Ms. Debbie Kelly	TCCP, Colorado State University U.S.A.
Mr. Raymond E.B. Ketchum	TCCP, Colorado State University U.S.A.
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Mr. Larry Klima	TCCP, Colorado State University U.S.A.
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Dr. Christy MacKinnon	TCCP, Colorado State University U.S.A.
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Dr. James Meiman	Colorado State University U.S.A.
Mr. Akbar Mohmand	TCCP, Colorado State University U.S.A.
Dr. Ludwig E. Müller	Centro Agronomico Tropical de Investigacion y Ensenanza Costa Rica
Dr. Riccardo Morpurgo	International Potato Center MORTURGO, Peru
Dr. Fathan Muhadjir	Bogor Research Institute for Food Crops, Indonesia

Name	Address
Mr. Anton Ben Musyimi	University of Nairobi Kenya
Dr. Murray W. Nabors	TCCP, Colorado State University U.S.A.
Dr. S. Shamshad Naqvi	Atomic Energy Agricultural Research, Pakistan
Dr. Eddy Noerhadi	Institut Technologi Bandung Indonesia
Dr. James H. Oard	University of California at Davis U.S.A.
Ms. Elizabeth Lee Olton	TCCP, Colorado State University U.S.A.
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Mr. Robert Oswald	TCCP, Colorado State University U.S.A.
Ms. Susana Pons	Instituto de Investigacion Agropecuaria de Panama - IDIAP Panama
Ms. Poungpet Poonsapaya	TCCP, Colorado State University U.S.A.
Mr. Sathish Puthigae	TCCP, Colorado State University U.S.A.
Dr. Shao Qiquan	Genetics Institute, Academia Sinica China
Dr. Azra Quraishi	NARC Pakistan
William M. Roca	CIAT Colombia
Francis A. Roth	Enginetics, Inc. U.S.A.
Dr. Milton C. Rush	Louisiana State University U.S.A.
Ms. Patricia Rustanius	Colorado State University U.S.A.
Dr. D.C. Sastri	ICRISAT India
Ms. Stephanie Schaal	TCCP, Colorado State University U.S.A.
Ms. Erin Scott	TCCP, Colorado State University U.S.A.
Ms. Sunitha Siriwardana	TCCP, Colorado State University U.S.A.
Dr. David Skeehan	Colorado State University U.S.A.
Ms. Supaporn Tang	Chulalongkorn University Thailand
Ms. Debbie Tono	Colorado State University U.S.A.
Dr. Trevor Thorpe	University of Calgary Canada
Dr. Leigh Towill	National Seed Laboratory Colorado State University U.S.A.
Mrs. Montakan Vajrabhaya	Chulalongkorn University Thailand
Dr. Thavorn Vajrabhaya	Chulalongkorn University Thailand
Ms. Mary Waters	TCCP, Colorado State University U.S.A.
Dr. Hartono Wirjodarmodjo	Manggala Wanabakti Blok IV Indonesia
Dr. Rapepun Wititsuwannakul	Prince of Songkla University Thailand
Ms. Patricia Yarmas	Dept. de Fisiologia Vegetal Estacion Experimental La Molina Peru

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First Annual Conference of The International Plant Biotechnology Network

The First Annual Conference of the International Plant Biotechnology Network was held in Fort Collins, Colorado on October 21-25, 1985. The Conference brought together scientists and research workers from countries of Asia, Africa, Latin and South America as well as from North America.

The Conference program included workshops, presentations of original research and roundtable discussions. The focus was on tissue culture biotechnologies and their application in agriculture and plantation crops. Technical sessions were conducted as workshops with extensive exchange of new advances in information and technology. Each session was introduced with the status of the technology followed by discussions on obstacles to their wider and efficient uses in crop plant species.

The leader and resource person for the session on protoplast technology was Dr. E.C. Cocking, Nottingham University, Great Britain. The session on somatic embryogenesis was lead by Dr. M.W. Nabors, the TCCP Project Director, Colorado State University. Dr. T.C. Thorpe of the University of Calgary, Canada conducted the session on mutation methods and lead a discussion on this topic with respect to tissue culture technologies. Dr. Karen Hughes, University of Tennessee, spoke on mutation selection and genetic transformation in plant cell and protoplast systems. The workshops were followed by verbal and poster presentations as well as sharing of original tissue culture biotechnology research by scientists representing research organizations from more than 20 countries.

Other important and successful features of the Conference were group discussions and subsequent recommendations on enhancing collaboration on research of mutual interest and developing more efficient systems of communication and exchange of information on new advances in knowledge and technology. The Conference was an exceptional opportunity for individuals to establish new contacts and explore where or with whom collaborative research might be of mutual benefit.

SUMMARIES OF RESEARCH PRESENTED AT THE FIRST CONFERENCE

1. Rice Somaculture
   Cau Jun, M.C. Rush, T.P. Croughan, Louisiana State University, Baton Rouge, LA 70503

A system was developed that allowed efficient regeneration of U.S. rice cultivars and several foreign varieties. More than 5000 plants have been regenerated and transplanted to the greenhouse in the last two years. About 700 lines from seeds produced on regenerated plants in the greenhouse were grown in the field at the Rice Research Station during 1985. These plants were evaluated for morphological variation, yield, and disease resistance. Line to line variation was observed in lines from the same cultivar for differences in height, days to heading, grain type, tiller number, tillering habit, leaf shape, leaf color, panicle size, and weight. Differences within the lines were observed for dwarfing, height, apiculus color, pubescence, awn production, hull color, and other characteristics. We observed apparent resistance in the field to narrow brown spot and sheath blight in several lines.

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2. Rice Callus Grown Under Salt Stress
   A. Quraishi, NACR, Islamabad, Pakistan

Callus production was observed in 75% of mature seeds of indica cultivars when cultured on modified MS medium. Callus was also obtained from root and nodal sections of rice plants. When 8p, N_6 and potato medium were tested on indica cultivars, there was no callus production. However, when the media were supplemented with an increasing level of sucrose and 2,4-D, callus was produced on 8p medium with 50 g/l of sucrose.

Callus tissue was cultured on media with sodium chloride and calcium chloride in a range from 0, 0.5, 0.75, 1.00 and 1-5%; growth was observed in 50% of the cultures when the stress was 1% and 6 to 9% when the level was 1.5%. A few plantlets were obtained.

3. Date Palm Micropropagation
   A. Quraishi, NACR, Islamabad, Pakistan

Sections of the apical region with few or no primordial leaves have been successfully used for direct regeneration of date palm plantlets. *In vitro* plantlets in different stages of growth have been obtained in medium containing NAA at 10 mg/l. Medium supplemented with NAA at 10 mg/l and GA_3 at 1 mg/l produced callus. Plantlets obtained in GA_3 at 5 mg/l have thin, flexible leaves and are easy to handle during subculturing and hardening in the pots. The techniques have been successful for the production of shoots but only a few with well defined root systems have been obtained.

4. Embryogenic Callus from Cultured Embryos of Wheat *(T. aestivum L.)*.
   H. Chlyah, Faculty of Science, B.P. 1014, Rabat, Morocco

The aim of this study was to define the role of the scutellum in somatic embryogenesis and to determine the zones of the embryo which have embryogenic potential. Immature and mature wheat embryos were used with or without the scutellum. The embryos were cut in sections transversely into two, three, or four explants, or longitudinally, with the scutellum either on one side or underlying the embryo; in these last two cases, one or two transverse sections made four or six explants.

All the explants from the three types of sectioning formed callus and somatic embryos. The higher the number of explants per cultured embryo, the greater the total number of somatic embryos formed. The longitudinal section of the embryo combined with transverse sections gave better results than transverse sections alone.

The presence of the scutellum was not indispensable to embryogenic expression. Explants from the root apex of the embryo, or in contact with the scutellum, generally formed more somatic embryos than those taken from shoot apex, or without scutellum.

5. Anther Culture of Rice
   N.H. Karim, A.K.M. Shahjahan, M.A.A. Miah, M.J. Haque, S.A. Miah, B.R.R.I., Joydebpur, Bangladesh

More than fourteen different media were tested to determine the combinations most suitable for culture of anthers. A medium designated M10 was suitable for both japonica and indica cultivars. It was also observed that rice anthers require 8-12 days at 8-10°C to yield maximum callus production. Culturing in the dark at 25°C for three weeks also enhanced callus production. Plants regenerated from the callus consisted of a high percentage of albino. Attempts are being made to find conditions to reduce the number of albino plants. The regenerated dihaploid green plants are being evaluated.

6. Potentials and Need for Biotechnological Research in Chickpea and Pigeonpea
   C.L.L. Gowda, ICRISAT, Patancheru P.O., Andhra Pradesh 502324, India

Chickpea *(Cicer arietinum)* and pigeonpea *(Cajanus cajan)* are the important grain legume crops in the arid and semi-arid tropics. The International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) has the mandate for improvement of these two crops along with sorghum, pearl millet and groundnut. Most biotechnology laboratories in developed countries are interested in temperate climate commercial crops, and in industrial applications of biotechnology. Considering the importance of these two crops as food sources and the promise and potentials of biotechnology in crop improvement, there is ample need and justification to conduct additional biotechnological research. A few wild species related to chickpea *(Cicer* spp.) and pigeonpea *(Atylosia* spp.) are known to possess genes for beneficial characters such as disease and pest resistance and high protein content. Some of these wild species cannot be easily crossed with the cultivated species. Therefore, embryo culture and ovule culture may be needed to obtain viable hybrids. Other areas where biotechnology research has potential for the two crops are: (i) anther and pollen culture techniques for developing haploids; (ii) a search for somaclonal variation to obtain useful novel plant characters; (iii) selection for tolerance to biological and environmental stresses by tissue and cell suspension culture; (iv) protoplast isolation and fusion techniques to help make somatic hybrids; and (v) rapid cloning techniques for vegetative propagation.

7. HMG-CoA Reductase in Latex and Leaves of *Hevea Brasiliensis*
   Rapepun Wititsuwannakul, Faculty of Science, Prince of Songkla University, Hat-Yai, Songkla 90112, Thailand

Our studies on variation of *Hevea* HMG-CoA reductase activity in latex, obtained from panel tapping, revealed that the reductase activity was closely related with the rubber content. The enzyme showed diurnal variation of activity during a 24 hour cycle. Peak activity occurred around sunset (1800 h) and was approximately two-fold above basal level during daylight period. Rubber content in the latex also varied diurnally in a pattern similar to that of the reductase. Two clonal types used in the study (RRIM 600 and KRS 21) showed the same variation patterns. The correlation suggested the importance of the enzyme in the regulation of rubber biosynthesis. Petiole rubber obtained from leaves detached at four-hour intervals showed a diurnal variation similar to that of the latex rubber content. The activity of HMG-CoA reductase in cell-free extracts of *Hevea* leaf tissue detached at four-hour intervals also varied diurnally, in a pattern similar to variation of the petiole rubber values. These studies suggested the potential role of HMG-CoA reductase in the regulation of rubber production in leaves as well as in the lacticiferous vessels in the bark area of the *Hevea* trees.

The studies are being extended to tissue culture. Callus will be used as tissue source for further studies on the relationship between HMG-CoA reductase activity and callus rubber content.

Comparative studies on levels of HMG-CoA reductase activity in leaves detached at 1800 h from various clonal types showed that the activity of the high-yielding clone was significantly higher than the low-yielding one. Furthermore, the activity level of HHG-CoA reductase from clone rRIM

600 which is known to be the highest yielder was consistently higher than PB 5/51 and PR 255 which are classified as high yielders.

The correlation between level of HMG-CoA reductase activity and rubber content of individual clones may lead to a possible use of HMG-CoA reductase activity as an indicator in evaluating rubber yields and superior rubber producing cultivars.

8. Effects of Sucrose and Coconut Water on Regeneration of Rice *(Oryza sativa)*

Thavorn Vajrabhaya, Montakan Vajrabhaya, Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok 10500, Thailand

This study was undertaken to determine the effects of sucrose and coconut water on inducing regeneration of plants from callus. It was found that omitting sucrose from the culture media not only increased the number of callus with greenspots but it also prolonged the life of callus and stimulated shoot development. Adding 10% coconut water to the media in the absence of sucrose intensified the effects stated above. It appears that the contents of coconut water have factors enhancing regeneration. The observations indicate that sucrose has a deleterious effect on certain stages of callus growth and differentiation. The roles of organic supplement nutrition, especially sugars and coconut water, on regeneration should be pursued further. Information on factors which enhance plant regeneration will help in speeding up stress tolerance selection through tissue culture.

9. Somatic Embryogenesis and Plant Regeneration from Anther Culture of *(Panax quinquifolius* (Ginseng)

Du Linge Inst. of Special Products, Zhojia, Jiln, China, Shao Qiquan, Li Ansheng, Genetics Institute, Academia Sinica, Beijing, China

Panax Quinquifolius is an important herb in China. Because of its long vegetative growing period, the plants usually do not flower until five years after planting. The normal flowers are produced seven years after planting. Ginseng improvement by conventional breeding technique is time consuming. We are employing tissue culture methods and have obtained preliminary results.

A large number of plants have been obtained from anther culture of ginseng. Several embryogenic cell lines were isolated from callus of anthers. Plants have been regenerated from the cultures for more than two years of subculture.

The media N6 and Miller were the best for callus induction from ginseng anthers. Addition of 2,4-D at 1.5 mg/l and kinetin at 1 mg/l were needed, sucrose was used at 3%. The best stage for inoculation of anthers was the middle stage of mononuclei pollen. The best callus induction from anthers was achieved at this stage. Pretreatment of flower buds at 69°C for 48 days significantly increased callus induction frequency.

The conditions for differentiation and regeneration of plants from calli were determined. More than 100 combinations of media were checked. Among them were 16 combinations which supported plant regeneration. The best combinations were: MS + IBA, 0.5 mg/l, + kinetin, 2 mg/l, gibberellic acid 2 mg/l, casein hydrolyzate 1000 mg/l, sucrose 3%. The frequency of plant regeneration was 6.0%.

Cytological observations showed that the plants induced from anther calli were haploid, indicating they originated from pollen. The frequency of haploid cells at the calli stage were 53% and 48% for root tip cells. Most of the plants regenerated from anther calli have normal leaves. Plants produced in test-tubes takes one month, by conventional methods in the field it takes more than one year. The flowering of plantlets in test-tube was induced under defined conditions. The plantlets flowered thirty days after transferring the somatic embryos into fresh medium. The experiment on artificial pollination in test-tubes is in progress.

10. Seedless Plants of Chinese Wolfberry *(Lycium barbarum)* obtained by Endosperm Culture

Shao Qiquan, Wang Li Shanxi Institute of Medicine, Taiyuan, China, Genetics Institute, Academia Sinica, Beijing, China

The fruits of Chinese wolfberry are widely used in Chinese medicine to reduce the side effects of cancer cure medicines and are also used in the food industry. The seeds are not desirable. We are trying to develop seedless Chinese wolfberry through tissue culture of endosperm. Chinese wolfberry has 2n=2x=24 chromosome. The plants induced from endosperm should be triploid 2n=3x=36 chromosomes. In that case we can expect to get seedless fruits. We have obtained plants from endosperm of wolfberry in 1982, using MS medium. They were planted in the field in the spring of 1984. Among them, four plants flowered and had fruits. The lack of seeds was verified and the plants were multiplied vegetatively.

Among these four endosperm plants, three of them were triploid. In the root tips the triploid cells made up more then 50%. The plants are designated endosperm plants No. 1, 2, and 3. One plant was diploid: endosperm plant No. 4. The endosperm plant No. 4 was induced from the same piece of callus as the other three endosperm plants.

The endosperm plants always have fruits with less seeds. For example, the number of seeds in endosperm plant No. 1 was eighteen seeds per fruit, in No. 2, thirteen seeds and for No. 3, twenty seeds, for No. 4, twenty-two seeds per fruit compared with control with 35 seeds per fruit. At the same time, most of the seeds in fruits of endosperm plants were shrivelled. In endosperm plant No. 1 the shrivelled seeds made up 93%, for No. 2 59%, for No. 4 14% and for control 2%. Thus the data shows that the number of viable seeds in fruits of the endosperm plants was significantly lower than in control plants.

The observation on meiosis of pollen-mother cells of endosperm plants shows that there are many abnormalities in meiosis in the endosperm plants. There are cases with monosomics without pairing, unequal division, lodging observations, which support the conclusion that the fruits were in effect seedless.

Beside the seedless feature, endosperm plants have at the same time other morphological characteristics that separate them from normal wolfberry. Endosperm plants usually have larger leaves and large stoma on the lower side of the leaves. The number of stoma of endosperm plants No. 1, 2, 3, 4 were 16, 17, 10 and 35, respectively, compared with 35 in the control. Endosperm plants usually have abnormal flowers. The abnormality in flowers for endosperm plants No. 1, 2, 3, 4, are 26%, 8%, 8%, and 0% respectively, against 0 in the control. Abnormal petals also were observed. Abnormal anthers in endosperm plants No. 1, 2, 3, 4, occurred at 2%, 6%, 5% and 0%, respectively against 0 in the control. The morphology and color of fruits of endosperm plants usually were the same as in the control. The size of fruits of endosperm plants varied greatly and the size of fruits in the same

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plant can differ by as much as three times.

11. Stress Physiology of Food Crops in Indonesia
Fathan Muhadjir, Department of Plant Physiology, Bogor Research Institute for Food Crops, Bogor, Indonesia

Conventional methods of screening for stress-tolerant crops such as rice, maize, and legumes have been done in Indonesia. The methods include stress selection for salt, aluminum, drought, and low temperature. The most important groups of soil to be considered for future expansion of agriculture in Indonesia are the Red Yellow Podzolic Soils, which occupy approximately 30% of the Indonesian land area. These soils have low salinity, low pH, high content of fixed phosphate and have shallow effective rooting zones. Drought is often a limiting factor to yield of tropical crops. It is also important to identify the optimal salinity levels used in screening and selection in order to employ tissue culture methods. It is essential to develop regeneration techniques for Indonesian varieties of rice, and apply them to screening and selection for sodium chloride- and acid-tolerant lines.

Methods of stress selection through tissue culture, methods for selecting cell lines tolerant to high levels of aluminum, and methods of selecting nitrogen-efficient cell lines are being explored in our research program.

12. The Effect of Lysine and its Analogs on the Enzyme Activity Changes During Development of Calli of Rice, *Oryza sativa L.* S.N. Widiyanto, M. Wirahadikusamah, E. Noerhadi, Dept. of Biologi, Institut Teknologi Bandung, Bandung 40132, Indonesia

Four variants of rice calli adapted to lysine (ly) 5 × 10^{-5} M, delta-hydroxylysine (HL) 10^{-5} M, thiolysine (TL) 10^{-5} M and no-treatment medium (Control) were used. The variants were identified as Ly, HL, TL and Control (CK).

The data indicated that peroxidase, catalase and glutamic-pyruvic-transaminase (GPT) activities in all of the variants increased when the adapted calli were subcultured on regeneration media. Peroxidase activity increased 30-70% and catalase activity increased 25-30%. The GPT activity appeared to be 300-400% higher than CK.

The results showed variations in peroxidase, catalase and GPT activity during the development of rice calli.

13. Plant Regeneration in Jute
A.S. Islam, S.M. Zahidur Rahman, Dept. of Botany, Dhaka University, Dhaka, Bangladesh

Jute fibers are extracted from cultivars of the two species of *Corchorus* — a genus belonging to the family of *Tiliaceae.* Since jute brings about 65 to 70% of the foreign exchange of Bangladesh, its improvement has become a national concern, particularly in the face of its competition with synthetic fibers. One limitation in bringing about genetic improvement is the reproductive biology of the cultivars. Cultivars of one species, namely *C. capsularis* are 100% self-pollinated and those of *C. olitorius* are only 10% cross-pollinated.

In the past, efforts were made to improve the varieties through irradiation, chemical mutagens, induced polyploidy and distant hybridization through interspecific crosses. Tissue culture research was initiated in 1980. Explants of stem, root, and leaves of seedlings were tried in MS and White's media supplemented with auxins and cytokinins in different proportions but differentiation of shoots took place rarely. The shoot regeneration capacity of different segments of germinating seeds of several cultivars of *C. olitorius* and *C. capsularis* has now been tested in MS medium using tyrosine and glutamine in addition to the usual supplements of auxins and cytokinins. The results of this experiment are reported.

The seventeen hour old germinating seeds of *C. capsularis* cv D154, CVL-1; *C. olitorius* cv 04 and C.G. and advanced generations of *C. olitorius* × *C. aestuans* were used in the experiment. The testa and the endosperm were removed. The seeds were divided into two lots after removal of root tips. From one lot, cotyledons were removed and in the second lot they were left intact. The length of the inoculum after root tip removal was 1 mm. The explants were transferred to MS medium solidified with 0.8% agar, supplemented with BA and tyrosine in various combinations and concentrations. The test tubes were placed at a twelve hour photoperiod with day and night temperature fixed at 28± 2°C and 23± 2°C, respectively. Callus developed from both lots within ten days. Within twenty days, fused multiple shoots were observed from all the material except C.G. The number of shoots was found to range from 10-30 depending upon the variety. The supplements found best for multiple shoot formation was 0.5 mg/l BA and 25 mg/l tyrosine. Since the fused multiple shoots had no roots, they were separated and each isolated plantlet was transferred to MS containing various concentrations of IBA and also a mixture of IBA and IAA in different ratios. The use of 0.2 mg/l IBA was found best for induction of rooting.

The transfer of test tube grown plantlets to pots was a difficult task because in the beginning almost all of them died following transplantation. Now almost all plants are saved from wilting by covering the freshly transplanted seedling with perforated polyethylene. The latter is removed on each successive day for increasing lengths of time until the transplanted plantlet was hardened enough to withstand wilting stress.

14. Micropropagation of Plantain and Banana
Susana Pons, Research Institute of Agriculture and Stockbreeding Panama (IDIAP), Panama 6A, Panama

Plantains and dessert bananas are major economic crops in tropical countries which consume plantain as a major source of carbohydrate and export fresh bananas as a dessert fruit. In Panama, *Musa* monoculture provides work for some 14,900 people. Plantain and banana production has been threatened since 1972 by a severe leaf spot disease caused by the fungus *Mycosphaerella fijiensis* var. *difformis,* commonly called Black Sigatoka. Black Sigatoka infestation of plantain has been the cause of the loss of 1632 ha in three years.

Research is in progress on dwarf Horn plantain micropropagation, and on techniques which will permit evaluation of the potential for *in vitro* production of novel genotypes through ovule culture and related methods.

Major problems of conventional breeding for plantain include not only low levels of true seed production, but the procedure is time consuming. There is also a lack of diversity in the germplasm of potential female breeding lines. Tissue culture, and especially ovule culture, may have potential for enabling the production of more and diverse germplasm by virtue of the enhancement of pollination effectiveness. Embryo rescue procedures may also have considerable potential value. Rescued embryos grown to maturity would represent germ lines which otherwise would not become available for conventional breeding.

Plans are in progress to develop the necessary protocols for plantain shoot tip and meristem culture, embryo culture from the perspective of "rescue" and other aseptic culture technologies which have promise for plantain improvement.

15. Clonal Propagation of Plantains
Musa spp.
Dr. E. Müller, Tropical Agriculture Center for Research and Training, Turrialba, Costa Rica

Plantain differ from dessert banana (AAA) due to the fact that its genome contains the so-called B *(M. balbisiana)* component in combination with A *(M. acuminata)*, either as AAB or ABB. Several diseases affect bananas and plantains, but in 1978 a new and very virulent disease started to spread in Central America, the so-called Black Sigatoka *(Mycosphaerella fijiensis* var. *difformis)*. In many areas plantain production was wiped out.

The project has several objectives. First, to propagate existing resistant material of two cultivars, 'Pelipita' and 'Saba' which are tolerant. A method was developed for rapid vegetative multiplication, using as explant the apical bud excised from corms. After the initial culture is established, serial subculturing is easy because of the formation of adventitious buds on a medium containing only BA which permits multiplication by a factor of ten to twenty.

The second objective was to find resistant or tolerant material among the commercial varieties. Preliminary trials were carried out to screen for differences in infection rate *in vitro*, using spores or mycelium of the fungus. Results indicated that this method may be useful as a method to select for tolerance. Large numbers of *Musa* plantlets, (bananas and plantains) are presently being screened for somaclonal variation.

16. *In vitro* Regeneration of Multiple Shoots and Somatic Embryos in *Brassica campestris* L.
H.K. Cheema, N. Sood, Dept. of Botany, Punjab University, Chandigarh, India

Investigations were undertaken to obtain plant regeneration from *in vitro* cultured organ explants of *Brassica campestris* by somatic embryogenesis. The results showed that multiple shoots and roots were produced directly in excised stem and leaf explants of *B. Campestris* var. Sarson without callus formation. Multiple shoots and roots were regenerated on Murashige and Skoog's medium (MS) without hormones or supplemented with NAA, and with kinetin. In addition to multiple shoots, green nodular structures were also produced near the basal cut end. On root and hypocotyl segments only roots were produced. The induction of embryogenic callus occurred on MS medium with 2,4-D. Globular and heart-shaped embryos were observed on cotyledon and bud callus grown on MS medium with 2,4-D.

Second IPBNet Conference To Be Held

The Second Annual Conference of the International Plant Biotechnology Network, co-sponsored by the Tissue Culture for Crops Project and Chulalongkorn University, will be held in Bangkok, Thailand, January 11-16, 1987. The Conference, entitled "Biotechnology in the Development of Stress-Tolerant Food Crops", will consist of four sessions: 1) Anther Culture and Somatic Embryogenesis, 2) Meristem Culture, 3) Gene Movement and Protoplast Technologies, and 4) Rapid Propagation.

Those planning to attend should send registration and abstract to the TCCP by September 1, 1986. For registration materials or further information, contact: Ms. Julie L. Fischer, Operations Director, Tissue Culture for Crops Project, Colorado State University, Fort Collins, CO 80523, USA.
TWX No. 9109309011 CSU CID FTCN Attn. Botany 1813

Training Continues At The TCCP

On March 3, 1986, the second session of the Tissue Culture Training Program began, hosting six participants from five countries. Each student has embarked on a special research project, applying the *in vitro* techniques they have learned to the crop of their choice.

Ms. Olivia Damasco (Philippines) is studying wheat anther culture, while Ms. Merlina Dionzon (Philippines) and Ms. Mussarat Raza (Pakistan) conduct studies on regeneration of rice and *in vitro* selection for salt-tolerant rice, respectively. Ms. Abeer Hassan (Jordan) is investigating meristem culture and virus indexing of potato, while Mr. Takaniko Ruabete (Fiji) and Mr. Simon Ndirangu (Kenya) study meristem culture of sweet potato and *in vitro* propagation of tea, respectively.

Applications are now being accepted for the Spring 1987 Training Program, commencing March 2, 1987. For further information, contact: Training Coordinator, Tissue Culture for Crops Project, Colorado State University, Fort Collins, CO 80523, USA.

Spring 1986 Training class. (left to right) Mussarat Raza, Abeer Hassan, Simon Ndirangu, Olivia Damasco, Takaniko Ruabete, Merlina Dionzon.

Recent Publications From The TCCP

Gamborg, O.L. Cells, protoplasts and plant regeneration in culture. In: Manual of industrial microbiology and biotechnology. A.L. Demain and N.A. Solomon (Eds.) Amer. Soc. Microbiol. Washington, D.C. pp. 263-273 (1986).

Gamborg, O.L., R.E.B. Ketchum and M.W. Nabors. Tissue culture and cell biotechnology for increased salt tolerance in crop plants. Proceedings of Biosaline Research Workshop, Karachi, Pakistan (1986).

Gamborg, O.L. and M.W. Nabors. Plant tissue culture biotechnology network for Thailand and support towards identified tissue culture projects, plant species, goals and locations in Thailand. National Center for Genetic Engineering and Biotechnology. Ministry of Science and Technology Thailand. BOSTID National Academy of Sciences, Washington, D.C. USAID/ Thailand. 67 pp. (1986).

Ghazi, T.D., H.V. Cheema and M.W. Nabors. Somatic embryogenesis and plant regeneration from two morphological types of embryogenic callus of soybean *(Glycine max* L.). In Press, Plant Cell Reports (1986).

The International Plant Biotechnology Network; Tissue Culture for Crops Project, Newsletter No. 5. J.L. Fischer (Ed.). Department of Botany, Colorado State University (1986).

MacKinnon, Christy, Glenys Gunderson and M.W. Nabors. High efficiency plant regeneration by somatic embryogenesis from callus of mature embryo explants of bread wheat *(Triticum aestivum)* and grain sorghum *(Sorghum bicolor).* In Press (1986).

MacKinnon, Christy, Glenys Gunderson and M.W. Nabors. Plant regeneration by somatic embryogenesis from callus cultures of sweet sorghum. In Press, Plant Cell Reports (1986).

MacKinnon, Christy, Glenys Gunderson, Kathleen M. Petersen and M.W. Nabors. Plant regeneration from salt-stressed sorghum cultures. In Press (1986).

Recent Visitors To The TCCP Facilities

Dr. Robert Conger	University of Tennessee, Knoxville, USA
Mr. Faraj	INRA, Rabat, Morocco
Mr. Marco Giacchero	FIAT, Turin, Italy
Dr. Don Hague	University of Oregon, Eugene, USA
Mr. Kacemi	INRA, Morocco
Dr. Albert Linck	Colorado State University, Fort Collins, USA
Mr. M. Mergoum	INRA, Morocco
Ms. Jill Tiffin	University of Zimbabwe, Harare
Dr. Joe Vaughan	Central State University, Edmond, Oklahoma, USA
Mr. Wu Yong Xiang	Northeast Agricultural College, Heilongjiang, P.R. of China
Mr. Zong-xiu Sun	China National Research Institute, Hangzhou, P.R. of China

Places Visited, Contacts Made: January - June 1986

Country	Organization
Bangladesh	Bangladesh Agricultural Research Council Bangladesh Jute Research Institute Bangladesh Rice Research Institute Dhaka University USAID/Dhaka
India	Indian Agricultural Research Institute Indian Council on Agricultural Research International Crops Research Institute for the Semi-Arid Tropics USAID/Delhi
Indonesia	Bogor Agricultural University Lembang Horticultural Research Institute USAID/Jakarta
Pakistan	Agricultural University, Faisalabad National Agricultural Research Center Nuclear Institute for Agriculture and Biology Pakistan Agricultural Research Council USAID/Islamabad
Philippines	International Rice Research Institute University of the Philippines, Los Baños USAID/Manila Visayas State College of Agriculture
Thailand	Chiang Mai University Chulalongkorn University Kasetsart University Khon Kaeu University Mahido University National Center for Genetic Engineering Prince of Songkla University USAID/Bangkok

For more information, contact: Tissue Culture for Crops Project
Colorado State University
Fort Collins, Colorado 80523, USA

TWX No.: 9109309011 CSU CID FTCN
ATTN Murray Nabors, Botany, 6996

Phone: (303) 491-6996

The International Plant Biotechnology Network

Tissue Culture for Crops Project

Newsletter #7 July 1987

Field Testing of Sorghum in Progress

Regenerated sorghum was field tested in 1986 at the Griffin Experiment Station in collaboration with Dr. Ronny Duncan and the University of Georgia. The R_1 lines resulted from sorghum callus cultures stressed on 0, 9 or 12 g/l NaCl for up to 15 months in culture with seven months on the stress media. Tissue culture methods are described in a recent publication from the TCCP (MacKinnon *et al.*, 1986).

The field plot was separated into a non-stressed environment and a high aluminum soil environment. The field studies were planted in single row plots 6.4 meters long with 91 cm between rows. Check plots consisted of non-regenerated seed from the five cultivars and were represented every ten plots. There were also regenerated check plots which were not stressed in tissue culture and were randomized within the plot. The non-stressed portion of the field test examined the R_1 lines on a limed, sandy-clay loam soil, with a pH of 5.5. Due to unusual drought conditions, there was an acidic subsoil interference which influenced the phenotypic expression; however, general trends were observed (Fig. 1). Uniformity was found within an R_1 line with little variation seen between R_1 lines regenerated on 0, 9 or 12 g/l NaCl. One hundred and twenty-three R_1 lines were selected for comparison on the aluminum field. Non-stressed R_1 lines of all five cultivars were represented; however, only Hegari had lines from 9 and 12 g/l NaCl stress cultures. Entries were planted in sandy-clay loam soil with 60% of the exchangeable cations being aluminum and a pH of 3.9 to 4.0.

Based on Dr. Duncan's rating system, 17 R_1 lines were found to have some degree of tolerance over their non-regenerated checks on the high-aluminum soils (Table 1). Due to the severity of the combined aluminum and drought stress, only four plants from RTx-430 R_1 lines were able to produce seed in the stressed plots (Fig. 2). All lines set seeds in control plots. Under these conditions, somaclonal variation for tolerance was found in three of the five cultivars tested.

Figure 1. *Regenerate Hegari (190) and non-regenerated (191) Hegari in non-stressed environment.*

Based on these preliminary observations, additional field studies will be conducted in 1987 to evaluate the extent of somaclonal variation for high-aluminum soil conditions.

***Field** continued on page 3.*

Figure 2. *The RTx-430 line which set seed in the high aluminum soil has the seed head bagged.*

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Colorado State University

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Department of Botany
College of Natural Sciences
Colorado State University
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Julie L.F. Ketchum, Editor, Operations Director
Murray W. Nabors, Project Director
Oluf L. Gamborg, Associate Director
Gary E. Hanning, Research Coordinator
Sunitha Siriwardana, Training Coordinator
Kerri L. Wright, Network Coordinator
Glen A. Hildreth, Lab Manager
Reagan M. Waskom, Greenhouse Manager

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Chief of Renewable Natural Resources, AID, Dr. Tejpal S. Gill

Cooperating Agencies include:

Colorado State University, Fort Collins, Colorado, USA
Colorado State University, Agricultural Experiment Station, Fort Collins, Colorado, USA

Participating Technical Staff:

Barbara Ashton, Leann Avery, Patricia Avissar, Gwendolyn Bane, Barbara Bucknam, Nancy Chiu, Julie Cotton, Theresa DeMersseman, Fariha Faizi, Susan Ferguson, Cynthia Flannery, Lori Fredrickson, Touba Ghazi-Moore, Hal Gibson, Melody Halpine, David Hewitt, Julie Hewitt, Harrison Hughes, Jeannette Hut, Christa June, Tina Kaven, Deborah Kelly, Larry Klima, Alanna Kulchak, Annette Lavoie, Clark Livingston, Heather Loomis, Ann Low, Alan Martinez, Marcus Murray, Lee Olton, Carol Ostrom, Melinda Paschal, Kathy Petersen, Gregory Pott, Scott Price, Monica Rusk, Stephanie Schaal, Peter Schuerman, Dennis Shippee, Debra Singleton, Kim Spitler-Nabors, Nitschka ter Kuile, David Timm, Michael Thompson, Susan Todd, Callie Vanderbilt, Kristin Vanderbilt, Mark Van Mouwerik, Alan Waring.

Graduate Students:

Francisco Gutierrez, Iteu Hidayat, Isaac John, Raymond Ketchum, Douglas McMurray, Amani Mnayer, Akbar Mohmand, Anna Novero, Poungpet Poonsapaya, Sathish Puthigae, Gaith Sasi, Nanan Widiyanto, Kerri Wright.

Visiting Scientists:

Suresh Kumar, Zong-xiu Sun, Sheila Vijayakumar, Yusuf Zafar.

Trainees:

Carol Rohrmann, Benchamas Silayoi.

For more information, contact:

Tissue Culture for Crops Project
Colorado State University
Fort Collins, Colorado 80523, USA
Phone: 303/491-6996
Telex: 3711418 TISCLT

Philippines Test TCCP Regenerant Rice Lines

In early August 1986, 387 regenerate R_1 lines of Calrose, Giza 159, IR-36, Pokkali and Mahsuri were delivered to Dr. Dely P. Gapasin at the Philippine Council for Agriculture and Resources Research and Development (PCARRD) in Los Baños, Philippines, for use in structured field tests in saline soils. The testing has been part of a collaborative program with Dr. Edwin Javier of the University of the Philippines at Los Baños (UPLB). In November, 58 R_2 lines from Texas seed increases and greenhouse hydroponic tests as well as 60 additional R_1 lines from the TCCP rice regenerate seed inventory were sent to the Philippines. The R_1 lines were increased (Fig. 3) and tested as R_2 lines in saline soils. Somaclonal varieties were observed in the R_1 lines (Fig. 4). The R_2 seed which was sent was evaluated on saline soil (Fig. 5).

Based on visual observations after six weeks on salt, several R_2 lines looked promising either in comparison to their non-regenerated check or by having outstanding individuals within the R_2 lines (Table 2). R_2 lines from the Texas

Figure 3. *R_1 seed increase at IRRI, March 1987.*

Figure 4. *Somaclonal variation for tiller number and growth habit in Pokkali at IRRI.*

field increase — six of 14 Calrose, five of 19 Giza 159, and one of four Mahsuri lines — looked promising. After selecting in a salt hydroponics, two of seven Giza 159 lines and four of nine IR-36 lines showed promise. From these preliminary results, the higher levels of salt stress *in vitro* — both salt level and length of time — will lead to increased number of R_3 lines which perform better than the non-regenerated checks.

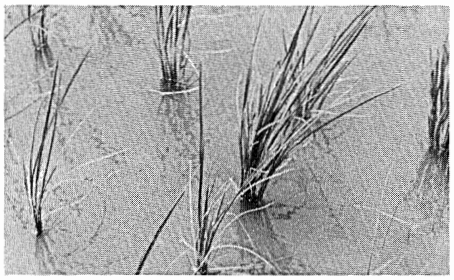


Figure 5. *R_2 lines of Calrose in salt evaluations.*

Table 2. *R_2 lines in salt evaluation trial in the Philippines*

Cultivar	Salt Stress (g/l)	Total Passage	Number of Lines: Sent	Number of Lines: Promising*
Seed from Texas				
Seed Increase				
Calrose	0	9	1	0
		11	1	0
	3	6	2	0
		7	4	3
		8	3	1
		7	3	2
Giza 159	0	17	1	1
	6	8	4	2
		9	3	1
		10	7	1
		11	2	1
	8	10	2	1
IR-36	0	8	1	0
	6	5	3	0
		12	1	0
	9	5	1	0
Mahsuri	0	9	1	0
	6	3	1	1
	8	3	2	0
Seed from Hydroponic Test				
Giza 159	8	10	7	2
IR-36	9	5	9	4

*Number of lines which had outstanding individuals or performed better than the non-regenerate check.

***Field** continued from page 1.*

Table 1. *R_1 lines planted on high aluminum soils in Georgia during the 1986 growing season.*

R_1 line(a)	Number of lines planted	Number of lines with tolerance	% of lines planted
Hegari	17	2	12
Hegari (9 g/l)	48	8	17
Hegari (12 g/l)	8	1	13
Keller	3	0	0
Rio	22	3	14
RTx-430	18	3(b)	17
RTx-7000	7	0	0

(a) R_1 lines were regenerated from non-stressed culture unless noted
(b) one line set seed.

Spring Wheat Tissue Culture

Tissue culture methodology has been developed for spring wheat (*Triticum aestivum* L.) cv. Pavon (MacKinnon *et al.*, 1986). Callus cultures were initiated from mature embryos on LS medium supplemented with 2 mg/l 2,4-D, vitamins, 2% sucrose and 1 g/l agar. Cultures were kept in a controlled environment under continuous light at 15 to 25°C and transferred to fresh media every four weeks. Embryogenic callus was identified within four passages and thereafter selected for transfer to fresh media. For plant regeneration, calli were moved to LS medium with 0.1 mg/l IAA plus 0.5 mg/l BAP for four to six weeks. The calli produced small plantlets with roots within seven days. Regenerated plants were transplanted into soil and grown to maturity in the greenhouse.

The Project now has focused on different types of selection methods to optimize the *in vitro* selection for NaCl tolerance. In this study, two methods of salt selection were used. In the first experiment, embryos were initiated on medium containing 0, 3, 6 or 9 g/l NaCl (Fig. 6). Embryogenic callus was maintained for up to eight passages on the salt stress and a portion of the callus moved to regeneration media starting after the fifth passage. In the second experiment, embryos were initiated and maintained on medium without NaCl for four passages; E callus was then moved to media containing 6 g/l NaCl. Every other passage, the salt level was increased 1 g/l until it reached 10 g/l. The E callus was weighed after each passage. Some callus was moved to regeneration media after the eighth passage. On medium without NaCl, about 90% of mature embryos plated will form E callus within 8 to 12 weeks. On medium containing NaCl, 25% of the embryos produced E callus in the same period of time. The number of plants regenerated decreased over time, but the number of plants per gram of tissue remained steady between passages five to eight on the control cultures (Table 3). The number of plants per gram and the number of plants regenerated varies in the stress cultures (Fig. 7). No plants were obtained from the 9 g/l NaCl cultures. In the stepwise selection, the number of plants per gram and the number of plants regenerated from non-stressed cultures decline from 8 to

***Wheat** continued on page 4.*

Wheat continued from page 3.

12 passages and these values vary on stress media (Table 4). Plants were obtained from 9 and 10 g/l stress cultures.

In the past year, over 2200 plants have been regenerated from cultures containing NaCl and over 1640 from control cultures. As of November, 1986, R_1 seed from 116 lines had been sent to Dr. A. Mujeeb-Kazi at CIMMYT for cytogenetic analysis and salt tolerance screening in Mexico and Pakistan. More seed will be sent as the plants mature.

Figure 7. *Plants regenerated from 0, 3, and 6 g/l NaCl.*

Table 3. *Plants regenerated from Pavon tissue cultures after 5 to 8 passages on NaCl stress media.*

Passage	Concentration Of NaCl	# Cultures on stress & nonstress	# Cultures moved to regeneration	Plants/gram of callus	Total # plants regenerated
5	0 g/l	14	7	88.13	141
	3	16	8	88	35
	6	25	12	145	34
	9	1	1	0	0
6	0 g/l	7	3	80	104
	3	8	4	166.6	50
	6	13	6	210	31
	9	0	0	0	0
7	0 g/l	4	2	95	19
	3	4	2	60	12
	6	7	3	30	3
	9	—	—	—	—
8	0 g/l	2	2	80	24
	3	2	2	20	2
	6	4	4	20	3
	9	—	—	—	—

Table 4. *Regenerated Pavon from the stepwise selection method.*

Passage	Concentration Of NaCl	# Cultures on stress & nonstress	# Cultures moved to regeneration	Plants/gram of callus	Total # plants regenerated
8	0 g/l	16	7	212	145
	7 g/l	50	26	94	176
10	0 g/l	9	3	227	54
	8 g/l	26	13	163	81
12	0 g/l	6	3	95	19
	9 g/l	13	6	16.6	3
14	0 g/l	3	3	35	7
	10 g/l	7	7	40	10

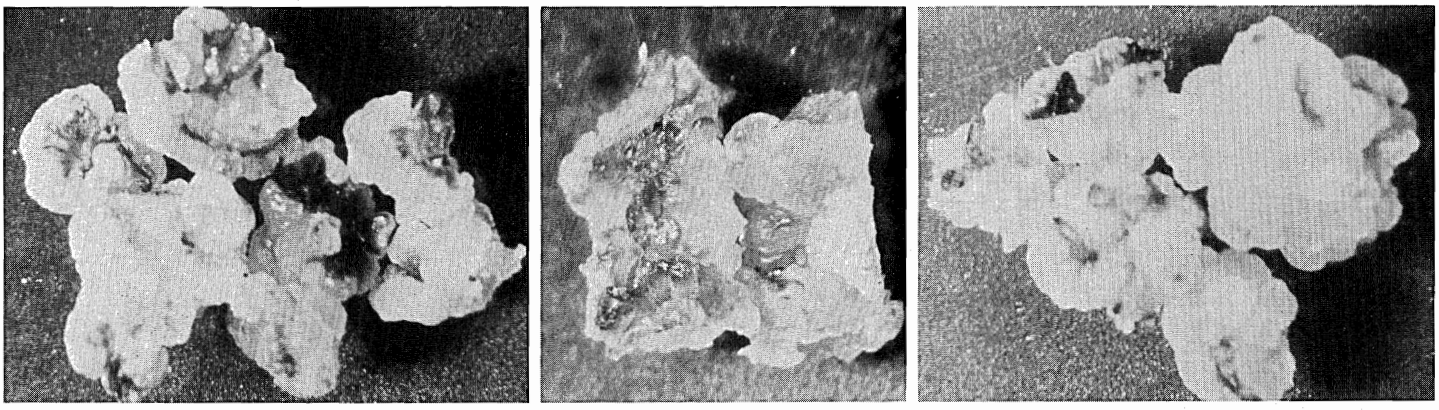


Figure 6. *E callus of Pavon growing on 0, 3, and 9 g/l NaCl at the end of second passage.*

Plants From Suspension Cultures of Drought-Tolerant Grain Legumes

Plant-regenerating, embryogenic cell suspension cultures are efficient systems for selection and isolation of somaclonal variants. Such suspension cultures are usually difficult to obtain, but have been successfully established in two species; (1) tepary bean *(Phaseolus acutifolius)*, and (2) moth bean *(Vigna aconitifolia)*. Both of these grain legumes are exceptionally drought-tolerant. The research in progress is designed to determine the level of salt tolerance of cultured cells of these two species. A salt-tolerant line obtained from these species could be used as sources of stress tolerance in important grain legume species.

The successful development of a plant regenerating suspension culture is the first report of such a system in a major grain legume. The suspension cultures were established from a callus produced from seedling leaf tissues in June 1986. Embryo-like structures were observed in two-month-old suspensions on modified MS medium (LM medium) with 10.0 mg different pore sizes (500, 250, 125 and 60 microns) for four to five subcultures to remove larger callus and single vacuolated and dead cells. The procedures resulted in the selection of fast growing cell lines with approximately twenty-eight and thirty-six hours doubling time of *Vigna* and *Phaseolus* spp. respectively.

Moth beans (VA-686) are now being maintained in LM medium with 1 mg/l of 2,4-D and tepary beans (TB-686) in LM with 2.25 mg/l of BAP. Green, embryo-like structures are formed when 2,4-D cultured cells of moth bean were transferred to LM medium with 1 mg while similar structures were observed from tepary beans on maintenance medium. Seventy to eighty percent of these structures produced somatic embryos when plated on LM agar medium with low concentration of BAP and GA_3. Continued culture of these embryos resulted in complete plant regeneration. A total of 147 moth bean and 56 tepary bean plants have been regenerated and are being grown to maturity.

Experiments are in progress to develop salt-tolerant cell lines of *Vigna aconitifolia* and *Phaseolus acutifolius* to produce embryogenic cell suspension cultures of major grain legumes such as pigeonpea, cowpea and dry bean.

Selection for Drought Tolerance in Winter Wheat

An investigation on improving the drought tolerance of Colorado winter wheat cultivars by tissue culture techniques, funded by the the Colorado State University Agricultural Research Station, was initiated at TCCP in 1986. The research has focused on developing regeneration techniques and using *in vitro* selection to develop drought-tolerant lines. Regenerated plants will be established in the greenhouse and screened for drought tolerance; selected germplasm will then be field tested. Five cultivars of importance to Colorado, Baca, Hawk, Vona, Tam 101 and Bennett were recommended by Dr. J. Quick, wheat breeder, Colorado State University, and are being screened for their regeneration potential. Mature embryos are being used as the explant source for callus induction. LS media supplemented with various concentrations of 2,4-D in the presence or absence of KIN or BAP were used as the initiation medium. The cultures were incubated in continuous illumination of approximately 2000 lux, and at 26 ± 1°C for passage length of six weeks.

Preliminary results indicate that responses observed are cultivar specific. In general lower concentrations of 2,4-D (1 mg/l) induce more callus (Fig. 8), but the cultures are very rooty. Of the levels tested, 2 mg/l 2,4-D was optimal. Cytokinins did not give significant increases in callus production except where BAP (Fig. 9) or KIN (Fig. 10) showed beneficial effects on Vona and Hawk, respectively.

Embryogenic callus has been identified for the winter wheat cultivars under investigation (Fig. 11). Its appearance is similar to that found in spring wheat cultivars and is smooth, compact, and pale yellow to cream in color. Differentiation can be observed as early as the first subculture. The ratio of E to NE callus increases when subcultured three times on media containing 2,4-D alone. At the same time, E callus begins to differentiate further, and green clefted areas, termed "embryoids" appear (Fig. 12). On 1 mg/l 2,4-D, the embryoids begin to develop into green shoots even without a change in the medium. The E callus, or embryoids, on 2 and 5 mg/l 2,4-D can be maintained for three subcultures with little or no differentiation. All five cultivars have been regenerated successfully to date, for a total of 1234 plants (Fig. 13). With the establishment of reliable methods for regeneration, we are now able to launch the *in vitro* screening phase of the experiment is ready to begin.

***Selection** continued on page 6.*

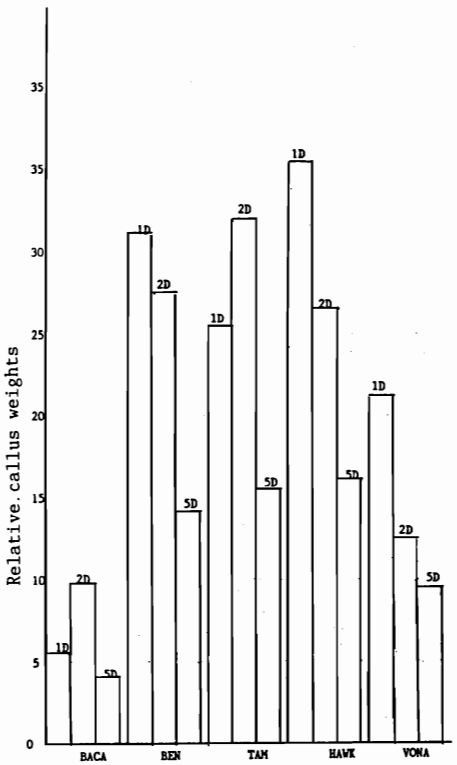


Figure 8. *Effect of 2,4-D on callus formation from mature embryos of five cultivars of winter wheat*

***Selection** continued from page 5.*

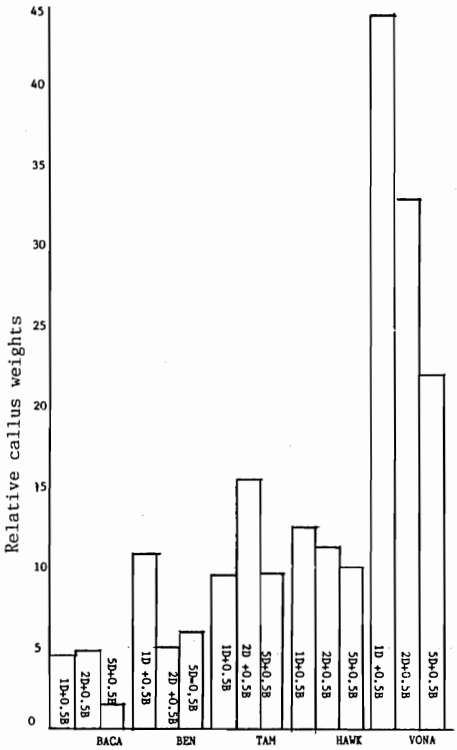


Figure 9. *Effect of BAP on callus formation from mature embryos of winter wheat*

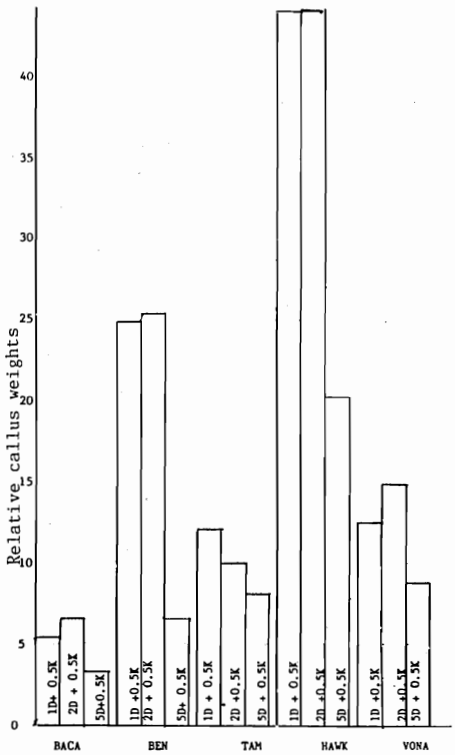


Figure 10. *Effect of KIN on callus induction from mature embryos of winter wheat*

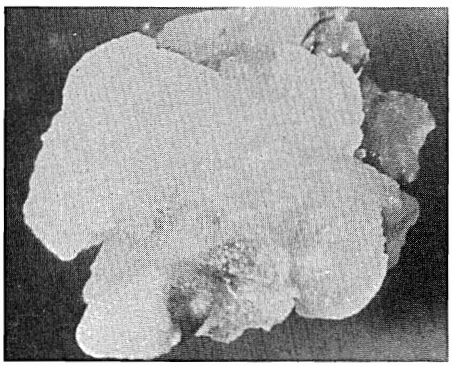


Figure 11. *Embryogenic callus (E) of Hawk*

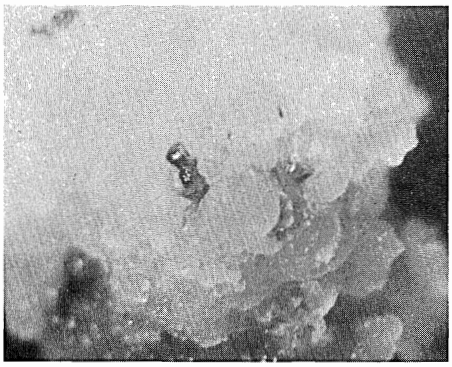


Figure 12. *Embryoid-like structures (EMB) surrounded by E callus*

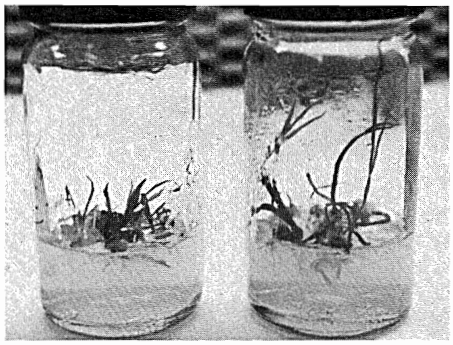


Figure 13. *Regenerated plants of winter wheat. The medium used contained IAA and BAP*

Screening for Salt Tolerant Cell Lines in Pigeonpea

Pigeonpea is a major grain legume of semi-arid tropics and constitutes a principal source of proteins in predominantly vegetarian diets. Although pigeonpeas have a wider adaptability to different climates than many other grain legumes, they were found to be sensitive to saline conditions (Joshi and Nimbalker, 1983). Development of salt-tolerant pigeonpeas holds great promise for extending the cultivation to salt-affected soils thus increasing the production *per se*, of this crop. The research program on pigeonpeas at TCCP was set-up to develop efficient plant regeneration methods by somatic embryogenesis and to produce salt-tolerant germplasm by *in vitro* screening and widecross hybridization methods.

A systematic approach has been undertaken to produce E callus and to achieve high-frequency plant regeneration from long-term cultures of pigeonpea. After experimenting with a wide range of media compositions, LM medium (Kumar *et al.*, in preparation) was found to be the most effective for producing somatic embryos on the surface of cotyledons. A green, shiny callus grew from excised cotyledons on LM medium with BAP. Several embryos with distinct hypocotyl, radicle and cotyledons were developed on the surface of this callus. These embryos were grown in culture and developed into complete plantlets on LM basal medium at half strength.

Since regeneration of the plantlets from long-term cultures is possible, *in vitro* selection and screening for salt-tolerant cell lines is in progress. The strategies for the selection of stress-tolerant cell lines have resulted in the regeneration of plantlets from the callus grown on 0.5, 0.75 and 1.0% NaCl. Plantlets are being grown to maturity in the greenhouse. Seeds from these plants will be screened in fields to determine the efficiency of *in vitro* screening. Experiments are in progress to isolate the cell lines which can tolerate prolonged culture at higher salinity levels.

Plants of related genera (*Atylosia scarabaeiodes, A. albicans* etc.) grow well in stress environments as compared with pigeonpeas. Attempts are being made to produce inter-generic hybrids with different degrees of tolerance to salt and drought. A reliable

method has been developed to produce the *Atylosia* x *Cajanus* inter-generic hybrids. The method uses a hormone treatment of the pollinated buds to prolong the bud retention. After fertilization, the embryos are excised and cultured for hybrid plant recovery. Several hybrid plants of the cross between ICP-7182 and *A. scarabaeiodes* and *A. platycarpa* have been produced. Experiments are in progress to produce more hybrids and to extend the embryo culture technique to other species of *Atylosia.*

Salt-Tolerant Rice through Protoplast Fusion

A collaborative project between the Tissue Culture for Crops Project (TCCP), and the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan, was set-up for the purpose of obtaining salt-tolerant Basmati rice *(Oryza sativa)* through protoplast fusion. Basmati is unmatched for its cooking quality, size, fineness, and unique aroma and flavor. It is second in importance to Pakistan as an export commodity and a source of foreign exchange.

Because of the rapid increase in accumulation of salts in irrigated lands of Pakistan and the relative sensitivity of Basmati to saline conditions, the development of salt-tolerant Basmati is very important. Protoplast and tissue culture techniques can be employed to produce hybrids of sexually incompatible plants. The plant selected for fusion with Basmati is Kallar grass *(Leptochloa fusca).* Kallar grass is a well-studied halophyte and is grown on large areas of otherwise unproductive saline soil as a green fodder for livestock.

The technology for isolating, culturing and fusing protoplasts in both these plants is being established at the TCCP. The initial experiments were set-up to demonstrate the plant regeneration capability in tissue cultures of Basmati rice and Kallar grass. Excised mature embryos of rice and grass seeds were used as explant sources. Each plant type has a specific nutrient requirement for the support of callusing and plant regeneration. Several media (N6, B5, MS) were tested with different combinations of auxins and cytokinins. For Basmati rice, the N6 medium with 4.0 mg/l NAA, 0.5 mg/l D and 2.0 mg/l BAP was found to be superior for callusing. Plant regeneration occurred in the same medium with 0.5 mg/l NAA and 2.0 mg/l BAP.

For Kallar grass, the B5 medium with 2.0 mg/l 2,4-D was found to be the most suitable. The N6 medium failed to support callus formation, but growth was observed on Blaydes, MS, Modified MS, and Schenk & Hildebrandt media. Regeneration was achieved in Kallar grass in B5 and MS media with 0.5 mg/l NAA and 2.0 mg/l BAP.

After establishing the conditions for regeneration, work began on establishing cell suspension cultures, since they would be the source of protoplasts. Chopped materials of friable callus, roots, and shoots of germinating seedlings were used as the starting material. An appropriate amount was placed in B5-1 (+ 500 mg/l casein hydrolysate), N6, MS and a B5-organic nitrogen medium (B5-AA) either alone or in combination (1:1) with the protoplast culture medium, without osmoticum. Cultures were placed in 125 ml Erlenmeyer flasks and kept on gyrarotary shakers at 100 rpm in continuous light at 22-24°C. Media were replaced every two weeks without reducing the cell densities. After four months of manipulations, a cell-line of Basmati rice (RB-187) was established. The B5-AA medium in combination with PCM was found to sustain the growth of cells in liquid media. Experiments are in progress to determine the plant regeneration ability in different media as well as the growth rates under various culture conditions.

The work on establishing the cell-line of Kallar grass was initiated from the chopped seedlings in the liquid media of AA, B5, MS, N6, and Modified MS alone or in combination with PCM. Further procedures are the same as described for Basmati rice.

Attempts are now being made to define the conditions for isolation, culture and plant regeneration from protoplasts of Basmati rice and Kallar grass. The source materials are the established cell suspension cultures. Current research involves evaluation of the different steps to obtain viable protoplasts with capability for sustained division and plant regeneration. The protoplast technology will then be utilized in the fusion experiments for the production of the Basmati rice-Kallar grass hybrids.

Research Reports from Network Members

Principal Investigator:
Dr. A. Mujeeb-Kazi
CIMMYT
Londres 40, Apdo. Postal 6-641
Col. Juarez Deleg. Cuauhtemoc
06600 México, D.F.
México

A collaboration between TCCP and CIMMYT was initiated in September 1985 when TCCP representative Nitschka S. ter Kuile began tissue culture experiments at CIMMYT in association with the wheat widecross program headed by Dr. A. Mujeeb-Kazi. This collaboration has given the TCCP access to the CIMMYT wheat and widecross germplasm and their cytological facilities while CIMMYT has gained access to TCCP technology to increase the speed of widecross gene transfer.

Experiments were started with the following objectives:

1. **Media Manipulation**
   Media manipulations were conducted for long-term callus cultures and regeneration potential of tetraploid and hexaploid wheat cultivars. The germplasm utilized was that which the widecross group used in F_1 hybrid production and in advanced backcross derivative.

2. **Alien Genetic Transfers**
   Inducing alien transfers in wheat/alien species hybrids in which recombination does not occur in F_1 hybrids. The study utilized an alien source *(Aegilops variabilis)* that was aluminum-tolerant, amenable to N-banding, and which possessed superior resistance levels to karnal bunt. This aspect shall be reported later.

This report provides results of the media manipulation for long-term callus cultures of tetraploid and hexaploid wheat cultivars. Five *Triticum aestivum* (2n=6x=42) cultivars were incorporated in the study. A similar number of *T. turgidum* (2n=4x=28) cultivars were used. The callus from both *Triticum* spp. was maintained until the seventh, one month passage. At each passage, some E callus (Fig. 14a) was

***Research** continued on page 8.*

***Research** continued from page 7.*

regenerated and plants were transplanted in the greenhouse. The greenhouse conditions were set at 22°C/14°C day/night temperatures, 15 hours natural light and approximately 55 to 60 percent relative humidity. Bread wheats *(T. aestivum)* were more prolific in producing E callus (Fig. 14b) and possessed a higher number of regenerated plants (370) than durum wheats *(T. turgidum)* (82) (Table 5). Figures 14b and 14c show the early and advanced regeneration stages for *T. aestivum.* There were several spike developmental abnormalities (Figs. 15a to d) that at the R_0 stage may well be transient changes. The heritable changes will be analyzed in the advanced generations.

Somatic root tip cytology was done on a limited number of plants of both wheat groups (255 for bread wheat and 45 for durum wheat) with some spikes being randomly sampled for meiotic analyses. Somatic root-tip count data predominantly expressed counts of 42 for *T. aestivum* and 28 for *T. turgidum* (Figs. 16a and b). The range for *T. aestivum* chromosome number was from 39 to 45 and from 28 to 56 for *T. turgidum* with one to two telocentric chromosomes. There was a single *T. turgidum* plant with 56 chromosomes (Fig. 14d) attributed to spontaneous doubling. The plant was male sterile but female fertile and has set seed upon backcrossing to *T. turgidum.* The meiotic analyses of a few 42 and 28 chromosome plants provided evidence of cytological variations where apart from the normal bivalent formation, meiocytes possessed several univalents, trivalents and quadrivalents. Consequently all R_0 derivatives shall be advanced as there exists the potential of selecting variants in advanced generations because of the observed intrinsic R_0 meiotic changes.

Seeds of the R_0 plants have been cut into an endosperm half and the embryo portion. The latter material is being advanced one generation under greenhouse conditions whereas the corresponding endosperm portion has formed the base for electrophoretic analyses.

To keep up with the widecross program needs, five new bread wheats have also been callused: CIANO 79, Seri, Buc "S," Buc "S"/Pvn"S," Dove"S"/Buc"S" and four durum wheats, Laru"S," CNDO/R143/ Ente"S"/Mexi"S," Memo"S"/Mexi"S," Rok"S"/Kmli"S".

More emphasis shall be given to meiotic analyses when regenerants are available from this study.

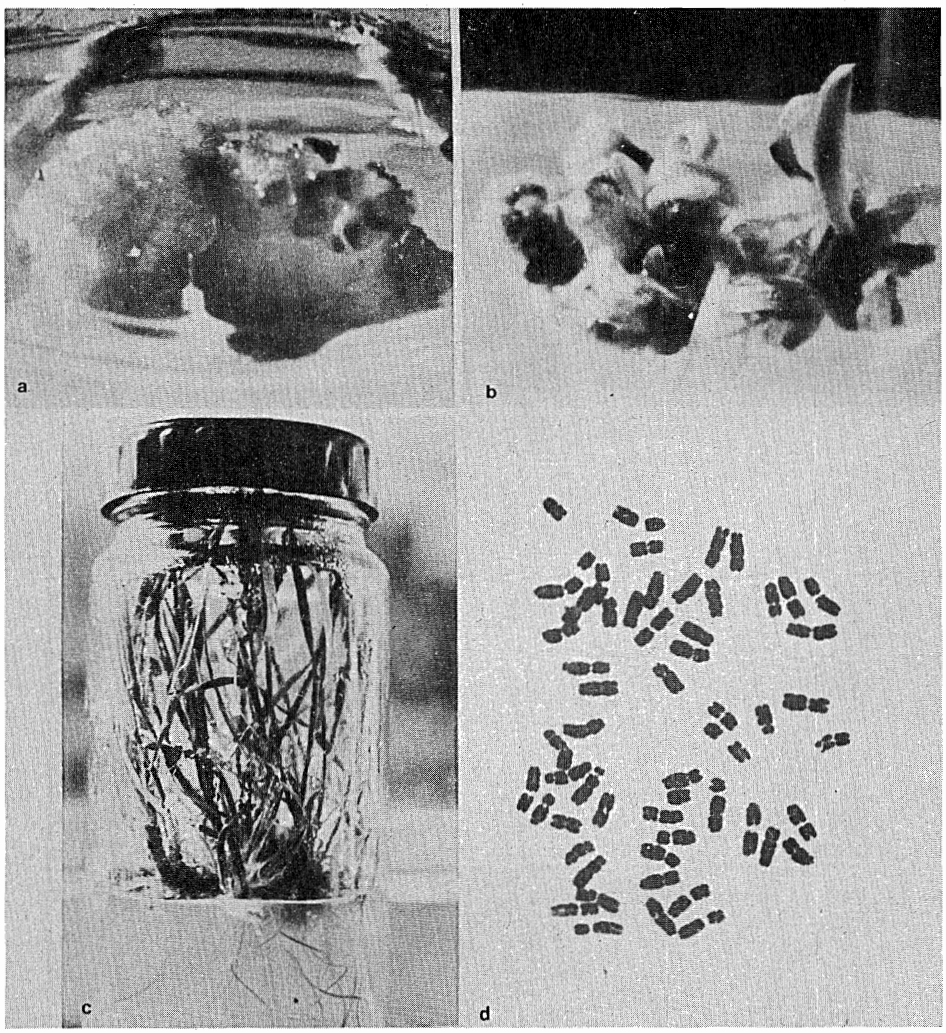


Fig. 14. (a) *Embryogenic callus formation in* Triticum aestivum *cultures* **(b)** *Early regeneration in* T. aestivum *cultures* **(c)** *Advanced regeneration in* T. aestivum **(d)** *A spontaneous doubled (2n=8x=56)* T. turgidum *somatic cell*

Table 5. *Regenerated* Triticum aestivum *and* T. turgidum *plant data from callus maintained up to seven months.*

	Months in callus					
Varieties	**3**	**4**	**5**	**6**	**7**	**Total**
	Number of Plants Regenerated					
BREAD WHEATS						
MRL"S"/BUC"S"	21	33	26	6	0	86
BOW"S"	1	17	12	0	0	30
ALD"S"/PVN"S"	24	36	21	15	11	107
GH"S"	30	68	33	6	7	144
PAVON	0	0	3	0	0	3
					Total	370
DURUM WHEATS						
CHEN"S"	0	8	0	0	0	8
LARU"S"	12	12	4	10	0	38
CNDO/R143/ /ENTE"S"/ MEXI"S"	9	0	0	0	0	9
MEMO"S"/MEXI"S"	22	3	0	0	0	25
ROK"S"/KMLI"S"	2	0	0	0	0	2
					Total	82
					Grand Total	452

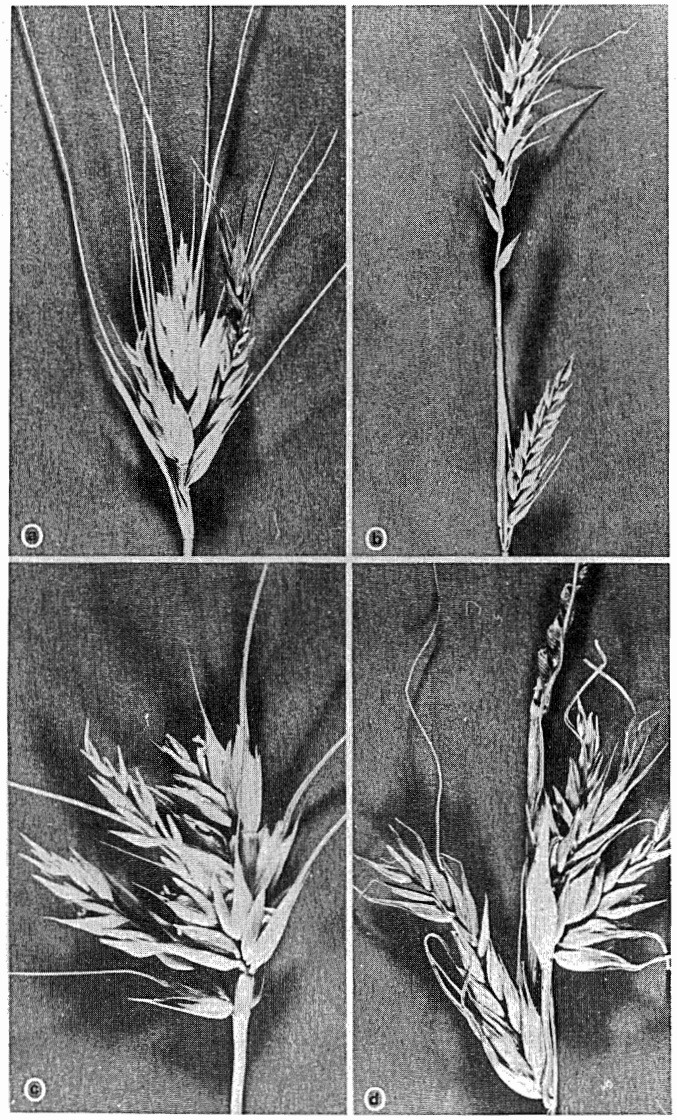


Fig. 15. (a) to (d) *Spike morphology variations in R_0 plants of* Triticum aestivum

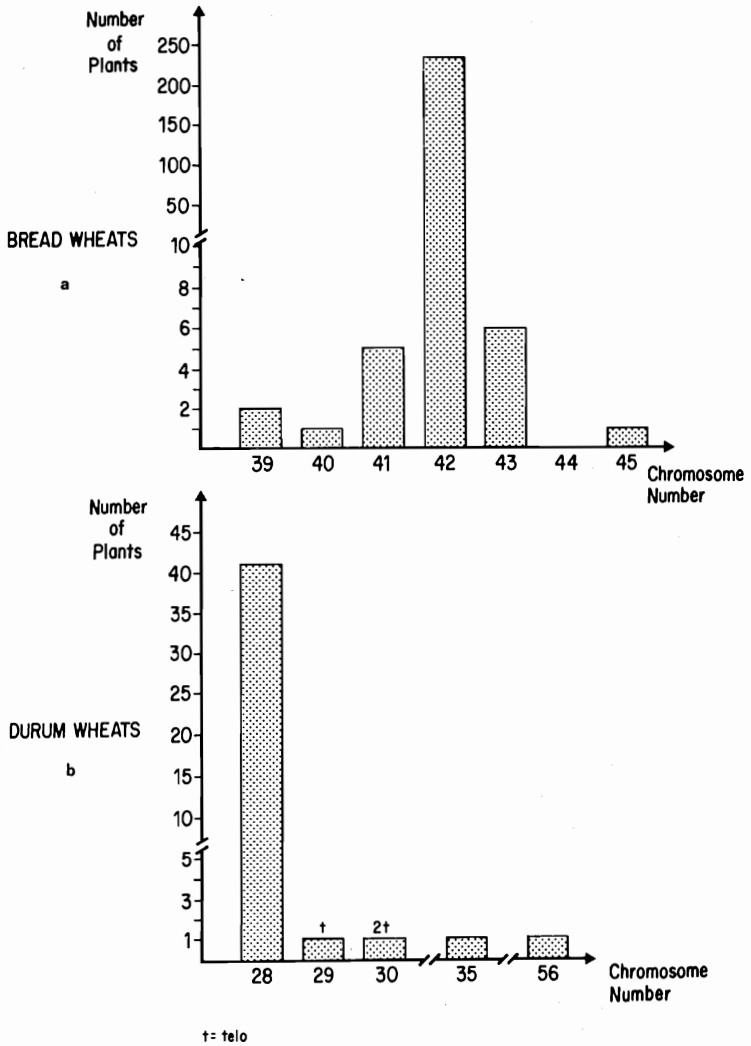


Fig. 16. (a) *Number of* Triticum aestivum *regenerated plants and their somatic chromosome numbers* **(b)** *Number of* T. turgidum *regenerated plants and their somatic chromosome number (t=telocentric chromosomes)*

Principal Investigator:
Dr. Pisit Voraurai
Department of Horticulture
Chiang Mai University
Chiang Mai 50002
Thailand

Dr. Pisit Voraurai, Department of Horticulture, Chiang Mai University, Thailand, is Director of the King's Initiative Project for the Lowlands of Northern Thailand. This article was submitted at the request of the TCCP staff.

Higher education institutions, especially universities in Thailand, have four functions, (1) teaching, (2) carrying out research, (3) providing academic services to communities, and (4) conserving the Thai culture. In these areas, the task of training graduates is considered a main role.

In order to produce graduates for a small country like Thailand, certain types of students are needed for country development. Besides "good brain," they must also have "good hand," and "good spirit," willing to help others who are poorer. It would be a great pity if one had the most clever graduate who was willing to work only for his/her own benefit. One way to build-up such "good spirit" in the graduates is to let them mix with villagers in the rural areas and allow them to learn the needs of and challenges facing a rural community. These first-hand encounters and learning experiences serve to provide the foundations for effective rural development programs.

A group of staff from Chiang Mai University (CMU), Thailand, are working on a project which blends tissue culture research with various rural development strategies. His Majesty The King's Initiative Project was started in 1980 and is now serving more than 70 villages with plant propagules and the appropriate technology transfer. The original research focused on several varieties of fruit trees, propagated through *in vivo* techniques, raised in a nursery, and then transferred to the various outlying villages.

Plant Propagation Centers were established in the identified target villages, and trees were planted in the backyards of the village participants. Later, group land was also established on the basis of cooperative ownership. The starting plant materials came from good varieties outside the villages. Guava, mango, pomelo, jackfruit and sapote were first used. Later, the staff of the tissue culture lab also introduced prop-

***Research** continued on page 10.*

***Research** continued from page 9.*

agated varieties of carnation as well as chrysanthemum for groups of the village youth to further propagate for cash crop purposes.

During the periods when the villagers are working in groups on their common land, a group of both students and university staff stay in the village, especially during field training. Through this, the group from CMU has the opportunity to mix and exchange opinions with the rural people, and to help train the villagers in special techniques such as mango grafting.

During the past seven years, we have had the opportunity to contact the rural communities, learn more about them, and contribute to effective technology transfer. Up to one million guava and mango plants have been distributed to the villagers' group. During the 1986 growing season, the villager's group produced more than 27 million gladiolus corms and cormels. This has successfully brought the participants from bare subsistence to cash cropping systems.

At this stage, our work has progressed to training the village youth in higher technologies by working with graduates from vocational schools and university graduates at the ratio of 25:3:2, respectively.

Principal Investigator:
Dr. M.C. Rush
Department of Plant Pathology and Crop Physiology
Louisiana State University
Baton Rouge, Louisiana 70803-1720 U.S.A.

In October, 1986, Dr. M.C. Rush entered into a sub-agreement with the TCCP to perform research on development of somatic cell culture systems for rice improvement.

Results to Date:

Objective 1: Improvement of existing somaculture systems

Comparative studies were conducted on the effects of explant source on callus formation and plant regeneration in rice. Explant sources tested included root tips, mesocotyl, mature embryos, endosperm, nodes, immature panicles, and panicles near exertion from the boot. Callus was induced from immature panicle explants primarily from floral primordia, but also from the peduncle, rachis, and panicle branches.

The medium used for these experiments was MS with 2 to 4 mg/l 2,4-D in the callus induction medium and either the combination IAA (0.5 mg/l) and BAP (0.8 mg/l) or NAA (2 mg/l) and KIN (1 mg/l) in the plant regeneration medium. The media had 3% sucrose. Explants were incubated in the dark at 28°C for callus induction and in light for plant regeneration.

Research was also conducted on suspension culture of rice cells. Callus derived from root tip explants was used to initiate suspension culture in liquid MS medium containing 0.1 mg/l 2,4-D. The cell suspensions were observed using SEM and light microscopy. Embryos derived from small, round cells were observed at several developmental stages. The most advanced developmental stages of the bipolar embryoids in culture germinated to form roots from coleorhiza. Shoots were not formed. On two occasions plants were formed from callus masses on the bottom of the flask.

When callus from immature panicles was used to initiate the suspension cultures, few embryoids were formed in the suspension culture. Callus masses with green points were formed on the flask bottom. One plant was regenerated by transferring these small calli to solid media. When suspension cells from these cultures were filtered and incorporated into regeneration medium, the cells or small cell clusters divided to form callus and several plants were regenerated. This procedure has great potential for treating cells in suspension with toxins or herbicides and regenerating resistant plants.

Objective 2: Somaclonal variation

Through November 1, 1986, somaclonal variation has been observed in our regenerated plants in the characters leaf blade angle, flag leaf angle, culm angle, leaf color, leaf pubescence, leaf senescence, culm strength, disease resistance, panicle type and size, panicle exertion, sterility, seedling height, mature plant height, sheath color, tiller number, leaf blade length, blade width, days to heading, panicle number, panicle weight, 100-seed weight, grain-type, grain quality, yield, apiculus color, and hull color.

In a yield trial during the 1986 season, the line Labelle 215 out-yielded the parent variety Labelle and the high-yielding commercial variety Lemont. However, grain quality was seriously reduced (head rice) due to breakage of the larger grain during milling. Lemont line #726 had out-yielded Lemont in a 1985 yield test, but yielded less than Lemont in the 1986 test.

Somaclonal variation observed in the field with somaclonal lines from Labelle and Lemont was great during both the 1985 and 1986 growing seasons. However, the cultivars Melrose, Tetep, Taipei 309, and Tadukan showed little or no variation. Somaclones from the cultivar Zenith showed several types of variation when planted in the field in 1986. This included variation in grain-type (changed from medium- to long-grain), hull color (straw-hulled to gold-hulled), height, resistance to sheath blight, and pubescence. It is clear that different rice germplasms show different responses as far as development of variation in somaculture is concerned. The cultivars Tetep and Tadukan have shown no variation to date. *If we had studied only these cultivars we would have concluded that rice does not show somaclonal variation.*

In 1986, we grew about 3000 somaclonal lines in the field. About 1100 lines were grown for the first time. In 1985 we identified two Labelle lines that were resistant to sheath blight. These lines were also resistant in 1986, although one line appeared to be segregating for resistance. In 1986, we identified two more Labelle somaclones and one Zenith somaclone with apparent increased resistance to sheath blight. Many Labelle lines appeared to vary in resistance to the rice blast disease when exposed to natural infection. Some lines appeared more resistant than the parent cultivar and some appeared to be more susceptible. About 30 lines were sent to Dr. M.A. Marchetti at the Texas A&M Rice Research Station in Beaumont, TX for evaluation of their blast resistance under controlled conditions.

Objective 3: Germplasm exchange utilizing embryo culture

Studies were conducted to develop a system to ship rice germplasm internationally without contamination by microorganisms. Areas of interest were the complete disinfection of seeds or embryos and development of a medium/container system that would allow survival of shipped embryos.

Treatment of dehulled seeds or detached embryos with 1000 ppm mercuric chloride for five minutes controlled seedborne microorganisms with the lease phytotoxicity to embryos. The effectiveness of mercuric chloride treatment was increased by pre-treatment of dehulled seeds in 0.5% sodium hypochlorite solution, containing 10 drops of Triton CS-7, at 55°C for 30 minutes. Sterilization of dehulled seeds increased

germination percentage when compared to untreated seeds.

The tissue culture growth media MS, Gamborg B_5, and Chaleff's R_2, containing the basic salts and vitamins but without growth regulators, supported seed or embryo germination and seedling growth. Transplanting survival percentage after 35 days of *in vitro* incubation in the dark was high. Fourteen days of incubation in darkness did not harm seedlings on tissue culture media.

Sterilized, dehulled seeds and detached embryos of the rice cultivars Bellevue, Calrose 76, Lebonnet, and Newbonnet were air shipped to the International Rice Research Institute in the Philippines and the International Institute for Tropical Agriculture in Nigeria. Seedlings from germinated seeds or embryos survived the shipment periods of 36 days to IITA and 11 days to IRRI. One hundred mm, disposable petri plates, containing Chaleff's R_2 medium without growth regulators, supported seedling growth during shipment. The visual detection and elimination of contaminated culture plates on arrival was easily accomplished. The survival rate of germinated seeds or embryos was high during shipment. Techniques developed and improved by this research may prove beneficial for international transfer of microorganism-free plant material as a source of germplasm.

Due to the potential toxicity of mercuric chloride to humans and the potential effect of this toxic compound on rice germplasm, we continued to study other disinfectants for sterilization of rice embryos for shipment in tissue culture media. Several more systems using sodium hypochlorite (Chlorox) were evaluated. None of the methods used gave complete sterilization. We will evaluate other disinfecting compounds in future research.

Articles for the Newsletter are welcome. Submit no more than three, double-spaced, typewritten pages. The Editor reserves the right to make appropriate changes.

Regional Training Center Established at CATIE

In collaboration with the TCCP, Dr. Ludwig Müller has set-up the first regional tissue culture training program at CATIE, Costa Rica. The three-month short course, which began April 23, consists of six weeks of lectures and lab sessions, after which students will carry-out individual research projects of their choice. The course emphasizes tissue culture of tropical crops, and is based on the more lengthy Tissue Culture Training Program at the TCCP. The CATIE course is in Spanish.

The CATIE Training Program currently hosts 20 participants from eight countries in Central and South America; over 50 applications were originally received.

To obtain information and an application for next year's Program, please write:

Dr. Ludwig Müller
Tissue Culture Laboratory
CATIE
7170 Turrialba, Costa Rica

Network Update

Since its inception in early 1985, the International Plant Biotechnology Network (IPBNet) has gained over 1000 affiliated members from 72 developing and 18 developed countries. The TCCP currently has ten signed Memoranda of Agreement (MOA) with institutions in eight countries in Africa, Asia and Latin America including four international centers. These agreements outline the terms of collaboration and include guidelines on exchange of information, field testing, germplasm exchange, technical assistance, training, graduate education, and personnel exchange.

The following provides a synopsis of the individual collaborations and agreements:

In Africa, the signed Memorandum of Agreement is with IAV Hassan, Morocco. Initial contacts have also been made with five other countries in Africa, and potential field testing sites have been identified for drought tolerance testing of sorghum and millet.

In Asia, the majority of MOA's exist in the southeast. In Thailand, collaborations with two universities have been initiated. A graduate student exchange program exists between Colorado State University and Chulalongkorn University. The TCCP hosted the first exchange student in the fall of 1985. In the Philippines, two MOA's have been signed; one with the Philippine Council for Agriculture and Resources Research and Development (PCARRD) and a second with the International Rice

***Network** continued on page 12.*

Graduates of the Fall 1986 session of the Tissue Culture Training Program. Top row (left to right): Renato Avenido, Philippines; Andrew Matibiri, Zimbabwe; Raed Bakheet, Saudi Arabia; Bishamber Gothwal, India. Bottom row (left to right): Ouafae Benlhabib, Morocco; Erlinda Rillo, Philippines.

***Network** continued from page 11.*

Research Institute (IRRI). Collaborations in the Philippines include subagreements for research in rice and corn tissue culture with Dr. N.T. Thanh-Tuyen of Visayas State College of Agriculture (ViSCA), heat tolerance in wheat with Drs. R. Barba and A. Zamora at University of the Philippines at Los Baños (UPLB), and field testing for salt tolerance in rice with Dr. E. Javier, also of UPLB.

Additional agreements in Asia have been established in Pakistan with the Nuclear Institute of Agriculture and Biology (NIAB), and in India with ICRISAT. Both collaborations have resulted in travel of research personnel to the TCCP. The agreement with NIAB consists of collaboration in producing a salt-tolerant rice grass hybrid by protoplast fusion. The agreement with ICRISAT is a collaborative project which applies tissue culture techniques for the improvement of chickpea. Dr. Yusuf Zafar and Ms. Sheila Vijayakumar are visiting scientists working on these respective projects.

In Central America, three MOA's exist with CIMMYT, CIAT and CATIE. The collaboration with CIMMYT involves the development of tissue culture techniques with application to the CIMMYT widecross program on wheat. Dr. A. Mujeeb-Kazi is the Principal Investigator of this project. Since January of 1986, a member of the TCCP staff, Ms. Nitschka S. ter Kuile, has been living and working at CIMMYT to assist with this research. The Project's collaboration with CIAT lies in field testing for acid tolerance in rice with Dr. Robert Zeigler. Finally, the extent of participation and collaboration with CATIE has led to establishment of a regional tissue culture training program, under the direction of Dr. Ludwig Müller, which combines the course material used in the TCCP's Training Program with the necessary crop modification for the tropics and Spanish translation instruction.

In addition to the various internationally focused collaborations, TCCP has agreements with various North American universities. Rice regeneration, field testing and seed increases are being performed by Dr. M.C. Rush at Louisiana State University; sorghum field testing for aluminum tolerance and seed increase are being performed at the University of Georgia by Drs. Ronny Duncan and David Isenhour, by Dr. Dwight Tomes at Pioneer Hi Bred, and at Texas A & M University by Dr. Fred Miller. Salt tolerance field testing of oats is taking place at the University of Saskatchewan, Canada with Dr. Alan McHughen. Drought tolerance field testing of millet is being performed at Kansas State University with Dr. Bill Stegmeir.

Substantial progress has also been made in the areas of training and graduate education. The Training Program has graduated 17 students to date and currently has two additional trainees, Ms. Carol Rohrmann from Guatemala and Ms. Benchamas Silayoi from Thailand, enrolled. Presently, the TCCP is supporting graduate candidates from India, the Philippines, Thailand, and the U.S. and hosts graduate candidates from Indonesia, Jordan, Libya, Mexico, Pakistan and the U.S. — thirteen students total.

The highlighted event this year was the second International IPBNet Conference held in Bangkok, Thailand. More detailed information can be found in the article entitled "TCCP Holds Second IPBNet Conference."

TCCP Holds Second IPBNet Conference

The Second Annual Conference of the International Plant Biotechnology Network was held at the Imperial Hotel in Bangkok, Thailand, January 11-16, 1987. Seventy-nine persons from eighteen countries attended. As expected, the major contingency was from Asia; eight Asian countries were represented with 36 persons from Thailand in attendance.

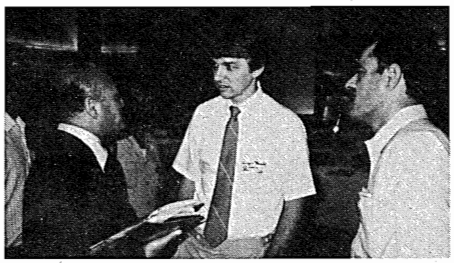
Thirty-one papers and over two dozen posters were presented on topics ranging from tea propagation to induction of smut resistance in sugarcane. In addition, keynote speakers Dr. Roger Beachy, Dr. Paiboolya Gavinlertvatana, and Dr. Colette Nitsch addressed the subjects of gene movement, rapid propagation, and anther culture, respectively. Dr. Kutty Kartha, who was scheduled to speak on meristem culture, was unable to attend due to illness.

IPBNet Conference participants.

An interesting feature of the Conference was a series of training films on *in vitro* techniques, breeding, and mutation induction furnished by IAEA in Vienna. The TCCP is currently working with IAEA to produce a series of films on TCCP techniques.

A Pre-conference book of abstracts is now available from the TCCP. Complete conference proceedings will be distributed later this summer to conference participants. Copies will be available to Network members upon request.

IPBNet Conference III is being planned for early 1989. Detailed information will be distributed later this summer.



***Clockwise from bottom left:** Dr. Paiboolya Gavinlertvatana; Dr. Colette Nitsche (left); Dr. Roger Beachy (center).*

IPBNet Directory to be Published

Network members represent a wide variety of research interests, ranging from selection of stress-tolerant varieties of tropical and sub-tropical agricultural crops to more advanced studies on the latest techniques in protoplast fusion, gene selection and other areas of plant biotechnology.

An important function of IPBNet is the dissemination of information on current plant biotechnology research. This enables members to exchange ideas on similar interests, and builds linkages within the Network which contribute to future research progress.

The Network does not function without input from its members, and its growth is determined by the amount of active participation. To encourage such participation, an IPBNet Directory will be completed and distributed later this year. This Directory will include names, addresses, research concentrations and crop emphases and will be available to all IPBNet members.

We need your cooperation in providing information for the Directory. Please complete and return the attached survey. Information received will be compiled and published for Network members' use.

Technical Note: Callus Terminology in Corn Tissue Culture

For most cereals "E callus" is understood to refer to a compact, white to yellow callus composed of non-vacuolated, meristematic cells which readily give rise to regenerated plants by somatic embryogenesis. "NE callus" is understood to refer to a loose, yellow to brown callus composed of vacuolated, large cells which does not give rise to either shoots or regenerated plants.

In corn, this terminology can be used as well, but it must be brought into line with the the terms "Type I and Type II" (C.L. Armstrong and C.E. Green. 1984. Establishment and maintenance of friable, embryogenic maize callus and the involvement of L-proline. Paper No. 13,094, Scientific Journal Series, Minnesota Agricultural Experiment Station.) Type I callus is the same as E

Corn continued on page 14.

Recent Visitors to the TCCP Facilities

Dr. Duane Acker	USAID, Washington, D.C., U.S.A.
Dr. J.M. Andersen	De Dankke Sukkerfabrikker, Denmark
Dr. Philip E. Austin	Colorado State University, Ft. Collins, CO, U.S.A.
Mr. Hamlin H. Barnes	Vipont Labs, Ft. Collins, CO, U.S.A.
Mr. Chris Bayley	University of Nottingham, U.K.
Dr. F. Brinkerhoff	Tegal Sci., Denver, CO, U.S.A.
Dr. K.P.S. Chandel	National Bureau of Plant Genetic Resources, New Delhi, India
Dr. Ralph B. Clark	University of Nebraska, Lincoln, NE, U.S.A.
Dr. Joel Cohen	USAID, Washington, D.C., U.S.A.
Dr. John H. Dodds	CIP, Lima, Peru
Dr. Ronny Duncan	University of Georgia, Griffin, Georgia, U.S.A.
Dr. Liwayway Engle	University of the Philippines at Los Baños, Philippines
Mr. Rolando Estrada	CIP, Lima, Peru
Dr. David Fanning	AAAS Fellow/KUNC, Greeley, CO, U.S.A.
Dr. Catalino I. Flores	INTSORMIL, CIAT, Colombia
Dr. Richard L. Foskett	Mono-Hy Seed Co., Longmont, CO, U.S.A.
Dr. David Galbraith	University of Nebraska, Lincoln, NE, U.S.A.
Dr. Jack Gallagher	University of Delaware, Newark, DE, U.S.A.
Dr. Tejpal S. Gill	USAID, Washington, D.C., U.S.A.
Dr. Clarence Gray	Virginia Polytechnic Institute and State University, Blacksburg, VA, U.S.A.
Dr. Shrish C. Gupta	University of Delhi, India
Dr. Saturnina Halos	Nat. Sciences Research Institute, Philippines
Dr. Hu Han	Academia Sinica, Beijing, China
Dr. Gary E. Hanning	University of Nebraska, Lincoln, NE, U.S.A.
Dr. Harvey J. Hortik	USAID, Washington, D.C., U.S.A.
Prof. Daofen Hu	Academica Sinica Beijing, China
Dr. Hans-Jorg Jacobsen	Bonn University, Germany
Dr. Gerry Jayawardana	CARI, Sri Lanka
Dr. Suk Seong Jeng	Institute of Forest Genetics, Korea
Mr. Koji Katada	Japan Economic Journal, Sapporo, Japan
Dr. A. Mujeeb-Kazi	CIMMYT, Mexico
Dr. Abdul Quyyum Kazi	MOST, Islamabad, Pakistan
Mr. Alastair Lindsay	Plantech, Inc., U.K.
Dr. Ming-Chin Liu	Taiwan Sugar Research Institute, Tainan, Taiwan, R.O.C.
Dr. Marziah Mahmood	University of Agriculture, Malasia
Mrs. Yupa Mongkolsook	Kasetsart University, Thailand
Dr. Ken Odhleof	Colorado State University, Ft. Collins, CO, U.S.A.
Dr. Kunihiko Ojima	Tohahu University, Sendai, Japan
Dr. Ogonnaya O. Okoro	Forestry Research Institute, Nigeria
Dr. P.E. Onnorah	National Center of Genetic Resources and Biotechnology, Nigeria
Mr. Gregory Robin	CARDI, Dominica
Dr. Walter G. Rockwood	USAID/Abidjan, Cote d'Ivoire
Mrs. Beverly K. Rockwood	USAID/Abidjan, Cote d'Ivorie
Mrs. Aurora del Rosario	University of the Philippines at Los Baños, Philippines
Dr. David Sands	Montana State University, Bozeman, MT, U.S.A.
Dr. Tatsuya Shiraishi	Murdoch University, Murdoch, Australia
Dr. M.T. Shirolon	Somali National University, Somalia
Dr. Khushnood A. Siddiqui	Agricultural Research Center, Tando Jam, Pakistan
Dr. Charles Y. Sullivan	University of Nebraska, Lincoln, NE, U.S.A.
Dr. Joseph Varner	Washington University, St. Louis, MO, U.S.A.
Dr. Warid A. Warid	University of Cairo, Egypt
Dr. Livy G. Winata	Bogor Agricultural University, Indonesia
Dr. Paul E. Wisdom	Colorado State University, Ft. Collins, CO, U.S.A.
Prof. Zhu De Yao	Jiangxi Academy of Agricultural Sciences, Nanchang, China
Dr. Yusuf Zafar	Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan

Trainee Program Participants September 1985-February 1987

Name	Country	Crop(s) of Interest
Mr. Renato Avenido	Philippines	Mungbean, Wheat
Mr. Raed Bakheet	Saudi Arabia	Wheat
Ms. Ouafae Benlhabib	Morocco	Tomato
Ms. Olivia Damasco	Philippines	Wheat, Corn
Ms. Merlina Dionzon	Philippines	Corn, Rice
Dr. Bishamber Gothwal	India	Wheat, Barley
Mr. Francisco Gutierrez	Mexico	Sweet Potato
Ms. Abeer Abu Hassan	Jordan	Potato, Citrus
Ms. Iteu Hidayat	Indonesia	Potato
Dr. Flordeliza Javier	Philippines	Apple
Mr. Rachmat Kartapradja	Indonesia	Tomato
Mr. Andrew Matibiri	Zimbabwe	Tobacco
Mr. Ben Musyimi	Kenya	Cassava
Mr. Simon Ndirangu	Kenya	Tea
Ms. Mussarat Raza	Pakistan	Peanut, Mungbean
Ms. Erlinda Rillo	Philippines	Coconut
Mr. Takaniko Ruabete	Fiji	Sweet Potato, Taro

Graduate Students at the TCCP

Name	Country	Degree/Project
Francisco Gutierrez	Mexico	Ph.D./Physiology of salt tolerance
Iteu Hidayat	Indonesia	M.S./Potato protoplast fusion
Isaac John	Pakistan	Ph.D./Salt tolerance mechanisms
Raymond Ketchum	USA	Ph.D./Rice/millet protoplast regeneration
Douglas McMurray	USA	Ph.D./Mung bean regeneration
Amani Mnayer	Jordan	Ph.D./Wheat regeneration
Akbar Mohmand	Pakistan	Ph.D./Drought tolerance in wheat
Anna Novero	Philippines	M.S./Nitrate reductase in rice
Poungpet Poonspaya	Thailand	Ph.D./Rice regeneration from suspension cultures
Sathish Puthigae	India	Ph.D./Conditioning factor in rice media
Gaith Sasi	Libya	Ph.D./Triticale regeneration
Nanan Widiyanto	Indonesia	Ph.D./Rice blast resistance
Kerri Wright	USA	M.S./Rice field testing

Recent Publications from the TCCP

Gamborg, O.L., R.E.B. Ketchum and M.W. Nabors (1985). Tissue culture and cell biotechnology for increased salt tolerance in crop plants. BioSaline Research Workshop, Karachi, Pakistan, September.

Ghazi, T.D., H.V. Cheema and M.W. Nabors (1986). Somatic embryogenesis and plant regeneration from embryogenic callus of soybean, *Glycine max* L. Plant Cell Reports 5:452-456.

MacKinnon, C., G. Gunderson and M.W. Nabors (1986). Plant regeneration by somatic embryogenesis from callus cultures of sweet sorghum. Plant Cell Reports 5:349-351 (1986).

International Plant Biotechnology Network; Tissue Culture for Crops Project, Newsletter No. 6 (1986). J.L. Fischer (Ed.). Department of Botany, Colorado State University.

Ketchum, R.E.B., L.J. Klima, F.L. Gutierrez, W.R.S. Warren and M.W. Nabors. 1987. The mechanisms and role of proline accumulation in suspension cell cultures of the halophytic grass *Distichlis spicata* L., submitted.

Kumar, A.S., O.L. Gamborg and M.W. Nabors. Somatic embryogenesis and plant regeneration from cell suspension cultures of *Vigna aconitifolia*. In preparation.

Tissue culture for Crops Project, Progress Report (1987). Department of Botany, Colorado State University. In Press.

Corn continued from page 13.

callus. Type II callus is not usually seen in the callus culture of cereals other than corn. It probably appears early in culture initiation, and could probably be produced in quantity if suitable medium manipulation experiments were carried out. Type II callus in corn looks superficially like NE callus, but it is even more loosely organized and produces somatic embryos on stalks. When discussing corn, the TCCP uses the term "Type O" to refer to NE callus.

The origin and fate of these three callus types in corn is not known. Type O and Type I seem to arise independently. Type II appears to revert rapidly to Type O (NE) unless maintained on the proper medium. But the exact relationships have yet to be worked out.

Technical Note: Hormone Stock Solutions

In making solutions of commonly-used plant hormones like auxins and cytokinins, a few important guidelines should be followed:

1. Cytokinins can be dissolved in dilute base, but this requires careful attention to adjustment of medium pH. Preferably, cytokinin stock solutions should be autoclaved to avoid possible pH problems; however, it is important to remember that concentrations greater than 50 mg/l will usually not stay in solution at room temperature and certainly not under refrigeration.

2. Auxins can also be dissolved in dilute base. At the TCCP this procedure is avoided for the reasons mentioned above. All auxins are easily dissolved either at room temperature or at temperatures near boiling if the solution is placed on a magnetic stirrer. Overnight stirring is required for solutions dissolving at room temperature whereas heated solutions dissolve in a few hours. Autoclaving is also a successful method of obtaining solutions. Stock solutions of 150 mg/l are easily maintained at room temperature.

3. Contrary to common rumor, auxins are not destroyed by light. Gradually auxin molecules dimerize in light, and the solutions turn brown. This can be reversed by boiling, or a new solution can be prepared.

4. Both auxin and cytokinin solutions will be invaded by bacteria and fungi if maintained under non-sterile conditions.

The International Plant Biotechnology Network

Tissue Culture for Crops Project

Newsletter #8 January 1988

Field Testing of Tissue Culture-Selected Plants Yields Promising Results

During the past year the TCCP, in collaboration with other institutions, has conducted a total of eight field evaluations. These plots are providing needed information concerning the value of *in vitro* selection techniques. In addition to the field plots under environmental stress, there were four generation advances to be used for future stress evaluation. Three — oats at Saskatoon, spring wheat at CIMMYT, and rice at the University of the Philippines at Los Baños — were for eventual evaluation on saline soils. The fourth rice plot is for testing an acidic soils at CIAT in 1988.

Millet

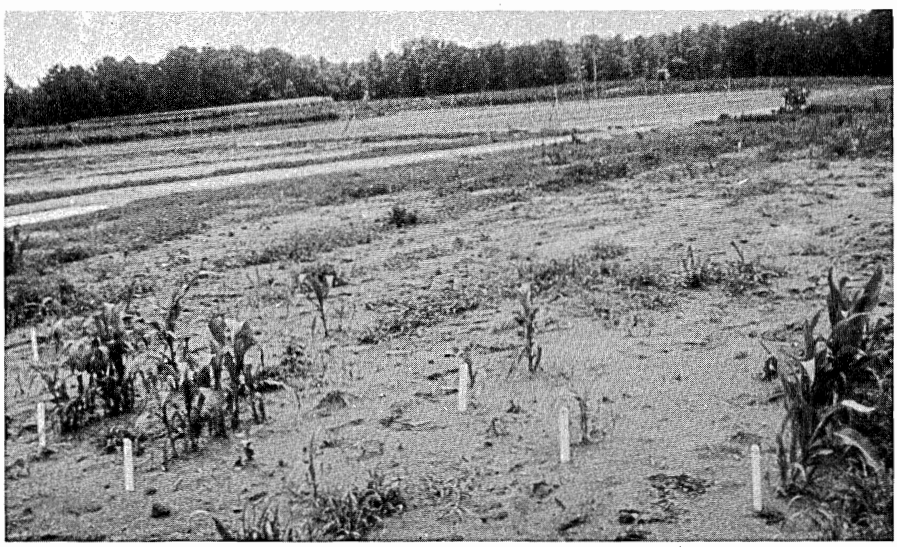
Millet R_1 lines in the TCCP's inventory are primarily derived from salt selection experiments. Millet R_1 lines were planted on June 20, 1987 on a clay loam soil at the University of Kansas Experiment Station, Hays, Kansas. Mr. Bill Stegmeier is our collaborator on these plots. Sixty plots representing five R_1 lines of Senegal Bulk and eight R_1 lines of HMP-559 derived from *in vitro* salt selection experiments were evaluated. Although this experiment was developed to select lines with increased drought tolerance, regular rainfall and relatively mild temperatures prevented drought conditions from developing. The regenerated lines exhibited more variation than non-regenerated checks for height, maturity, panicle morphology, leaf architecture, and growth habit.

Rice

In January, Dr. Edwin Javier at the University of the Philippines at Los Baños (UPLB) planted over 500 R_1 and R_2 with the R_2 lines being grown in saline conditions. That information was presented in IPBNet Newsletter #7. Since that time the seed has been increased and will be evaluated in saline conditions this coming season.

A second, saline field site has been developed with Dr. Kauser Malik of NIAB in Pakistan. Sixty R_1 and R_2 lines were sent to Pakistan for evaluation. These lines were planted in July 1987 and should be harvested by late November.

Field - *continued on page 2*



Field evaluation of R_2 sorghum lines on pH 4.0-4.3 soil near Griffin, Georgia. Line on the far right is Sc283, an acidic soil-tolerant line. On Sc283's left is non-regenerated Hegari, and further to the left are regenerate lines from Hegari.

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Colorado State University

Published by the Tissue Culture for Crops Project (TCCP)
Department of Biology
College of Natural Sciences
Colorado State University
Fort Collins, Colorado 80523, USA

Julie L.F. Ketchum, Editor, Operations Director
Murray W. Nabors, Project Director
Oluf L. Gamborg, Associate Director
Gary E. Hanning, Research Coordinator
Sunitha Siriwardana, Training Coordinator
Kerri L. Wright, Network Coordinator
Glen A. Hildreth, Lab Manager
Reagan M. Waskom, Greenhouse Manager

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Cooperating Agencies include:
Colorado State University, Fort Collins, Colorado, USA
Colorado State University, Agricultural Experiment Station, Fort Collins, Colorado, USA

Participating Technical Staff: Barbara Ashton, Leann Avery, Gwendolyn Bane, Barbara Bucknam, Laurie Clauss, Theresa DeMersseman, Kim Ellis, Jan Emming, Fariha Faizi, Lauren Fesler, Lori Frederickson, Touba Ghazi-Moore, Hal Gibson, David Hewitt, Julie Hewitt, Michelle Hunt, Jeannette Hut, Christa June, Deborah Kelly, Larry Klima, Alanna Kulchak, Heather Loomis, Marcus Murray, Kris Muscarella, Lee Olton, Elizabeth Orle, Carol Ostrom, Kathy Petersen, Gregory Pott, Scott Price, Catherine Rivera, Stephanie Schaal, Peter Schuerman, Dennis Shippee, Debra Singleton, Kim Spitler-Nabors, Robert Tengler, Jeff Tjaden, Nitschka ter Kuile, Michael Thompson, David Timm, Michael Tinker, Callie Vanderbilt, Kristin Vanderbilt, Brian Wood.

Graduate Students: Isaac John, Raymond Ketchum, Douglas McMurray, Akbar Mohmand, Anna Novero, Poungpet Poonsapaya, Sathish Puthigae, Gaith Sasi, Nanan Widiyanto.

Visiting Scientists: Ahmed Al Naggar, Suresh Kumar, Sheila Vijayakumar, Deng Wanyin, Yusuf Zafar

Training Program Staff: James Colbert, Oluf Gamborg, Harrison Hughes, Clark Livingston, Sunitha Siriwardana, Leigh Towill

For more information, contact:

Tissue Culture for Crops Project
Colorado State University
Fort Collins, Colorado 80523, USA
Phone: 303/491-6996
Telex: 3711418 TISCLT

Field - *continued from page 1*

In an earlier, whole plant screen in the greenhouse, an R_1 line of G-159 showed a yield potential of nearly twice that of the non-regenerated check. This line was sent to Dr. Neil Rutger at the University of California at Davis for field evaluation. The plants have been harvested but yield data have not been determined.

Sorghum

The TCCP's greatest success in sorghum has been in evaluation on low pH, high aluminum-containing soil. Dr. Ronny Duncan, University of Georgia Experiment Station, planted on a high aluminum soil with a pH of 4.0 to 4.3. On May 25, 1987, there were over 1400 plots of R_2 Hegari, Rio and RTx-430 on this acidic soil. A pH of 4.2 normally eliminates growth in even the most tolerant lines.

Approximately 2% of the plots survived and grew in this environment. Survivability was higher than last year partly due to the plants being from the R_2 rather than an R_1 evaluation. Heads were bagged to ensure self pollination, but a dry period persisted during heading and thus seed production was low. Seeds from this trial have been sent to Puerto Rico for increase and will be grown in Georgia again next year for further evaluation.

Sorghum's evaluation has extended to saline soils at Yuma, Arizona. The testing was a collaboration with Dr. Dwight Tomes of Pioneer HiBred Co., Iowa, Dr. Robert Voight of the University of Arizona and Mr. Bill Blackledge of the Yuma Valley Experiment Station. The field site consisted of 1000 plots of the cultivars Keller, RTx-430, and Hegari. They were planted on June 22, 1987 and irrigated five times during the growing season with saline water. The salt concentration in the soil was measured at 2-4 Ec, a relatively low salt stress. There were, however, noticeable differences between cultivars exhibiting salt damage. There was also variation for salt tolerance within regenerated plants of a particular cultivar. The data has not been fully analyzed. There was also considerable insect infestation, but it was sporadic within the field and no difference could be statistically determined.

In addition, ten lines were sent to Dr. John Clark, INTSORMIL, Niamey, Niger and were planted on July 2, 1987. Emergence was good but seedling survival was poor due to infertile, acidic soils (pH of 4.0), sand blasting, drought, and high soil temperatures (50°C).

Bonus Is Gained From Sorghum Field Evaluations

Unexpected results can be quite rewarding. For the TCCP, an unexpected but significant accomplishment has been made with the occurrence of insect tolerance in sorghum. There were two evaluations for insect resistance at Tifton, Georgia with Dr. David Isenhour; one for fall armyworm (FAW) and the second for sorghum midge. Both are serious pests in the southern areas of the United States as well as other areas of the world. Little tolerance has been found for FAW or for the sorghum midge in the world collection of exotic sorghum material. During the 1986 seed increase at Griffin, Georgia there was a relatively heavy, natural infestation of FAW. Nine lines showed reduced feeding and were selected to be used in a more stringent field evaluation at Tifton in 1987.

The evaluation in 1987 was based on both open pollinated and selfed head selections from the 1986 trial. When the sorghum plants were approximately 1 m tall, each plant was infested two times each with 20 FAW larva. One R_2 line from salt-selected callus showed significant tolerance to the FAW. The observed resistance is being confirmed in greenhouse studies this fall at Tifton and will be retested in field studies in several locations in Georgia next year.

Evidence of tolerance to Fall armyworm in line 2442 at the R_2 generation. R_3 generation is in progress to confirm the tolerance.

The resistant R_2 line, designated 2442, was in culture a total of 11 passages and on 9 g/l NaCl for five of those passages. The trait could be an escape, but the observations in the R_2 generation point to a valid FAW tolerance.

Sorghum midge damage is rated late in the season by determining the percent seed set. The same lines used in the FAW test were evaluated for midge tolerance. Two open pollinated R_2 lines from salt-selected callus showed some tolerance. Both lines, 2354 and 2435, came from a total of 11 passages in culture with either four or five passages on 9 g/l NaCl. These will be reevaluated next year to confirm these findings.

Damage incurred from artificial infestation of Fall armyworm.

Photos contributed by Dr. David Isenhour.

Plants Regenerated From Rice Suspension Cultures

TCCP's objectives in developing a plant regeneration system for rice and other species includes the use of suspension cell lines as a selection system and a source for protoplast isolation. Modifications of published protocols have been used to develop an embryogenic cell suspension regeneration system in rice. The improved method will then be tested in other rice varieties. Protoplasts from reported research on Taipei 309 (Abdullah *et al.*, 1986) were not regenerated from suspensions.

The TCCP's methods are now being developed by Ms. Poungpet Poonsapaya, Thailand, for her Ph.D. dissertation. Mature seeds of rice (*Oryza sativa* L. var Taipei 309) were dehusked and surface sterilized with 20% Clorox solution. The seeds were then cultured on LS medium (Linsmaier and Skoog, 1965) with 2.5 mg/l 2,4-D and 4% sucrose for callus induction. Cultures were maintained at 25±2°C in the dark.

Cell suspension cultures were initiated by inoculating embryogenic calli derived from mature seeds into liquid LS medium. The suspensions were cultured at 25±2°C on a gyratory shaker at 120 rpm in the dark. Subcultures were made by replacing the original medium with fresh medium every one to two weeks for three to four subcultures. Then the medium was changed to a modified AA medium supplemented with 2.5 mg/l 2,4-D after which subcultures were made every two to three weeks. The modified AA media contains the major salts of LS minus NH_4NO_3 and KNO_3, LS minor salts, LS vitamins, glutamine, aspartic acid, arginine and glycine. Within two months, cell clusters and small callus clumps of 1-4 mm in diameter were formed. These clumps gave rise to embryoid-like structures.

After three months in culture as suspensions, 2-4 mm callus clumps were transferred and cultured on solid LS medium with or without 0.5 mg/l BA and 0.05 mg/l NAA. Within two to three weeks, greenish cell sections developed and eventually regenerated into shoots and complete plantlets. More than 100 plants have been regenerated and transferred to the greenhouse to be grown to maturity. These plants were regenerated from three- to five-month-old suspension cultures.

Future plans are to repeat and improve this technique, then begin testing other varieties for their ability to regenerate plants from similar embryogenic cell suspension cultures.

Tissue Culture For Chickpea Improvement

A collaborative project between the Tissue Culture for Crops Project (TCCP) and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India, was set-up to develop reliable procedures of tissue culture techniques for the improvement of chickpeas.

Chickpea (*Cicer arietinum* L.) is an important grain legume crop on the Indian subcontinent, parts of Asia, Africa, Europe, and South and Central America. It is valued both as food and fodder, and the seeds constitute a major source of dietary protein.

A reliable procedure for micropropagation was developed using shoot cuttings from mature *Cicer* plants. Shoot cuttings (2-3 cm long) from chickpea genotypes and wild *Cicer* species produced roots when placed on rooting medium containing 0.1 mg/l NAA and 0.2 mg/l IBA, respectively.

Shoot tip explants from chickpea seedlings grown *in vitro* were used for clonal propagation. Multiple shoots were produced from each explant when placed on L-6 or B5 medium containing 2.0 mg/l BA and 0.5 mg/l IAA. Callus production was higher in B5 medium. The shoots were excised and placed on rooting medium containing 0.5 mg/l IBA for root induction. The plants will be grown to maturity in the greenhouse.

Research is in progress to develop procedures for plant regeneration by somatic embryogenesis and to produce interspecific crosses of wild *Cicer* species with chickpea genotypes by embryo culture. Immature (6, 9, 12 and 15-day-old) embryos of the female parent *C. arietinum* genotypes germinated on MS or L-6 medium containing 2.0 mg/l BA and 0.1 mg/l IBA. The media will be used to culture the hybrid embryos.

Research Reports From IPBNet Members

Researchers:
Dr. Nguyen T. Thanh-Tuyen,
B.C. Padvano, M.N. Dionzon
Tissue Culture Laboratory
Department of Horticulture
Visayas State College of Agriculture
ViSCA, Leyte 7127-A, Philippines

Objectives:

To increase drought and acid soil tolerance of important local cultivars of corn and rice using tissue culture techniques. To develop reliable plant regeneration methods for the cultivars.

Results:

Five cultivars of upland rice were used (Kinadang Patong, UPLRi 7, IR 43, IR 13754-5-2, and IR 12721-1-1-3) with IR 36 used as a check cultivar. Seeds were used to initiate callus on LS medium containing 1 mg/l 2,4-D, 0.5 mg/l KIN, and 50 mg/l TRP in both light and dark. The results are shown in Table 1.

Kinadang Patong and IR 12721-5-1-1-3 produced less callus than the check cultivar. The initiation was revised to contain 0.5 mg/l 2,4-D, 0.5 mg/l KIN, and 100 mg/l TRP. After six weeks on initiation medium, E callus was transferred to medium with 0.5 mg/l 2,4-D and 0.25 mg/l KIN. All cultivars except Kinadang Patong and IR 12721-5-1-1-3 did well on this medium. The latter two cultivars did well if continued on their revised initiation medium. Table 2 shows plant regeneration after ten weeks on initiation and maintenance media.

Three cultivars of corn (Improved Tiniguib, ViSCA 8311, and IPB 2) were used. Immature embryos of 1.5 mm were best for callus initiation in all cultivars. An MS medium with organic constituents replaced by 0.5 mg/l thiamine and 1 mM L-asparagine produced more total callus and a larger percent E (Type I) callus in light than regular N6 medium. Both media were supplemented with 2% sucrose, 1 mg/l 2,4-D, 5 mM proline and were solidified with 0.6% agar. Callus initiation on young leaf segments was poor compared to that on immature embryos, and contamination levels were higher.

The callus were maintained for a few passages on initiation medium before transfer to N6 medium with 6% sucrose and no 2,4-D for embryo development. They were then transferred to modified MS medium with 2% sucrose and no 2,4-D for plant regeneration.

On initiation medium, scutellar callus production increased several folds in the first two passages of four weeks and subsequently declined. Percent E callus increased slowly up to the fourth passage and started to decrease afterwards (Fig. 1).

Plant regeneration was observed in the second passage, but was rare after three passages on initiation medium. However, when E callus (Type I and II) was transferred to N6 medium with 6% sucrose and lacking 2,4-D, the development of somatic embryos was reduced, and no plants were regenerated on transfer to germination medium containing modified MS formula with 2% sucrose and no 2,4-D.

The regenerated plants from cultured embryos of IPB 2 were potted and grown to maturity in our screenhouse. A few plants had no tassels, or had shelled or naked kernels and protruded embryos. Seeds with protruded embryos were fertile and germinated.

Researchers:
Endang Gati[2], Ika Mariska[3], and Fathan Muhadjir[4]
2) and 3) Bogor Research Institute for Spice and Medicinal Crops, Bogor, Indonesia 4) Bogor Research Institute for Food Crops, Bogor, Indonesia

Table 1. Production of E and total callus (E + NE) from mature rice seeds of six cultivars on LS initiation medium containing 1 mg/l 2,4-D, 0.5 mg/l KIN and 50 mg/l TRP under light and dark incubation for six weeks.

CULTIVAR	Light Incubation		Dark Incubation	
	Fresh weight (mg)		Fresh weight (mg)	
	E callus	Total callus (E+NE)	E callus	Total callus (E+NE)
Kinadang Patong	6.7	105.4	273.2	453.5
UPLRi 7	185.5	233.2	28.1	139.5
IR 43	431.4	555.8	48.6	144.4
IR 13754-5-2	654.5	786.5	132.2	312.0
IR 12721-5-1-1-3	3.2	65.9	4.2	132.3
IR 36	38.4	120.1	431.6	552.1

Sample size was 20 seeds per cultivar

Table 2. Rice plantlets regenerated from 100 mg E callus maintained for two weeks on media containing either IAA, BA, TIBA singly or a combination of BA and TIBA.

CULTIVAR	Number of plantlets (0.5 - 4.0 cm long)			
	0.5* BA	0.5* BA	0.5* TIBA	1.0* BA + 0.5* TIBA
Kinadang Patong	0	0	0	0
UPLRi 7	7	11	13	0
IR 43	9	5	7	4
IR 13754-5-2	22	25	5	14
IR 36	12	15	3	5

*mg/L
The data are average number of plantlets from 10 vials of 100 mg E callus per cultivar.

Abstract:
Red ginger (*Zingiber officinale* Rosc.) is used as a source of essential oil. The method of rapid propagation of red ginger through tissue culture is being developed in Indonesia. Explants were derived from rhizomes. The data indicates that (MS) medium with KIN fails to induce bud formation. The use of MS with 10 mg/l BA with 1 mg/l NAA was found to induce callus followed by formation of adventitious buds and shoots. The shoots were then induced to root. Bacterial contamination was eliminated by using 70% alcohol, 0.5% $HgCl_2$, 5.0% sodium hypochlorite, followed by three rinses of sterile, distilled water.

Researchers:
Dr. P. Gavinlertvatana and
Dr. R.C. Barba
Plantek International (PTE), Ltd.
Unit 04-01A, Block 14
The Maxwell
Science Park Drive
Singapore 0511

Objectives:
Micropropagation research and application for horticultural and plantation crops.

Results:
Micropropagation has become the most widely applied tissue culture technique. Large numbers of plants are now being produced and utilized in horticulture, agriculture and forestry. The paper outlines an update on current production worldwide and provides information on technologies in use for specified crops. The major portion of the paper is concerned with orchids, flowering and foliage crops, fruit crops, tropical fruits, palms and beverage crops.

Copies of the paper can be obtained by contacting TCCP.

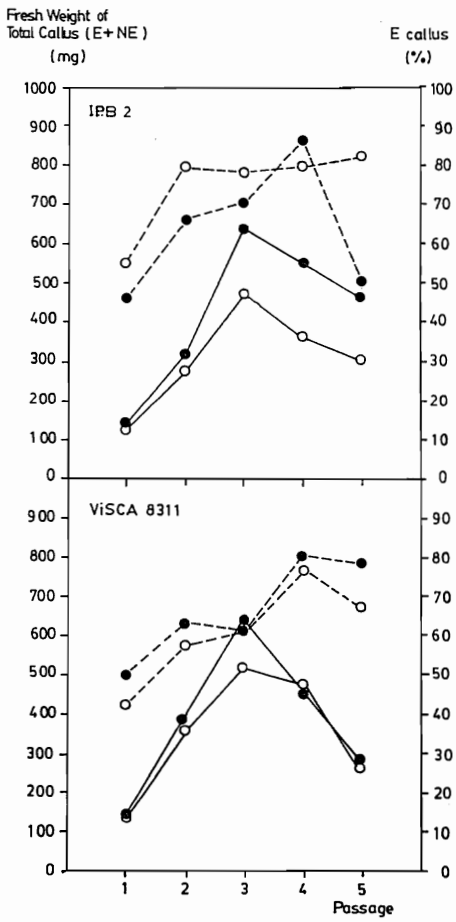

Figure 1:
Production of callus from scutellum of immature corn embryos cv. IPB 2 and ViSCA 8311 on MS (shaded circles) and N6 (open circles) medium. (_____ Total callus, ----- E callus)

Unique Tissue Culture Training Available

The Tissue Culture for Crops Project (TCCP) laboratory has an established, comprehensive Training Program in tissue culture and related biotechnologies. The Program covers topics in callus and suspension culture methods, plant regeneration, screening *in vitro*, embryo and anther culture, meristem procedures as well as protoplast techniques.

The Training Program consists of lectures and laboratory exercises to introduce and give trainees hands-on skills in the most recent methods of tissue culture and other biotechnology procedures that can be applied to crop plant improvement and production.

The unique feature of the Training Program is the research project. After six to eight weeks, the trainees are required to prepare a research proposal under the guidance of the Coordinator and an instructor. The research must apply one or more techniques acquired during the early part of the course to a crop of importance in the country of the respective trainee. The research is then performed and results presented in writing and orally upon the completion of the training period. The total program enables the trainee to return to their respective institution and be able to set-up and implement the technologies. Graduates will also be able to instruct others in the acquired procedures and skills.

More detailed information on the Training Program can be obtained by contacting the Training Coordinator, TCCP, Biology Department, Colorado State University, Fort Collins, CO 80523, USA.

Announcement

The Royal Nepal Academy of Science and Technology is organizing a conference/symposium on plant cell and tissue culture.

Interested parties should contact:
Dr. Vishwanath P. Agrawal, Director
Research Lab for Agricultural
Biotechnology and Biochemistry
P.O. Box 2128
Kathmandu, Nepal

Update On Tissue Culture Training Programs At International Agricultural Research Centers

Recently a survey was sent to the various International Agricultural Centers to gather information on the tissue culture training courses available. The following is a synopsis of the information received:

CIAT: Primary training activities concentrate on in-service internships for a limited number of scientists (up to six per year). Formal training courses in collaboration with CIP and IITA are also ongoing. Training subjects include:

a. *In vitro* techniques for disease elimination, micropropagation, germplasm exchange and conservation in cassava and tropical pasture grass species.
b. Anther culture for achieving rapid homozygocity in rice.
c. Embryo/shoot tip culture for micropropagation and recovery of special genotypes in common bean and cassava.
d. Callus/cell culture and regeneration techniques being developed for cassava, common bean, rice, and some tropical pasture legume species (*Stylosanthes*).
e. Characterization of germplasm by electrophoresis for cassava and common bean.

For more information regarding costs, scholarships, etc. contact:

Coordinator, Training Program
CIAT
Apartado Aereo 6713
Cali, Colombia

CIMMYT: Six-month training sessions of externally-supported national program researchers exist in the general area of widecrosses. Training includes hybridization, embryo culture, cytology, field-operated electrophoresis, and callus culture of hybrids (intergenic and interspecific) in wheat. Tissue culture aspects are an offshoot of areas initiated by TCCP, specifically, alien genetic transfers mediated by tissue culture in wheat. For trainee and research fellowships, contact:

Dr. A. Violi
Head, Training Program
CIMMYT
Lisboa 27, Apdo. Postal 6-641
Deleg. Cuauhtemoc 06600
Mexico, D.F.

CIP: Training courses relating to potato are regional (international) or in-country and are carried-out as joint activities with national programs. Participants are selected by CIP's regional representatives in collaboration with the national potato program leaders. This past year, CIP held courses in Cuba, Venezuela, China and Peru. For further information, contact:

Senior Training Specialist
CIP
P.O. Box 5969
Lima, Peru

IITA: Offers individual two-month training for national programs who have or anticipate tissue culture work in tropical root and tuber crops, plantain, and banana. Training programs funded by UNDP. Interested national programs should send their nominations to:

Director, Training Program
IITA
Oyo Road
PMB 5320
Ibadan, Nigeria

IRRI: Does not have a formal tissue culture training course; applicants are accepted on a case to case basis. Training focuses on rice anther and somatic cell cultures and design and performance of specific experiments. Training periods average six months. The trainee/institution is responsible for finding donors for financial support. Contact:

Director for Training and Research
IRRI
P.O. Box 933
Manila, Philippines

ICRISAT: No organized tissue culture course exists, but applications for post-doctoral fellows, in-service fellows, visiting scientists or apprentices should apply to:

Training Officer
ICRISAT
Patancheru P.O.
Andhra Pradesh 502 324
India

IBPGR: Currently, IBPGR is placing emphasis on development of appropriate technology for slow growth and cryopreservation. No organized training course exists at this time.

For funding information, please consult the 1987-88 IPBNet Directory.

Plant Tissue Culture Researcher Wanted

The Tissue Culture for Crops Project, a U.S. Agency for International Development-funded project at Colorado State University, is seeking a full-time Research Associate for work in cereal tissue culture. The successful candidate must have at least a B.S. in plant science or a related field and experience in embryogenic monocot tissue culture. The person should be interested in stress physiology *in vitro* as well as in whole plant testing and in supporting the efforts of international plant research.

Application deadline March 1, 1988. Position available July 1, 1988. Non-tenure track. Salary $14,000-$18,000. Must be a U.S. citizen or permanent resident. Send resume and three letters of recommendation to:

Dr. Gary E. Hanning
Research Coordinator
Tissue Culture for Crops Project
Department of Biology
Colorado State University
Fort Collins, CO 80523
Phone: (303) 491-1813

Colorado State University is an EEO/AA employer. EO Office: 314 Student Services Building

Research Summaries Requested

If you would like to see reports about your lab in the IPBNet Newsletter, please prepare them and mail to the Editor. Reports should be double-spaced, typed, and no more than two pages long. Reports should have enough details to give an informative, concise summary of both results and current research.

The Editor reserves the right to shorten and edit if necessary.

TCCP Publications and Reports

Dykes, T. 1987. Report on IPBNet regional training course, Center Agronomico Tropical de Investigacion y Ensenanza, Turrialba, Costa Rica.

Hanning, G.E. 1987. Brief on insect studies in Georgia. TCCP, Colorado State University, Fort Collins, Colorado.

Hanning, G.E. 1987. Brief on regenerate sorghum plants tolerant to acidic soils. TCCP, Colorado State University, Fort Collins, Colorado.

Hanning, G.E. 1987. Brief on results from regenerate rice evaluation of saline soils. TCCP, Colorado State University, Fort Collins, Colorado.

Ketchum, J.L.F., O.L. Gamborg, G.E. Hanning, and M.W. Nabors (Eds.). 1987. Progress Report. TCCP, Colorado State University, Fort Collins, Colorado.

Ketchum, J.L.F. (Ed.). 1987. IPBNet Newsletter No. 7., TCCP, Colorado State University, Fort Collins, Colorado.

Kumar, A.S., O.L. Gamborg, and M.W. Nabors. 1987. Plant regeneration from cell suspension culture of *Vigna aconitifolia*. Plant Cell Reports, submitted.

MacKinnon, C., G. Gunderson, K.M. Petersen, and M.W. Nabors. 1987. Plant regeneration from salt-stressed sorghum cultures. Life Science Advances 6: No. 2.

MacKinnon, C., G. Gunderson, and M.W. Nabors. 1987. High efficiency plant regeneration by somatic embryogenesis from callus of mature embryo explants of bread wheat (*Triticum aestivum*) and grain sorghum (*Sorghum bicolor*). *In vitro* Cellular & Developmental Biology 23:443-448.

Siriwardana, S., and G.A. Hildreth. 1987. Handbook for plant tissue culture laboratory organization and management. Colorado State University, Fort Collins, Colorado.

Tissue Culture for Crops Project. 1987. Proceedings of the Second Annual Conference of the International Plant Biotechnology Network (IPBNet). Colorado State University, Fort Collins, Colorado.

Wright, K.W. 1987. Trip report — Kenya. TCCP, Colorado State University, Fort Collins, Colorado.

Recent Visitors to the TCCP Facilities

Christine Alang	University of Agriculture, Malaysia
Supat Attathom	Kasetsart University, Thailand
Pierre-Paul Bove	Pasco Industries, Colorado, USA
Ed Bresch	Pasco Industries, Colorado, USA
Yin Congnong	Shandong Provincial Hydrology Service, P.R. of China
H.S. Dhaliwal	Punjab Agricultural University, India
Jay Dowling	Kellogg Corporation, Colorado, USA
Harold Eddleman	USA
Unver Ekmekei	Gunes Tohum Islah A.S., Turkey
Lloyd Frederick	USAID, Washington, D.C., USA
Ditalamane Hebie	University of Quagadougou, Burkina Faso
Pedro Hernandez	Colorado State University, USA
Steve Hoffman	Somatogenetics Intl., Colorado, USA
Bob Hutchinson	CSURF, Colorado State University, USA
Hans Jobert	University of Arizona, USA
John Keenan	University of Wyoming, USA
Wayne Keim	Colorado State University, USA
Bob Kelley	Danbury USA, Inc., Colorado, USA
Iqrar Khan	University of California, Riverside, USA
Masanori Kimura	All in One, Inc., Japan
Lisbeth A. Levey	AAAS Office of International Science, Washington, D.C., USA
Gezo Marai	Agricultural University, Hungary
Brad Masi	USA
JoAnne Masi	USA
S.L. Mehrutra	CSIR, New Delhi, India
David Mok	Oregon State University, USA
B. I. Muruli	University of Nairobi, Kenya
Suresh Patil	University of Hawaii, USA
Allyson Porter	World Technology Group, Inc., Colorado, USA
Hasan Rhadra	Royal Scientific Society, Amman, Jordan
Mario Salazar	Colorado State University, USA
Charles Scoggin	Somatogenetics Intl., Colorado, USA
Yang Shoufa	Ministry of Water Resources and Hydroelectric Power, P.R. of China
Hao Shui	Northeast Normal University, P.R. of China
Shree P. Singh	CIAT, Colombia
Porntip Thanutong	Khon Kaen University, Thailand
S. A. Tjaja	Batan, Jakarta, Indonesia
Marion Verzea	Research Institute for Cereals and Industrial Crops, Romania
Xiao Yuquan	Institute of Water Conservancy and Hydroelectric Power Research, P.R. of China
Hu Zunfeng	Chinese Hydrology Society, P.R. of China

TCCP Training Program Continues

The Tissue Culture for Crops Project has recently graduated two trainees who completed the comprehensive training program in biotechnology procedures. These graduates of the spring 1987 session of the Training Program are Ms. Benchamas Silayoi of Kasetsart University, Thailand, and Ms. Carol Rohrmann of Guatemala. They were presented with diplomas for their achievements in August 1987, after presenting the results of their respective research. Ms. Silayoi presented her results on the successful regeneration of banana plants from callus tissue derived from leaf explants. Her results were one of the first reported, successful attempts to regenerate plants from callus cultures of banana. Ms. Rohrmann developed a regeneration media for tissue culture of potato.

Five trainees are currently participating in the fifth session of the TCCP Training Program. They have completed the basic and formal part of the course and will focus their research on topics and crop species integrated with the ongoing research.

Seven candidates have been accepted for the spring 1988 session, representing Bulgaria, China, India, Indonesia, Kenya, Nigeria, and the Solomon Islands. We look forward to another successful training session beginning March 7, 1988.

Third International IPBNet Conference To Be Held In Nairobi, Kenya

The Tissue Culture for Crops Project (TCCP) has two major functions. The principal function is to develop, apply, and demonstrate the value of plant tissue culture technologies in the development of plant germplasm with increased stress tolerance. The other function is to facilitate transfer and exchange of technology, information, and materials through communication and collaboration with individuals and research centers worldwide. The latter functions are carried out through the International Plant Biotechnology Network (IPBNet), a significant feature of which is the IPBNet conferences.

The TCCP will sponsor the Third IPBNet Conference January 8-12, 1989. Nairobi, Kenya was chosen as the site, with the goal of strengthening collaborations and linkages with African scientists. The Conference will cover topics of importance for Sub-Saharan Africa and the role of crop improvement through breeding, tissue culture, and genetic technologies.

The Conference will begin on Sunday evening with a reception and lecture. Monday through Thursday will consist of morning plenary sessions and afternoon or evening sessions for oral and poster presentations. Abstracts should be sent with registration. There will be 34, 20-minute oral presentations accepted, so early submission of abstracts is crucial. Posters will also be accepted.

Additional information and a detailed agenda will be mailed in May.

Conference Plan

Sunday, January 8

5:00 - 7:00 pm	Registration
7:00 pm	Reception and Lecture on International Agricultural Development Dr. M.S. Swaminathan, Director General, IRRI

Session I

Monday, January 9

9:00 - 12:00 am	Topic: Tissue Culture Technologies Tentative Keynote Speakers: Dr. Irwin Y.E. Chu, Twyford Plant Laboratories Dr. Gary E. Hanning, TCCP
1:30 - 5:00 pm	Paper and Poster Presentations

Session II

Tuesday, January 10

9:00 - 12:00 am	Topic: Plant Breeding and Genetics for Stress Tolerance Tentative Keynote Speakers: Dr. Stephen Baenziger, University of Nebraska Dr. Ronnie Duncan, University of Georgia
7:00 - 10:00 pm	Paper and Poster Presentations

Session III

Wednesday, January 11

9:00 - 12:00 am	Workshops on Practical Applications of Biotechnology
7:00 - 10:00 pm	Paper and Poster Presentations

Session IV

Thursday, January 12

9:00 - 12:00 am	Topic: Novel Biotechnologies Tentative Keynote Speakers: Dr. Roger Beachy, Washington University Dr. Oluf L. Gamborg, TCCP
1:30 - 5:00 pm	Paper and Poster Presentation
7:00 pm	Farewell Banquet

Optional Activity

Friday, January 13 - Sunday, January 15

Safari to Masai Mara Game Reserve. Adjoining the Serengeti park in Tanzania, Mara lies 270 km south of Nairobi in the Great Rift Valley. It is known for the black-maned lion, elephant, leopard, cheetah, rhinoceros, and its huge herds of wildebeest.

Cost: $200 per person (approximately). $100 per person, non-refundable deposit due with registration. Balance due December 15.

Includes lodging (double occupancy), meals, transportation, park fees, and guide.

Limit: 25 persons

Accommodations

Conference Site: Silver Springs Hotel
Valley Road
P.O. Box 61362
Nairobi, Kenya

Registration Packages Available:

(1) Conference Registration and fees—includes materials, reception, banquet, and breaks

Before 10/1/88	$100 non-student $ 75 student
After 10/1/88	$125 non-student $100 student

(2) Conference Registration, fees, hotel (five nights), and two meals (breakfast, plus lunch or dinner, Sunday - Thursday)

Before 10/1/88	$295 non-student, double occupancy $325 non-student, single occupancy $270 student, double occupancy $300 student, single occupancy
After 10/1/88	Add $25 per person

Add $31 (double), $37 (single) per night if you plan to stay more than five nights.

Those who prefer other accommodations must make their own arrangements.

The International Plant Biotechnology Network

Tissue Culture for Crops Project

Newsletter #9 July 1988

IPBNet Conference Update

Registrations are being accepted for the upcoming IPBNet Conference entitled "The Role of Tissue Culture and Novel Genetic Technologies in Crop Improvement." The Conference will be held at the Silver Springs Hotel in Nairobi, Kenya, January 8-12, 1989 and will focus on topics of importance to Sub-Saharan Africa.

The deadline for registration fees and abstract submission is October 1, 1988. *A late charge of $25 is due with registrations after October 1.* Abstract and registration forms are included in this newsletter.

Two packages are available:

1) *Registration fees only* - includes materials, reception, banquet, and refreshment breaks.
   Cost: $100 non-student
   $ 75 student
2) *Registration fees and hotel* - includes all in Package 1, plus five nights hotel accommodation and two meals per day.
   Cost: $295 each non-student, double
   $325 non-student, single
   $270 each student, double
   $300 student, single
   Additional nights (beyond five) $31 each (double), $37 each (single) *Breakfast only included.*

After 10/1/88 add $25 per person.

The optional Safari to Masai Mara Game Reserve is filling-up fast. Be sure to send your $100 deposit along with your registration. *We cannot hold places without deposits.*

More detailed information will be sent upon receipt of registration fees.

Conference Program

Sunday, Jan. 8	**Registration** **Reception. International Agricultural Development,** Keynote Speaker: Dr. M.S. Swaminathan
Session I Monday, Jan. 9	**Plant Breeding & Genetics,** Speakers: Dr. B. McCarter, Maize breeding for stress tolerance Dr. Y.L. Nene, Prospects & problems in grain legume breeding Dr. R. Duncan, Sorghum breeding for Africa Oral and Poster Presentations.
Session II Tuesday, Jan. 10	**Tissue Culture Technologies,** Speakers: Dr. V. Villalobos, Root crops; status & prospects for application Dr. I.Y.E. Chu, Micropropagation; commercial application Dr. G.E. Hanning, Selection & plant regeneration in cereals & grain legumes. Oral and Poster Presentations.
Session III Wed., Jan. 11	**Practical Applications of Biotechnology** Workshops, Round-table. Oral and Poster Presentations.
Session IV Thurs., Jan. 12	**Novel Biotechnologies,** Speakers: Dr. H.J. Dodds, Biotechnology applied to potato improvement Dr. O.L. Gamborg, Genetic approaches to stress tolerance Dr. R.N. Beachy, Applications of gene transfer to stress tolerance Oral and Poster Presentations. Banquet.

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Colorado State University

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Department of Biology
Colorado State University
Fort Collins, Colorado 80523, USA

Julie L.F. Ketchum, Editor, Operations Director
Murray W. Nabors, Project Director
Oluf L. Gamborg, Associate Director
Gary E. Hanning, Research Coordinator
Sunitha Siriwardana, Training Coordinator
Kerri L. Wright, Network Coordinator
Glen A. Hildreth, Lab Manager
Reagan M. Waskom, Greenhouse Manager
E. Lee Olton, Office Manager

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Chief of Renewable Natural Resources, AID, Dr. Tejpal S. Gill

Cooperating Agencies include:
Colorado State University, Fort Collins, Colorado, USA
Colorado State University, Agricultural Experiment Station, Fort Collins, Colorado, USA

Permanent Research Staff: Barbara Ashton, Gwendolyn Bane, Fariha Faizi, Suresh Kumar, Daniel Miller, Carol Ostrom, Catherine Rivera Smith.

Participating Technical Staff: Amy Beth, Barbara Bucknam, Laurie Clauss, Kim Ellis, Jan Emming, Lauren Fesler, Hal Gibson, David Hewitt, Julie Hewitt, Michelle Hunt, Jeannette Hut, Christa June, Monica Kieber, Larry Klima, Alanna Kulchak, Heather Loomis, Jolanta Menert, Marcus Murray, Kris Muscarella, Elizabeth Orle, Gregory Pott, Scott Price, Peter Schuerman, Susan Sidinger, Debra Singleton, John Tamulonis, Robert Tengler, Nitschka ter Kuile, David Timm, Michael Tinker, Kristin Vanderbilt, Brian Wood.

Graduate Students: Iteu Hidayat, Isaac John, Raymond Ketchum, Douglas McMurray, Amani Mnayer, Akbar Mohmand, Anna Novero, Poungpet Poonsapaya, Sathish Puthigae, Gaith Sasi, Nanan Widiyanto.

Visiting Scientists: Ahmed Al Naggar, Paul Chavarriaga Aguirre, Sheila Vijayakumar.

Training Program Staff: James Colbert, Oluf Gamborg, Harrison Hughes, Clark Livingston, Sunitha Siriwardana, Leigh Towill

For more information, contact:

Tissue Culture for Crops Project
Colorado State University
Fort Collins, Colorado 80523, USA
Phone: 303/491-6996
Telex: 3711418 TISCLT

New Cultivar of Pearl Millet Is Evaluated

Previous *in vitro* experimentation at the TCCP has focused primarily on several outcrossed cultivars of pearl millet *(Pennisetum americanum)* cvs. HMP-559 and Senegal Bulk. Tissue culture methodology has now been developed for an inbred line of pearl millet cv. Tift 23 DBE. A media study was conducted on Tift to develop a media conducive to E callus growth and formation.

Callus cultures were initiated from mature caryopses on LS medium supplemented with 1.1% agar, 4% sucrose and on nine media combinations utilizing concentrations of 2,4-D from 3 to 7 mg/l and from 1 to 3 mg/l IAA plus .3 mg/L KIN for callus induction and maintenance. In addition, IAA concentration from 0 to .5 mg/l with .5 mg/l BA or .5 mg/l IBA were studied as components for regeneration. Embryogenic callus fresh weights from each culture vessel were determined by placing the E callus on a petri plate and weighing with a Mettler analytical balance.

At the end of the first subculture, embryoids were virtually non-existent, making it difficult to identify E callus. Therefore, the entire mass of callus, minus shoots and caryopses, was transferred onto fresh media at the end of the first passage. Non-embryogenic callus growth was rapid during the second subculture and was dissected away and discarded after the second passage. Embryoids and various other callus types were obvious following the third transfer (Table 1). Yellow, hard, nodular, compact callus was often the most prevalent callus type formed followed by the production of a green, hard, compact, nodular, type. In most cases the "true" embryogenic, creamy, white, compact, callus was the least amount formed. All three callus types were weighed by cell line. Four of the seven media studied produced a substantial amount of E callus. Of the four media that produced E Callus, three contained 7 mg/l 2,4-D (Figure 1).

For the production of whole plants, E callus was subcultured on regeneration media. An average amount of .1 g of callus was placed on 20 mls of regeneration media to allow formation of shoots. When shoots reached a height of 1 1/2 to 2 inches, they were placed on media containing 3 mg/L IBA to in-

crease root development. Regenerates with an established root system were then transplanted to a soil-less mix and placed in the greenhouse for seed increase. By the ninth passage, four media had produced plants which have been established in the greenhouse (Table 2). The media with 3 mg/l 2,4-D, 2 mg/l IAA and .3 mg/l KIN had produced the most shoots by the ninth passage. This media also had the highest percentage of jars forming shoots and the highest estimate of shoots produced after the eleventh passage which are still on regeneration media.

Table 1. *Percent of callus types formed by media type over 3 passages.*

Media			E callus	Yellow callus	Green callus
2,4-D	IAA mg/l	KIN			
3	1	.3	00	27	69
5	1	.3	01	53	46
7	1	.3	17	75	09
3	2	.3	15	44	42
5	2	.3	11	63	25
7	2	.3	04	79	19
3	3	.3	17	39	44
5	3	.3	02	53	30
7	3	.3	44	46	10

Technical Note

Acid soils are very complex with many interacting phenomena. In Georgia, two prominent acid soil types have a pH between 4.4 and 4.8 with possible Mn toxicity or a pH between 3.9 and 4.3 with high Al saturation. Dr. Ronny Duncan's breeding program at the University of Georgia (Fig. 2) uses the higher pH soil to initially screen material for tolerance to acid soil. The population which is selected at the higher pH is then placed on the lower pH acid for further testing.

Table 2. *Plant regeneration data for Tift DBE 23.*

Media			Plants Regenerated by 9th passage	% of Jars producing plants	# of Jars in culture	Estimate of the # of shoots in culture
2,4-D	IAA mg/l	KIN				
3	1	.3	0	0	0	0
5	1	.3	0	0	1	5
7	1	.3	63	26	8	50
3	2	.3	101	54	15	200
5	2	.3	0	0	0	0
7	2	.3	0	0	4	35
3	3	.3	55	36	14	100
5	3	.3	0	0	5	30
7	3	.3	31	13	29	125

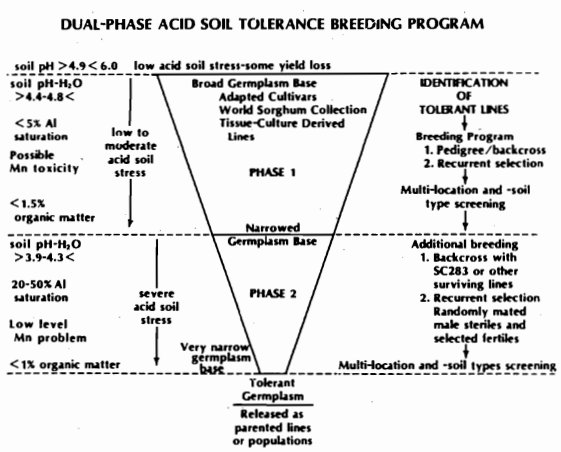

Figure 2. *Schematic of acid soil constraints, narrowing of germplasm base during development, and weeding approaches during the dual-phase program. (Contributed by Dr. Ronny Duncan.)*

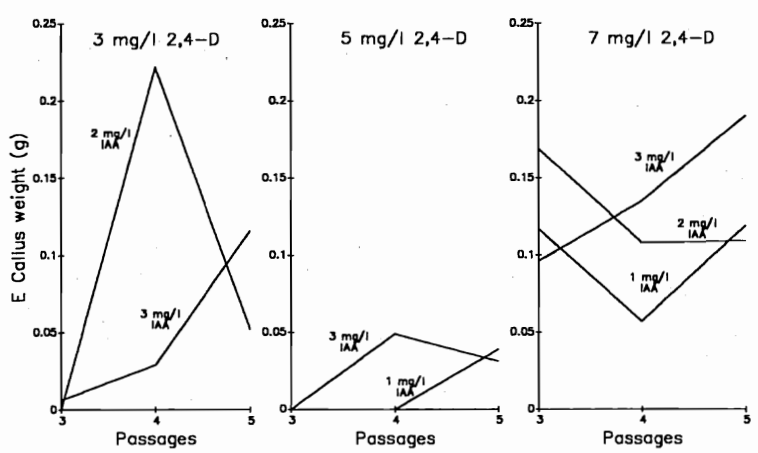

Figure 1. *Embryogenic callus weight on 3, 5 or 7 mg/l 2-4,D and 1, 2, or 3 mg/l IAA. All combinations include 0.3 mg/l KIN.*

Reports from IPBNet Members

I. Dr. C. S. Prakash and Dr. B. A. Thieger, Department of Forestry, University of Kentucky, Lexington, Kentucky 40546-0073, USA.

Background.

Eastern cottonwood *(Populus deltoides Marsh.)* and related species and hybrids are fast growing trees planted for fuelwood, pulpwood, lumber, veneer and as windbreaks and ornamentals. The major topics of research include the development of plant regeneration methods for poplar and the use of RFLP to study tissue culture induced variation.

1. **Plant regeneration.**

Studies in our lab have focused on tissue culture regeneration of plantlets by employing leaf segments from juvenile shoots as explants from clones of *P. deltoides* cv. K417 and *P.* X *euramericana* cv. I-488. Good callus formation was seen around the cut ends of leaf segments incubated in darkness on a woody plant (WP) or LP medium supplemented with 1 μM NAA and 1 μM BA. Maximum shoot regeneration occurred when callus was transferred to a medium without auxin but supplemented with 1 μM BA. Thidiazuron (0.1 μM) was also highly effective in inducing adventitious shoot regeneration from the callus. Excised shoots were subsequently cultured on WP medium with 0.5 to 0.1 μM BA to achieve internode elongation and rooted on WP medium (0.01 μM BA) or in peat plugs (Techniculture, Salinas, CA) kept under intermittent mist. Using this protocol, we have generated many plantlets of cultivars K417 and I-488 and have observed excellent growth of these somaclones under greenhouse conditions; however, other *P. deltoides* genotypes appear to differ in their response to regeneration *in vitro.*

2. **Analysis of somaclonal variants.**

The somaclones are currently being tested for stability through analysis of nuclear and organellar DNA by the RFLP approach and by isozyme analysis. We also intend to test these somaclones for possible changes in leaf rust (*Melampsora medusae* Thum) resistance or susceptibility. This protocol for regeneration is also being employed to obtain transgenic plants through *Agrobacterium* - Ti plasmid transformation.

II. Dr. C. Sivarama Reddy, Tissue Culture Lab, Department of Genetics and Plant Breeding, A.P. Agricultural University, Rajendranagar, Hyderabad 500 030, INDIA.

The tissue culture laboratory was established in June 1985. Three new courses entitled 1) Plant Tissue Culture-I. Principles and Methods (2+1, crs), 2) Plant Tissue Culture-II. Genetic Manipulations and Applications (2+1), and 3) Biotechnology-Genetic Engineering Principles and Methods (3+0) are being offered for graduate students during the fall and spring of every academic year. So far, seven M.Sc. (in rice, chickpea and pigeonpea) and one Ph.D. (in rose) have completed their research. Two M.Sc. (rice anther culture, Oil Palm and Coconut) and two Ph.D. (in rice and chile pepper) students are working in tissue culture at present.

1. **Tissue culture of rice.**

Explants of mature seeds and embryos of Tellahamsa and MTU-2077 cultivars produced good callus while that of Tetraploid (Basmati x Tellahamsa) produced moderate callus. Immature panicles of Tellahamsa cultivar produced callus while the other two cultivars did not. No callus was produced from leaf, stem or root explants of all cultivars. MS medium containing 2 mg/l 2,4-D yielded best response in terms of callus induction and growth compared to LS medium and other hormonal combinations. Calli from immature panicles of Tellahamsa showed a higher frequency of plantlet regeneration in MS medium with 4 mg/l KIN and 1 mg/l IAA (90%) or NAA (80%) and in LS Medium with 2 mg/l KIN and 1 mg/l IAA or NAA (60% each) compared to other hormonal combinations. The presence of 2,4-D in both media reduced plantlet regeneration but induced good callus growth and root formation. About 60% of the regenerated plants survived when transferred to the field after hardening and all had normal flowering and seed set. Tissue culture of chickpea callus was initiated from leaf, stem and cotyledon explants of Annigeri, ICCC-32 and ICCC-42. B_5 medium was better for callus induction and growth compared to MS medium. The root explants of all varieties did not produce any callus. Callus initiation occurred more quickly in leaf explants (6-7 days) compared to cotyledon and stem (15-20 days).

Explants of Annigeri responded by good callusing in all the treatments compared to ICCC-32 and ICCC-42. In general, 5 mg/l 2,4-D and 1 mg/l KIN induced good callus growth from all explants of all varieties. But, further increase in KIN concentration (2 mg/l) inhibited callus growth and development. Different concentrations of 2,4-D and IAA (0.5, 1.0 and 2.0 mg/l) with 4 mg/l KIN were tested with MS and B_5 media for morphogenesis from stem and cotyledonary callus. The combination of 4 mg/l KIN plus 2 mg/l 2,4-D promoted good callus growth and formation of small green embryo-like structures. Using 4 mg/l KIN plus 2 mg/l IAA induced rooting in both MS and B_5 media. No plant regeneration was achieved from callus of the cultivars.

2. **Tissue culture of rose, pigeonpea, chile pepper, oil palm and coconut.**

In vitro propagation of rose through nodal bud culture has been standardized and after three sub-cultures each nodal explant produces as many as 25-30 shoots in MS medium with 2 mg/l BA. The *in vitro* shoots produced roots in MS medium with 3 mg/l IBA in about 15 days. All the operations, from the excision of explants to the transfer of *in vitro*-produced rose plantlets into the field, take about four months. About 80% of the plantlets survived in one field and produced flowers which were true to type.

Work is also in progress for standardization of anther culture techniques for production of haploids in chickpea, pigeonpea, rice and chile pepper and tissue culture of oil palm and coconut has been initiated. Other projects include the development of embryo culture techniques for rescuing hybrid embryos in inter-specific crosses in chickpea and pigeonpea.

3. **Isozyme studies in callus cultures.**

A larger number of peroxidase isozymes were observed in different calli than in the original explant tissue of both rice and chickpea. Specific peroxidase isozyme bands were observed in each explant and the callus of different cultivars of chickpea and rice. In chickpea, the band with Rf 0.81 (in Annigeri), 0.51 (in ICCC-32) and 0.39 (in ICCC-42) were specific only to callus cultures of stem and cotyledon explants.

Electrophoretic studies of differentiating callus cultures of immature panicles of Tellahamsa rice revealed a

larger number of peroxidase isozymes in callus during shoot formation, plant formation, and root formation stages compared to the explant. Five specific bands were found at the shoot formation stage, one specific band at root formation stage and three bands at plant formation stage. There were seven bands common to both shoot and root formation stages, while only two bands were common to all three stages. The occurrence of common and specific bands at different stages of differentiation suggest that a specific marker pattern exists.

III. Dr. J. P. Moss, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru P.O., 502 324, A.P., INDIA.

The Cytogenetics Unit was initiated in April 1978. The initial emphasis in the laboratory was to transfer desirable genes from wild *Arachis* species into tetraploid *A. hypogaea*-like lines which can be used as parents in breeding programs. With the formation of the Legumes Program in 1986, work started on Chickpea, *Cicer arietinum*, and pigeonpea, *Cajanus cajan*.

The Cytogenetics Laboratory is now one of the units working on Biotechnology in the Legumes Program, and studies have started on regeneration from a range of explants and on wide hybridization in pigeonpea and chickpea.

Cytogenetics and Tissue Culture of Groundnut.

In collaboration with the ICRISAT Genetic Resources Unit, we have assembled a collection of wild species of *Arachis* from existing collections in North and South America and maintained the collections and distributed seed to a number of countries. Dr. J. P. Moss has participated in two collecting trips on behalf of the Genetic Resources Unit. Accessions are evaluated for desirable characters, especially disease and pest resistance, in collaboration with ICRISAT Groundnut Pathologists and Entomologists.

Species in section *Arachis* have been cytogenetically analyzed to develop a better understanding of species relationships and the evolution of *A. hypogaea*. This information has been utilized in planning the most effective routes for gene transfer. A range of interspecific hybrids, from diploid to hexaploid, with several combinations of genomes, have been produced and backcrossed to *A. hypogaea*. Many have produced stable tetraploid derivatives. Hybrids and derivatives are screened in the field against rust and late leafspot, the two major yield reducing factors around the world. Selected lines were further backcrossed to produce uniform, resistant lines. They have been agronomically characterized and several high yielding lines have been entered into yield trials of the All India Coordinated Research Project for Oil seeds (AICORPO). These lines are resistant to *Puccinia arachidis* (rust) and *Phaeoisariopsis personatum* (late leafspot), and have good pod and haulm yield, as they retain green leaves until harvest. They have also been supplied to cooperators in and outside of India on request.

In 1987 a new crossing program was initiated for the transfer of early leafspot resistance caused by *Cercospora arachidicola*, another major yield-reducing factor for which a stable source of resistance within *A. hypogaea* is lacking. Accessions like *A.* sp. 30085, *A. chacoense* and *A.* sp. 30003 have been identified with genes conferring resistance to early leafspot. The genomes of these species have been combined with *A. hypogaea*.

The majority of species in the genus are not compatible with *A. hypogaea*. Application of hormones such as GA3 after pollination substantially increases peg numbers. These pegs do not set pods consistently, but a sequential application of auxins and cytokinins at different stages of fruit development increases the number of pods and the size of the ovules. Seeds are usually about 2mm long - rarely about 4mm - and have embryos which are not properly differentiated and/or are generally smaller than 0.5mm. These pods rarely mature and therefore, ovules or embryos are cultured *in vitro* to allow further growth and/or some callus and plantlets. Hybrid calli and plantlets have been produced in five cultivars of *A. hypogaea* crossed with *A.* sp. P. I. 276233, *A. glabrata*, *A.* sp. Coll. No. 6949 (all of Eurhizomatosae). Transfer of these plantlets to soil has not been very encouraging and therefore a technique is being standardized to sustain such rare hybrids by grafting the hybrid shoots onto a parent *A. hypogaea* stock.

Techniques are being developed to regenerate plantlets at high frequency from a range of explants of both cultivated and wild groundnuts. This enables us to multiply certain genotypes, and produce large numbers of hybrids, as well as giving access to cellular and molecular biology to produce new variants through parasexual means.

In 1987, work also started on anther and microspore culture to produce haploids.

IV. Nguyen T. Thanh-Tuyen, Tissue Culture Laboratory, Department of Horticulture, Visayas State College of Agriculture, ViSCA, Leyte 6521-A, Philippines.

The first trial of the study on somatic embryogenesis from six genotypes of corn which include two commercial varieties and four promising lines in response to six culture media adopted from Fahey *et al.* (1986) has been completed. The embryogenic response or percent explants which produced embryogenic (E) callus, including both type I and type II, varied widely with the genotype and culture medium. The overall response was highest in V8426 and V8346 (77.3% and 76.9%, respectively) followed by Improved Tiniguib, V8341 and IPB 2 (57.8%, 55.8% and 38.1%, respectively), and lowest in V8311 (9.75%). Among the culture media, MS medium with 25mM proline added was most favorable for IPB2, V8311 and V8341 while PHL medium with proline was most effective for Improved Tiniguib and V8426. For V8346, the cultured embryos were responsive almost equally to all the tested media. Medium YP-Rap produced very poor embryogenic response in IPB2 and V8341 while RMM6 was ineffective for V8311. In terms of percent increase in fresh weight of E callus after two weeks on initiation medium, V8341 yielded the largest increase (395%), followed by V8346, V8426, V8311 and IPB 2 (in the range of 123-133%) and Improved Tiniguib (90%).

The regeneration ability of cultured embryos of six genotypes varied widely on six media. In general, the regeneration of all genotypes except V8346, as reckoned by percent cultures with shoots (S) and plantlets (P) and number of S and P per zygotic embryo was very poor on YP-Rap medium. Likewise, RMM6 was ineffective for shoot and plant regeneration in most genotypes except V8426. The same trend of variation was observed in percent regenerating cultures and number of S and P regenerated per implanted embryo on the effective media with MS Pro most favorable for IPB2, V8311, PHL for V8346 and PHL Pro for V8341, V8426 and Improved Tiniguib.

In spite of the relatively high regen-

***Report** continued on page 6*

***Report** continued from page 5*
eration potential as measured by average number of S and P per zygotic embryo plated on favorable media, the overall number of plantlets per treatment which survived until potting was quite low. The observations point to the possible problems related to development of shoots into plantlets and growth of the *in vitro* plantlets. It has been commonly observed that the type of plantlets correlates closely with their survival. The erect type survived better than the spreading type with curved leaves. Several factors possibly related to the growth and development of regenerated shoots and plantlets are being investigated.

The effects of arginine on corn somatic embryogenesis have been investigated. Percent embryogenic response of V8341 was highest at 1 mM arginine added to MS (76.7%) and N6 (100%). Likewise, more culture regenerated shoots and plantlets on the medium containing 1mM arginine.

The addition of osmoticum, particularly mannitol (3%) and sorbitol (3%) to MS medium containing 25mM proline has been tried with the aim to retain the regenerative ability of corn callus. Partial results have shown that sorbitol is more effective than mannitol in maintaining the embryogenic condition of the callus.

The R_2 generation of IPB2 plants derived from a corn embryo plated on N6 medium in an earlier experiment was field planted and hand pollinated. All the R_2 plants flowered within a 10-day period at about 62 days after sowing. Plant height at flowering ranged from 190-225 cm. No abnormality of the reproductive structures were observed in R_2 plants. About one half number of R_2 plants produced two ears, 33% produced one ear and 17% had three ears. Nevertheless, seed production was more abundant on number one ear in most of the plants. The R_3 generation has been planted in the field at the present time.

Recent Visitors to the TCCP Facilities

Abdullah Al-Niemi	University of Mosul, Iraq
Yank Banowitz	Ft. Collins, Colorado, USA
John Call	Gilroy Foods, USA
Joel Cohen	USAID/Washington, USA
Ronny Duncan	University of Georgia, USA
Robert Finch	University of Nottingham, England
Kenneth Frey	Iowa State University, USA
Dely Gapasin	PCARRD, Philippines
Tejpal Gill	USAID/Washington, USA
Tom Gorell	Colorado State University, USA
Jack Hautaluoma	Colorado State University, USA
Ken Keegstra	University of Wisconsin, USA
Wayne Keim	Colorado State University, USA
Raymond Kitchell	Alexandria, Virginia, USA
Kauser Malik	Nuclear Institute for Agriculture and Biology, Pakistan
James Mattoon	Colorado College, USA
A. Mujeeb-Kazi	CIMMYT, Mexico
Toshio Murashige	University of California, Riverside, USA
R. S. Paroda	ICAR, India
Donald Plucknett	CGIAR Secretariat, USA
Joy Reese	Denver, Colorado, USA
William Roca	CIAT, Colombia
Otto Silberstein	Gilroy Foods, USA
Guinko Sita	University of Ouagadongou, Burkina Faso
Rodrigo Tarte	CATIE, Costa Rica
Victor Villalobos	CATIE, Costa Rica
Ian Wardlan	CSIRO, Australia
Yongyuth Yuthavong	NCGEB, Thailand

Technical Note: Preparing Solid Media for *In Vitro* PEG Screening

A major barrier in using polyethylene glycol (PEG) in solid media is the inability of agar to solidify with 3300 - 8000 PEG at concentrations above 12%. Recently, a method has been developed by S. Siriwardana, Training Coordinator, and M. Purohit, a participant in TCCP's Training Course, to overcome this difficulty. Gel-Gro™[1], a gellan gum, is used as the solidifying agent. Gel-Gro needs monovalent or divalent cations to solidify and has a higher gelling temperature than agar. Optimal concentration for media without PEG is .3% Gel-Gro. For media containing up to 18% of 8000 PEG a concentration of .45% Gel-Gro is needed.

Procedure for preparing 500 ml of 0.45% Gel-Gro medium containing 18% PEG.

1. Measure out all media components from stock solutions[2] required to prepare 500 ml of full strength in a total volume of 265 ml.
2. Add sucrose and other ingredients. Adjust pH and add 2.25 g Gel-Gro (0.45%). Autoclave the Gel-Gro solution (A).
3. Weigh out 90g PEG and place in measuring cylinder or graduated beaker and dissolve in 145 ml deionized, double distilled water. NOTE: The amount of water added to the PEG = 235 ml - volume of PEG (90 ml).
   Autoclave the PEG Solution (B).
4. After autoclaving and while still very warm, mix the A and B solutions aseptically in a laminar flow hood. Keep the solutions warm on a hot plate or water bath at about 60-C until dispensing into the culture vessels is completed.

[1]Obtained from ICN Biochemical Co., P.O. Box 28050, Cleveland, OH 44128

[2]X10 major stock solutions and X100 micro/trace stock solutions have been used in this example.

Whole Plant Testing Update

Two international site visits were conducted during the spring of 1988 to evaluate TCCP's regenerated material in stress environments. In late March, Dan Miller visited the Philippines to observe a rice study and in early April, Reagan Waskom visited Botswana to evaluate sorghum plots.

Dan Miller traveled to the Philippines on March 25th to observe the progress of the TCCP regenerated rice salinity field test, conducted by Drs. Hernandez and Panningbatan of the University of the Philippines, Los Baños (UPLB). Three hundred and ninety-six R_2 and R_3 lines of IR36, Mahsuri, and Pokkali lines were entered in a saline-prone, irrigated lowland rice trial for salt tolerance evaluations. Data from this test is presently being compiled by Dr. Hernandez and will be analyzed by TCCP in the near future. During his stay in the Philippines, Mr. Miller also visited the tissue culture labs of Dr. Nguyen T. Thanh-Tuyen at the Visayas State College of Agriculture (ViSCA) and Dr. Alfinetta B. Zamora at the Institute of Plant Breeding, UPLB.

Reagan Waskom travelled to Gabarone, Botswana on April 3rd to observe field trials of regenerated sorghum conducted by Dollina Malepa of the Botswana Department of Agriculture. The sorghum trials were composed of ten R_3 lines of *in vitro*-selected Hegari which was replicated four times in two locations. Five different sorghum genotypes were used as drought-tolerant and susceptible checks. One aluminum-tolerant check was also included in the study. All regenerated materials survived to produce seed. As of publication time, the material has been harvested but yield data has not yet been compiled. This collaboration will be expanded next year to include other sorghum lines as well as some millet, tepary bean, and moth bean lines from tissue culture.

Opportunity for Technology Exchange

The IPBNet Newsletter is intended as a forum to provide exchange of new information and technologies. Frequently someone has developed a new or more efficient procedure. Another person, and that person could be you, may need the particular method. A short note in the IPBNet Newsletter would communicate to a wide geographic area.

IPBNet members are strongly encouraged to submit information on a new technology and/or new research data obtained by using tissue culture or other biotechnologies, including micropropagation, plant regeneration, or cell and molecular genetics, for publication in the Newsletter.

Submit a title, a short summary (a maximum of two double-spaced, typewritten pages), your name and address to the TCCP Editor. The information will be published in the next issue of the Newsletter.

Training Program Graduates

Graduates of the fall 1987 Training Program (from left to right): Indira Singh, Rosemarie Bressani, Gabriela Casas, Madhumati Purohit, and Marziah Mahmood.

Short Training Courses

Specialized short-term courses will be held at the TCCP on micropropagation of woody and herbaceous species and on tissue culture and *in vitro* screening for stress tolerance of cereal crops. Both courses will be offered November 29 to December 20, 1988 and April 3 to 28, 1989. Costs for the fall course are $750 for tuition and $500 for living expenses. For the spring course the tuition is $1,000 and living expenses are $600. Submit resumé, statement of interest, proof of financial support, and two letters of reference by August 15 for the fall 1988 course and by January 1 for the spring 1989 course.

For more detailed information contact the Training Coordinator, TCCP, Department of Biology, Colorado State University, Ft. Collins, CO 80523 (Telex 3711418 TISCLT).

The following article was presented at the Science Advisors PSTC Conference in Washington, D.C. June 6-9, 1988, by Dr. A.S. Kumar of the TCCP.

Increased Salt Tolerance in Grain Legumes Through Tissue Culture Techniques

A.S. Kumar, O.L. Gamborg and M.W. Nabors

Grain legumes are second only to cereals in importance as human food. The major objectives of TCCP are to develop and apply the tissue culture technologies to produce plants with increased stress tolerance.

Efficient plant regeneration methods have been developed for three grain legumes, viz., moth bean (*Vigna aconitifolia*), tepary bean (*Phaseolus acutifolius*) and pigeonpea (*Cajanus cajan* L.) for the first time from cell suspension cultures.

The cells were cultured in L-6 medium with 2,4-D or NAA. A critical step was the selection of a particular size of cell aggregates obtained by filtering procedures. The cell fraction with densely cytoplasmic and isodiametric cells were cultured on L-6 medium with 2,4-D and zeatin and plants were regenerated on agar medium containing either BA or zeatin, NAA and GA. The plant regenerating capability of *Vigna* and tepary bean cell lines has remained relatively constant for more than 22 months.

Salt-tolerant cell lines were obtained by growing the normal cell lines in medium with 2.0% NaCl, and selecting the surviving cells. The cells were then multiplied in control medium and again cultured in the saline medium. Sodium chloride-tolerant cell lines were obtained by repeating the above procedure for three more cycles. The tolerant cell line of *Vigna* has been maintained in L-6 saline medium with 2.0% NaCl since December 1986, and plants have been regenerated at regular intervals on sodium chloride-containing L-6 agar medium. The salt-tolerant tepary bean cell line was maintained in L-6 medium with 1.0% of NaCl and plants were regenerated at regular intervals.

The regenerated plants and their progeny are being screened in hydroponics. The progeny of regenerated plants will be field tested on salt-affected soils of Asia and Africa.

TCCP Collaborates with ICRISAT on Tissue Culture and Field Testing

Ms. Sheila Vijayakumar has recently completed one year of research at the TCCP on the application of *in vitro* technologies to chickpea breeding. She has returned to ICRISAT where she has joined the biotechnology group and the chickpea breeding program.

Whole plant testing of pigeonpea lines selected *in vitro* for salt tolerance at the TCCP is also in progress at ICRISAT. Salt tolerance screening is being performed under controlled greenhouse conditions on a portion of the seeds. The remainder of the seeds (R_1) are being multiplied under field conditions to produce the R_2 generation.

TCCP Collaborates with CIAT

Collaboration has been established between the Biotechnology Research Unit at CIAT, under the direction of Dr. W. Roca, and the TCCP. Mr. Paul Chavarriaga Aguirre, Research Assistant from CIAT, is currently a guest worker at TCCP with Dr. Suresh Kumar, who developed the regeneration procedures for tepary bean (*Phaseolus acutifolius*). The suspension cell lines from which plants can be regenerated after more than one year in culture have been used for salt tolerance selections. Mr. Chavarriaga Aguirre is currently applying the procedures to *P. vulgaris* (dry bean) lines from several countries.

Correction

In IPBNet Newsletter No. 8 it was erroneously stated that "Protoplasts from reported research on Taipei 309 (Abdullah *et. al.*, 1986) were not regenerated from suspensions." The sentence should have read, "Research on Taipei 309 (Abdullah *et. al.*, 1986) did not produce plants from suspensions but did produce plants from suspension-derived protoplasts."

The TCCP regrets the error.

TCCP Publications and Reports

Ghazi, T.D., G.E. Hanning, and M.W. Nabors. 1988. Plant regeneration from soybean callus cultured on media containing NaCl. Soybean Genetics Newsletter 15:93-95.

Hanning, G.E. 1987. Brief on insect studies in Georgia. TCCP, Colorado State University, Fort Collins, Colorado.

Hanning, G.E. 1987. Brief on regenerate sorghum plants tolerant to acidic soils. TCCP, Colorado State University, Fort Collins, Colorado.

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Ketchum, J.L.F., O.L. Gamborg, G.E. Hanning, and M.W. Nabors (Eds.). 1987. Progress Report. TCCP, Colorado State University, Fort Collins, Colorado.

Ketchum, J.L.F. (Ed.). 1988. Newsletter No. 8. TCCP, Colorado State University, Fort Collins, Colorado.

Kumar, A.S., O.L. Gamborg, and M.W. Nabors. 1988. Plant regeneration from cell suspension culture of Vigna aconitifolia. Plant Cell Reports 7:138-141.

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MacKinnon, C., G. Gunderson, K.M. Petersen, and M.W. Nabors. 1987. Plant regeneration from salt-stressed sorghum cultures. Life Science Advances 6: No. 2.

MacKinnon, C., G. Gunderson, and M.W. Nabors. 1987. High efficiency plant regeneration by somatic embryogenesis from callus of mature embryo explants of bread wheat *(Triticum aestivum)* and grain sorghum *(Sorghum bicolor)*. *In vitro* Cellular and Developmental Biology 23:443-448.

Siriwardana, S., and G.A. Hildreth. 1987. Handbook for plant tissue culture laboratory organization and management. TCCP, Colorado State University, Fort Collins, Colorado.

Tissue Culture for Crops Project. 1987. Proceedings of the Second Annual Conference of the International Plant Biotechnology Network (IPBNet). Colorado State University, Fort Collins, Colorado.

The International Plant Biotechnology Network

Tissue Culture for Crops Project

Newsletter #10 June 1989

Stress-Tolerant Sorghum Regenerates Identified in Field Trials

Three R_3 lines of sorghum have demonstrated statistically improved field tolerance over their respective non-regenerated controls. Two RTx430 sister lines, 2075-46-1 and 2075-44-6, identified originally from 1986's acid soil evaluation and one Hegari line, 2021-4-1, identified in 1987's acid soil evaluation, were confirmed for acid soil tolerance in 1988 (Fig. 1a). These lines were also evaluated in Yuma, Arizona under severe drought and salt conditions and were among the top lines.

The acid soil site is in collaboration with Dr. R. Duncan, University of Georgia. This field site has a pH range of 4.2 to 4.4 with a high level of exchangeable aluminum. In the 1988 evaluation, none of the 13 non-regenerated Hegari plots survived while all three plots of 2021-4-1 survived and set seed. This is a significant difference for survival based on a Chi-square analysis. RTx430 is very susceptible to acid soils and it is remarkable that in 1986 a RTx430 line set seed. This line is the parent of the two lines, 2075-46-1 and 2075-44-6, both of which set seed in 1988.

The drought site was in collaboration with Dr. R. Voigt, University of Arizona. Drought conditions were imposed by limiting irrigations. Salt accumulation was a secondary stress. The two RTx430 lines were identified in 1987 as being more tolerant than the non-regenerated check. The 1988 site confirmed that observation. Both lines were rated statistically higher for late season vigor and were significantly taller at heading. Line 2075-46-1 was also rated significantly higher for early season vigor and had greater head weight (Fig. 1b). Line 2075-46-1 was also very vigorous under non-stress conditions (Fig. 1c). The Hegari line, 2021-4-1, was planted in Arizona after being identified in 1987 on acid soil. This line was rated significantly higher in 1988 than the non-regenerated Hegari check for late season vigor and early maturity.

This data indicates that somaclonal variants can be identified for environmental stresses and that, in these lines, stress tolerances may be related.

Figure 1. Plots of RTx430 R_3 families 2075-46-1. a: Acid Soil. b: Yuma. c: Seed Increase

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Colorado State University

Important Networks Available to the Scientific Community

As a result of panel discussions during the Third IPBNet Conference in Nairobi, Kenya, (January 8-12, 1989) delegates requested that information be made available on existing plant biotechnology research networks. These programs are primarily affiliated with crop research programs located at the International Agricultural Research Centers (IARC's), universities, and private foundations. They are viewed as means of enhancing collaborative research efforts through personnel exchanges, training, and technical and information assistance.

The following information provides an overview of the types of networks available. Please contact the appropriate person for additional information. If you would like to contribute information on programs not listed for inclusion in future Newsletters, please contact Kerri Wright, IPBNet Coordinator, at the TCCP.

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Department of Biology
Colorado State University
Fort Collins, Colorado 80523, USA

Julie L. F. Ketchum, Editor, Operations Director
Murray W. Nabors, Project Director
Oluf L. Gamborg, Associate Director
Gary E. Hanning, Research Coordinator
Sunitha Siriwardana, Training Coordinator
Kerri L. Wright, Network Coordinator
Reagan M. Waskom, Greenhouse Manager
E. Lee Olton, Office Manager
Jolanta L. Menert, Research Associate, Greenhouse
Daniel R. Miller, Research Associate, Whole Plant Testing
Carol S. Ostrom, Research Associate, Tissue Culture

Technical Staff: Camella Boyd, Adam Boyle, Barbara Bucknam, Jennifer Clark, Laurie Clauss, Dawn Dow, Jan Emming, Michelle Fink, Hal Gibson, Lynn Herrmann, Jeannette Hut, Priscilla Johnston, Heather Loomis, Robert McComb, Ron Monroe, Marcus Murray, Debbie Neubaum, Dana Nichols, Scott Price, Matt Ragsdale, Michael Temple, Robert Tengler, Nitschka ter Kuile, David Timm, Kristin Vanderbilt, Julie Vanderkolk, Lynn Van Male, Brian Wood.

Graduate Students: Raymond Ketchum, Douglas McMurray, Amani Mnayer, Poungpet Poonsapaya, Sathish Puthigae, Gaith Sasi, Nanan Widiyanto.

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Cooperating Agencies include:
Colorado State University, USA
Centro Agronomico Tropical de Investigacion y Ensenanza (CATIE), Costa Rica
Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT), Mexico
IAV Hassan II, Morocco
Philippine Council for Agriculture, Forestry and Natural Resources Research and Development, Philippines
University of Arizona, USA
University of Georgia, USA
University of the Philippines at Los Banos (UPLB), Philippines
Visayas State College of Agriculture (ViSCA), Philippines

For more information, contact:

Tissue Culture for Crops Project
Colorado State University
Fort Collins, Colorado 80523, USA
Phone: 303/491-6996
Telex: 3711418 TISCLT
FAX: 303/491-2293 (Attn. TCCP)

Asian Grain Legumes Network (AGLN)
Groundnut, Chickpea, and Pigeonpea Network of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)

The AGLN began in 1986 as a network within ICRISAT's Legumes Program. It is designed to ultimately assist regional farms by facilitating the interchange of material and information between grain legume scientists at ICRISAT and in Asian countries. Network objectives include:

- Produce a directory of AGLN cooperators
- Operate an information bank for cooperators
- Support identification of adapted grain legume lines
- Promote training of legume scientists from AGLN countries
- Assist in special research projects to support the AGLN

The AGLN is currently active in the following regions and countries: South Asia (Bangladesh, Burma, Nepal, Pakistan, and Sri Lanka); and Southeast Asia (People's Republic of China, Indonesia, the Philippines, and Thailand). A special feature of the AGLN is the coordination of its activities with those of other regional and international research organizations. This is aimed at minimizing overlap of effort.

To fulfill a primary objective, the AGLN is developing a list of cooperators in AGLN countries directly associated with research in groundnut, chickpea, and pigeonpea. This list will include scientists and administrators.

If you are interested in becoming a cooperator in the AGLN, contact:

Coordinator
Asian Grain Legumes Network
ICRISAT, Patancheru
Andhra Pradesh 502 324 India

Cooperative Cereals Research Network (CCRN) of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)

This network serves to provide an effective link among sorghum improvement programs around the world. The main objectives are to:

- Make the world's elite breeding lines, cultivars, and hybrids available to sorghum scientists in existing breeding programs.
- Provide sorghum scientists with

an opportunity to assess the performance of theirown advanced breeding lines and released cultivars in various climatic, soil, disease, and insect conditions.

- Identify lines with stable resistance to major diseases, insects, and other stresses
- Serve as a center for information on sorghum genotype performance over a range of environments.

For more information contact:
Coordinator
Cooperative Cereals Research Network
ICRISAT, Pantancheru
Andhra Pradesh 502324 India

Rice Tissue Culture at IRRI

In nearly ten years, tissue culture at IRRI has progressed from utilizing anther culture to include other techniques such as isolated pollen culture, somatic cell culture, and protoplast culture. Studies on rice transformation, using the technology on isolated pollen and protoplast cultures, are being initiated. Anther culture is being utilized for the immediate production of homozygous lines from F_1 crosses for tolerance to adverse conditions such as cold, salinity, drought, and blast in collaboration with various national programs and IRRI scientists. Research on somatic cell culture provides scientists with the opportunity of exploring its utilization as a method of mass production of planting material for hybrid rice. Research on protoplast culture at IRRI has been successful in regenerating plants for Japonica (Taipei 309 and Taipei 177) and Indica (Telep) cultivars. Current research focuses on increasing the efficiency of various tissue culture techniques, and is geared towards the development of protocol for somatic hybridization and gene transfer.

For additional information contact:
Dr. Francisco J. Zapata
Tissue Culture Specialist
The International Rice Research Institute
College, Laguna, Philippines

IRRI accepts trainees in rice tissue culture on a case-to-case basis, with priority given to participants from national programs. Interested parties may send requests for additional information to:
Dr. M. D. Pathak
Director for Research and Training
IRRI
P.O. Box 933
Manila, Philippines

CIP Collaborative Research Networks

The International Potato Center (CIP) has created its own model for collaborative research networks involving intercountry planning, joint implementation of research, and monitoring of mutual regional problems. Over the past decade, CIP has promoted the initiation of five collaborative research networks - - three in Latin America, one in East Africa, and one in South East Asia -- dedicated to the improvement of the potato crop through research and transfer of technology.

1. Programa Regional Cooperativo de Papa (PRECODEPA)

This is the first of CIP Networks to be founded, beginning in 1978. Member countries include Costa Rica, Cuba, Dominican Republic, El Salvador, Guatemala, Haiti, Honduras, Mexico, Nicaragua and Panama. The cooperating source for thisnetwork is the Swiss Development Cooperation (SDC). The Program Coordinator is Ing. Roberto Rodriguez (Panama).

2. Programa Andino Cooperativo de Investigacin en Papa (PRACIPA)

This network was formed in 1982 and consists of primarily the Andean countries: Bolivia, Colombia, Ecuador, Peru and Venezuela. The cooperating source for this network is the International Development Research Centre (IDRC) of Canada. The network coordinator is Dr. Pedro Leon Gomez (Columbia).

3. Programa Cooperativo de Investigaciones en Papa (PROCIPA)

This network, also formed in 1982, includes Argentina, Brazil, Chile, Paraguay and Uraguay. Since its initiation, this network has received direct support from CIP, both technically and financially. An alternative donor is currently being explored, with hopes of financial support by the end of 1989. The network is coordinated by Dr. Oscar Hidalgo (CIP).

4. Southeast Asian Program for Potato Research and Development (SAPRAD)

Research and development in potatoes in South Asian countries is often limited by a shortage of resources, both human and financial. In countries where food systems have been traditionally based on rice, collaborative research in potato is necessary to maximize resource efficiency. The SAPRAD was formed in 1982 and consists of Indonesia, Malaysia, Papua New Guinea, Philippines, Sri Lanka and Thailand. The funding for the network is sponsored by the Australian Development Assistance Bureau (ADAB), in conjunction with CIP. The network coordinator is Dr. Ponciano Batugal (CIP).

5. Programme Rgionale d'Amlioration de la Culture de Pomme de Terre en Afrique Centrale (PRAPAC)

In 1981, CIP scientists in Africa were approached by representatives of the Communite du Pays du Grands Lacs (CPLG), a regional development authority of the francophone countries bordering the Great Lakes: Burundi, Rwanda and Zaire. A network was formed to assist in the design and preparation of potato production, identification of research priorities, and to provide in-service training for national technicians. Later, Uganda was admitted as the fourth member. This program is supported by the United States Agency for International Development (USAID) and coordinated by Mr. Jeroen Kloos (CIP).

For further information concerning the CIP Collaborative Research Networks, contact:
Dr. Fernando N. Ezeta
Network Specialist
International Potato Center (CIP)
Apparrado 5969
Lima, Peru

East and Southern Africa Root Crops Research Network (ESARRN)

The ESARRN was formed in 1985 when the heads of national root crops programs of East and Southern Africa agreed that their common problems warranted a regional approach to supplement their national program activities.

Its member countries are: Burundi, Ethiopia, Kenya, Madagascar, Malawi, Mozambique, Rwanda, Tanzania, Uganda, Zanzibar, and Zambia.

The ESARRN's mission is to strengthen collaboration between national agricultural research programs and IITA, and among national programs themselves, and to facilitate the transfer and exchange of technologies on improved varieties, agronomicpractices, post harvest, and other related improved technologies of root crops. It also supports staff development of national agricultural research programs from East and Southern Africa through training.

Through collaboration in research and training, ESARRN assists national agricultural research programs in strengthening their research capabilities in root crops and in their efforts to address specific problems that will improve food production, farmers' income, and contribute to food self-sufficiency.

For further information, contact:
The Co-Coordinator
ESARRN
Chitedze Research Station
P.O. Box 158
Lilongwe, Malawi

Joint Division of FAO (Food and Agricultural Organization of the United Nations) and IAEA (International Atomic Energy Agency)

A main feature of the FAO/IAEA activities is the research contract program designed for promoting technology development and refinement. Coordinated Research Programs having specific objectives comprise ten to twelve research institutes from developing and developed countries. Programs in developing countries receive some cost-sharing research support (US$ 3000- 5000 per year).

Networks continued on page 4

Networks continued from page 3

Examples of objectives of such coordinated research networks are:

- improvement of grain protein content in cereals
- improvement of nitrogen fixation in grain legumes
- improvement of tropical root and tuber crops
- improvement of disease resistance using *in vitro* toxin screening.

Funds available to the Joint FAO/IAEA Division permit the simultaneous operation of two to three networks; however, with additional funds from donors, more can be accomplished. Results of such work are published in the FAO/IAEA Mutation Breeding Newsletter, in technical documents, and in the form of books.

Each year, the Plant Breeding Unit of the Laboratory at Seibersdorf hosts a six- week international training course which provides instruction to researchers from developing countries within the framework of the IAEA Program of Technical Assistance and Cooperation.

For more information, contact:
Dr. Alexander Micke, Head
Plant Breeding and Genetics Section
Joint FAO/IAEA Division
P.O. Box 100
A-1400 Vienna, Austria

The International Board for Plant Genetic Resources (IBPGR) International Database

The IPBGR *In Vitro* Conservation Databases have been operating since 1980. Four surveys, carried out every two years, were used to gather information from scientists in over 70 countries worldwide. The Databases hold information on the *in vitro* culture of a wide range of crops, but concentrate on those that have a genetic conservation problem due to vegetative propagation and sterile or recalcitrant seed production. As a result of the most recently completed survey (1987/88), 506 species in 270 genera are covered. The survey acquired information on the following areas:

- field of interest/application
- explant used
- clonal propagation procedure
- operational and biological problems
- characterization of cultures and plants
- disease indexing
- storage of cultures in normal and in slow growth
- cryopreservation
- *in vitro* collection and exchange/distribution.

The plant material categories covered in the Databases include root and tuber crops, industrial crops, fruits, and others including medicinal plants and spices, legumes, cereals and grasses, ornamentals, and woody species.

The Databases can be searched using very general or very specific inquiries. For example, they can provide information on all scientists working in a particular country or records of the successful cryopreservation of a named species. Searches are carried out at no charge to users. The Databases are currently under review to determine their usefulness to the scientific community. Scientists are invited to submit comments on the value of research databases as aids to remaining up-to-date on developments in biotechnology and *in vitro* culture. Requests, detailing the exact information needed, should be sent to:

Ms. Shelagh Wheelans
IPBGR *In Vitro* Conservation Databases
Dept. of Agriculture and Horticulture
School of Agriculture
Sutton Bonnington
Loughborough LE12 5RD
United Kingdom

For additional information on the IPBGR *In Vitro* Conservation Research Programme, contact:

Dr. Lyndsey Withers
IPBGR
C/O FAO of the United Nations
Via delle Terme di Caracalla
00100 Rome, Italy

International Network for the Improvement of Banana and Plantain (INIBAP)

An international network for banana researchers has recently been formed containing four regional components, each with a regional coordinator. The regions are: Latin America and the Caribbean, West Africa, East Africa and Asia. The INIBAP receives financial support from several conventional donors.

The network has several thematic activities and workshops, ranging from taxonomy to pathology and biotechnology.

For more information, contact:
Dr. E. DeLanghe, Director
INIBAP
Avenue du Val de Monferrand
B.P. 5035
34032 Montpellier Cedex, France

International Cassava-Trans Project

The Cassava-Trans project is focused on two viral diseases of cassava; the cassava common mosaic virus which is present in South America, and the African cassava mosaic virus which infects most of the African cassava cultivars. Research involves transferring the coat protein genes of these viruses via transformation. This is predicted to confer to the transgenic plants a new type of monogenic resistance specific to these viruses, based upon the results of other experiments. The project is a result of collaborative efforts of the French research organization "ORSTOM," and Washington University of St. Louis, Missouri which houses the project in the laboratory of Dr. R. N. Beachy. The program is currently funded by the ORSTOM and the Rockefeller Foundation. The Cassava-Trans Project, composed of three French researchers and one German tissue culturist, is linked to European research laboratories and International institutes working with cassava, namely IITA in Nigeria and CIAT in Colombia.

In addition to the Cassava-Trans Project, ORSTOM is also assisting in the organization of the IVth International Plant Virus Epidemiology Workshop focusing on "Resistance to Viruses and Vectors -- Temperate and Tropical Plants," in Montpellier, France, September 3-8, 1989.

For more information contact:
Dr. Claude Fauquet
Washington University -- ORSTOM
Department of Biology
Campus Box 1137
One Brookings Drive
St. Louis, Missouri 63130 USA

Dr. Roger N. Beachy, Director
Center for Plant Science and Biotechnology
Campus Box 1137
One Brookings Drive
St. Louis, Missouri 63130 USA

Rockefeller Foundation's International Program on Rice Biotechnology

The Rockefeller Foundation's International program on rice biotechnology is an integrated set of research, training, and capacity-building activities structured to produce improved rice varieties for developing countries. Program objectives are: 1) Using basic science to insure that the techniques of biotechnology are developed for rice; 2) creating the capacity to conduct rice biotechnology research in rice- dependent developing countries; and 3) understanding the consequences of agricultural technical change and determining priorities for biotechnology applications.

Early in the program an assessment was made of priority needs. Support was provided to a group of the world's leading plant molecular and cellular biology laboratories, to move rice biotechnology research to a status comparable to current biotechnology programs in the major cereals of the developed world. Drawing upon the knowledge base, rice biotechnology tools, and training opportunities provided bythe advanced laboratories, the program is now emphasizing research on agronomic traits, technology transfer, and establishing the capability to utilize the new technologies at the international

centers and in developing countries. In addition, in those developing countries having an adequate scientific establishment, the Foundation will help build the scientific capacity and fundamental research programs necessary to manage future development of rice genetic improvement technologies.

For further information contact:
Dr. Gary H. Toenniessen
Associate Director for Agricultural Sciences
The Rockefeller Foundation
1133 Avenue of the Americas
New York, New York 10036 USA

DNA Bank-Net: A Network of DNA Banks for the Conservation and Utilization of DNA

Although still in the formative stages, DNA Bank-Net will be a network of DNA Banks (repositories) at institutions on every continent. During the next ten years, DNA Bank-Net will give preference to the acquisition of DNA from rare and endangered species. Each DNA acquisition will be documented by an herbarium voucher, and information on the source, collector, date of acquisition, etc. will be kept in a database. Additional DNA banks will be started in cooperating countries as a safety net and backup system, and to provide easier access to DNA in developing countries. Institutions from the following countries will participate: China, Colombia, Costa Rica, England, Ethiopia, India, Japan, Kenya, Mexico, Nepal, New Zealand, Scotland, Swaziland, Thailand and the U.S.A. The DNA Bank-Net Center at Baylor University will also develop a program to train international colleagues in techniques for extracting, curating, and amplifying genomic DNA. The DNA-Bank Net is planning its first international workshop, hosted by the Royal Botanic Garden in Kew, London, in the spring of 1990.

For additional information, contact:
Dr. Robert P. Adams, Director
DNA Bank-Net/Baylor
Baylor University
BU Box 7372
Waco, TX 76798 USA

The International Association for Plant Tissue Culture

The International Association for Plant Tissue Culture (IAPTC) is an international organization which facilitates continuous communication between parties interested in plant tissue culture and associated technologies. The IAPTC publishes a quarterly newsletter which reports on research activities in specific laboratories, provides news on conferences, and announces recent publications.

Every fourth year, the IAPTC holds an international Congress. The 7th Congress will be held from June 25-30, 1989 in Amsterdam, The Netherlands. Questions regarding the Congress should be directed to:

Organization Buro Amsterdam
IAPTC Congress Europaplein
1078 GZ Amsterdam
The Netherlands

Membership in the IAPTC has grown from 2740 in 1986 to 3990 in 1988. The current membership fee is $9.00 A membership list is in preparation, and will be sent to members. Anyone interested in joining the IAPTC should contact either their country's National Correspondent, or write directly to the IAPTC secretary:

Dr. Ad J. Kool
Zaadunie Research
Plant Biotechnology Division
P.O. Box 26
1600 AA Enkhuizen
The Netherlands

Visitors to the TCCP Laboratory

Alberto Acevedo	Instituto Nacional de Tecnologia Agropecuaria, (INTA), Argentina
Tom Bartlett	Colorado State University, USA
Bob Briggs	Colorado Greenhouse Growers Assoc., USA
Carlos B. Carpio	PCA-ARC, Philippines
Jerry Dodd	University of Wyoming, USA
Frank Ellison	University of Sydney, Australia
Chris Ells	Kitayama Brothers, Colorado, USA
Bonnie Ennis	Adams County Extension, Colorado, USA
Marco Flores	Colorado State University, USA
Junzo Fujigaki	Tokyo University of Agriculture, Japan
Yuri Y. Gleba	Institute of Botany, UAS, USSR
Hironoto Goda	All-In-One, Inc., Japan
Hiroki Hamada	Okayama University of Science, Japan
Leo Hartman	Veldkamps Greenhouse, Colorado, USA
Kuminori Honda	All-In-One, Inc. Japan
Marcelo Huarte	Instituto Nacional de Tecnologia Agropecuaria, (INTA), Argentina
Mohamed Abdo Ismail	Mogadisho, Somalia
Jing-fen Jai	Lanzhou University, China
Carl Kaspar	Pikes Peak Greenhouse, Colorado, USA
Jeanette Katuuk	IKIP, Indonesia
P.P. Khanna	National Bureau of Plant Genetic Resources (NPBGR), India
Toyomi Kokubun	All-In-One, Inc. Japan
Josefina Lantican	Bureau of Agricultural Research, Philippines
Zaida Lentini	Cornell University, USA
H. R. Lerner	The Hebrew University, Israel Ludi Li Huazhong Agricultural University, China
Willie L. McCuistion	NARP/ARC, Egypt
Hiroshi Mitami	All-In-One, Inc., Japan
Mohamed Fauad Mohamed	Egypt
Sama S. Monde	Rice Research Station, Sierra Leone
A. Mujeeb-Kazi	CIMMYT, Mexico
Musa Mohamed Musa	Agricultural Research Corporation, Sudan
Yasnori Nagaya	All-In-One, Inc. Japan
Julius Odongo	Namulonge, Uganda
G. M. Reddy	Osmania University, India
Satyapal	United Nations Development Program (UNDP), USA
Laxman Sherchaud	Ministry of Agriculture, Nepal
Bhola Meher Shrathe	Ministry of Agriculture, Nepal
Shambhu L. Shrestha	NARSC, Nepal
Veronica Silversmith	Navajo Agricultural Products Industry, USA
B.P. Singh	NPBGR, India
Ponpimon Suriyajantratong	Khon Kaen University, Thailand
Dave Swift	NREL/Colorado State University, USA
R. J. Swuntz	Tagawa Greenhouses, Colorado, USA
Moshe Tal	Ben Gurion University, Israel
Assa Tawfik	Egypt
Jun Termi	All-In-One, Inc. Japan
Yoshiaki Tozama	All-In-One, Inc., Japan
Dale Wirth	BIA, USA
Dirayos Wititsuwannakul	Mahidol University, Thailand
Rapepun Wititsuwannakul	Prince of Songkla University, Thailand
Heidi Yamamoto	Denver, Colorado, USA
Koichi Yanagita	All-In-One, Inc., Japan
Abdelouahhab Zaid	INRA, Morocco

Graduates of TCCP Training Course Find Uses for Their Skills

Ms. Iteu M. Hidayat
Lembang Horticultural Research Institute
P.O. Box 587
Bandung, Indonesia

Tissue culture in Indonesia has taken on more importance since the Government of Indonesia instituted a policy of increasing non-petroleum exports, reducing imports, and promoting the agriculture sector which supports the industrial sector. This policy has encouraged many agrobusiness companies from the private sector to establish businesses for production of crops for export and import substitution. Horticultural crops in high demand include potato, asparagus, garlic, onion, and bamboo shoot.

The Lembang Horticultural Research Institute has been given the task of providing farmers with the right varieties and good quality seeds, and developing cultural practices to improve horticultural crops production. At the Institute, a tissue culture laboratory is in a position to support the plant breeding program.

Meristem culture, micropropgation, and rapid multiplication techniques for virus-free seed potato production have been routinely applied. Based on the techniques for potato, similar procedures are being adapted for garlic, onion, and asparagus. Germplasm collection, *in vitro* storage, and maintenance of virus-free stocks of those crops is under study as well.

The application of tissue culture techniques for crop improvement has been limited by facilities and financial problems; however, anther culture to produce haploid plants of local hybrid cabbages and somaclonal variation to produce genetic variability in garlic are under study.

There are also some other problems that tissue culture can help resolve, such as selection for drought tolerance in beans, or selection for resistance to diseases, heat, mineral toxicity and acidity in other important horticultural crops. These will be taken into consideration in breeding programs in the near future.

Dr. Flordeliza B. Javier
Assistant Professor
Department of Horticulture
UPLB, Los Banos
College, Laguna
Philippines 4031

Dr. Flordeliza B. Javier, a graduate of the fall 1985 session of the TCCP Training Program, has established the Tissue Culture Laboratory of the Fruit Crops Division, Department of Horticulture, at the University of the Philippines at Los Banos (UPLB). Dr. Javier teaches three classes at UPLB, including a team-taught course in Plant Tissue Culture. She also taught two short courses in May 1989, one on tissue culture of banana.

Dr. Javier is currently conducting a study of several banana cultivars (*Musa* sp.) to determine the effect of different cultural systems on shoot regeneration and proliferation.

Ongoing research also includes *in vitro* clonal propagation of exotic fruits such as rambutan (*Nephelium lappaceum* L.), mangosteen (*Garcinia mangostana* L.), and durian (*Durio zibethinus* L.). Research on *in vitro* propagation of Calamandarin (*Citrus* sp.) is also in progress. Calamandarin is a root stock plant used for grafting to citrus varieties in the Los Banos area, and is resistant to drought and disease; however, there are few plants available for propagation purposes and seed production is highly seasonal. Another promising root stock plant, *Feronia limonia* L. Swingle, which is reportedly resistant to drought and diseases as well as being suited to highly acidic soils, is being used for *in vitro* grafting to *Citrus* sp.

Graduates of the Spring 1988 session of the TCCP Training Program (left to right): Anastasios Bouraimis, Stanley Ndungu, Ruth Daniels, Todor Gubatov. Instructors: Sunitha Siriwardana and Harrison Hughes.

Mrs. Erlinda P. Rillo
Philippine Coconut Authority
Agricultural Research and Development Branch
Albay Research Center
Banao, Guinobatan, Albay
Philippines

Following the request of the Philippine Government, the Ministry for Economic Cooperation of the Federal Republic of Germany agreed to fund a coconut tissue culture development project and commissioned the Deutsche Gesellschaft fur Technische Zusammenarbeit to plan, implement and monitor the project. The three-year implementation phase began in February 1989. Depending on the results obtained during the implementation and succeeding main work phases, the project has a programmed horizon of 15 to 20 years until the technology is developed to a commercially viable level.

Mrs. Erlinda P. Rillo of the Philippine Coconut Authority (PCA), Albay Research Center, is the Senior Filipino Scientist of the project and a fall 1986 graduate of the TCCP Training Program. Together with three other researchers, Mrs. Rillo will work in close collaboration with the project consultant, Dr. Andreas W. Ebert, and senior consultant Dr. Jennet Blake who is based at Wye College, University of London.

The project aims to contribute to higher and earlier coconut yields attained by more rapid production of high yielding planting material of reduced variability. Specific goals include the development of the most effective medium for callus culture and embryogenesis. Further research will include a comparison of plantlet development and growth in a range of media, humidity, light, etc.

IPBNet Conference Held in Nairobi

The Third IPBNet Conference, which attracted 92 persons from 30 countries, was held in Nairobi, Kenya, January 8,-12, 1989. Program topics included plant breeding, tissue culture, and novel genetic biotechnologies. Nine invited speakers presented research in their respective areas of expertise. Plenary sessions were followed by oral and poster presentations by Conference participants.

The keynote speaker was Dr. Murray W. Nabors, Director of the TCCP. He spoke of current obstacles to agricultural development and food production including soil salinity, soil acidity and drought, which affect large portions of agricultural land. He emphasized plant biotechnology as a component of the solution to these problems and noted the importance of collaboration among scientists with limited access to technological information and resources. Dr. Nabors stressed the need for a "basic/applied interface" to move technologies from the lab to the field and, ultimately, to the people.

Exchange of ideas at various levels proved once again to be a valuable asset of the Conference. Participants voiced strong support of IPBNet as a means of keeping scientists informed. Enthusiasm was also expressed for expansion of regional tissue culture training programs. Two such programs, offshoots of the six-month "Tissue Culture Technologies and Laboratory Practices" course at the TCCP, already exist in Costa Rica at CATIE and in Morocco at IAV Hassan II. The Egyptian participants also expressed an interested in hosting an Arabic-speaking tissue culture short course.

Aside from the plenary sessions, one morning was devoted to Round Table discussions led by experts in the respective topics. These discussions, which covered the areas of National Policies, Applications of Biotechnologies, Networking, and Financial Resources, were a unique feature of the Conference which allowed participants to voice their concerns and ideas with respect to the future of plant biotechnology research. Various obstacles, such as lack of funding, supplies, equipment, electricity, water, appropriate and affordable technologies, training, and information -- including scientific references and current research being conducted -- were discussed.

Recommendations were made by each Round Table panel as to the possible solutions to these problems. Networking, particularly IPBNet, was seen as a vital component of the solutions in-so-far as it is able to provide training, technical assistance, and information to researchers and administrators of research programs.

One tangible result of the Round Table discussions was the formation of an African biotechnology network, which will be coordinated by Dr. A. Ian Robertson of the University of Zimbabwe.

Participants of the Third Conference of the International Plant Biotechnology Network (IPBNet).

Financial support for the IPBNet Conference was provided by over 30 donors, including 18 government agencies, eight private organizations, and four International Centers. In particular, support for plenary speakers was provided by CIP, ICRISAT, McCormick and Company, Monsanto, Pioneer Hi-Bred, Pioneer Overseas, Twyford Laboratories, and USAID.

The Fourth IPBNet Conference is planned for January 1991 in Costa Rica. Details will be available in the next issue of the IPBNet Newsletter.

TCCP Affiliates Receive Advanced Degrees

The staff of the TCCP extends its congratulations to four recent graduates. Ms. Iteu Hidayat, who was supported by Winrock International, completed her Master of Science in Botany in October 1988 and presented her thesis on "Heat Screening and Plant Regeneration from Suspension Cultures and Protoplasts of Diploid *Solanum* Species." She has since returned to the Lembang Horticultural Research Institute in Indonesia where she is writing proposals to work on haploid production of *Brassica*, drought tolerance in beans, somaclonal variation in garlic, and to continue her research on potato. Ms. Hidayat is also a graduate of the TCCP Training Program.

Dr. Akbar Mohmand completed his Ph.D. in Botany and returned to Pakistan in March. Dr. Mohmand, who was supported by FAO during his program at Colorado State University, presented his dissertation on "Stress Selection, Osmoregulation, Somaclonal Variation, and Long-Term, High-Frequency Plant Regeneration in Wheat."

Ms. Anna Novero, a TCCP-supported graduate student who completed her M.S. in Botany this spring, presented her research on "In Vitro Selection for Improved Nitrate Reduction in Rice. Ms. Novero plans to return to the Philippines to do research either at IRRI or UPLB.

Also completing a Masters degree this spring was Ms. Kerri Wright, IPBNet Coordinator for the TCCP. Ms. Wright, who received her degree in Agronomy, presented her professional paper, entitled "Evaluation of a Short Term Tissue Culture Training Course," in March.

Ms. Wright surveyed 25 graduates of the "Tissue Culture Technologies and Laboratory Practices" training course offered by the TCCP to determine the effectiveness of the Course in transferring tissue culture technology to developing countries. Results showed that 72 percent of the respondants are currently engaged in research and/or advanced degree programs which utilize the skills they obtained in training. Upon immediate return to their home countries, 60 percent of the respondents returned to the same institution and position they held prior to training. Various socio-economic factors were also surveyed to gauge how training had helped in career advancement. Thirty-two percent of those responding reported increased job responsibilities as a result of their training, while 40 percent reported no significant changes in job status.

Overall, the Training Course received a positive evaluation from the respondants, with 88 percent reporting that their expectations were met and 80 percent answering that the Course prepared them for the problems and challenges encountered in their current research. Recommendations were also made by the respondents for future program changes and incorporated into the study as a basis for continued program development.

Effect of pH on $AlCl_3$-Stressed and Non-Stressed Rice Tissue Cultures

Many regions of the world have acidic soils. In acidic soils, heavy metals which are usually insoluble dissolve into soil solution, and thus become available for plant uptake.[1] Aluminum is a constituent of soil clay materials. Therefore, aluminum toxicity is theoretically possible in most, if not all, soils and occurs when soil pH decreases to levels where the clay mineral structure decomposes.[2]

Tissue culture has been praised as a means of performing selections in petri dishes that would require hundreds of acres at the whole plant level.[3] Recent experiments at the Tissue Culture for Crop Project involved testing IR- 36 rice embryogenic callus cultures on four different treatments, consisting of two different pH levels (4.2 and 5.5), and two levels of $AlCl_3$ stress (0 and 100 ppm) in a factorial arrangement of treatments. Seeds were initiated directly on the four media treatments and were maintained for three passages on stress and two on regeneration (each passage equals four weeks).

A continuing problem with initiating cultures is fungal contamination. Soaking Clorox-sterilized rice seeds in a 0.1 percent Benlate fungicide overnight reduced the contamination rate to zero (Table 1). This proved very useful, for it allowed several seeds to be initiated on one petri plate without contamination.

Callus initiated from seed directly on stress showed that statistical differences existed among the passages (Table 2). Mean embryogenic callus weight was expected to increase consecutively with each passage. The low mean for Passage 4 was attributable to an overheating of the culture room during in the third passage of this experiment.

Treatments were not significantly different but were fairly close ($Pr > F = 0.0825$). The trend seen was as expected with the control (pH=5.5 $AlCl_3$=0) having the highest embryogenic callus weight (Table 3). The lowering of the pH or the addition of 100 ppm $AlCl_3$ reduced the callus weight similarly. The combination of the lower pH and the $AlCl_3$ reduced the callus weight the greatest amount.

This experiment indicates that the effect of $AlCl_3$ on callus growth is enhanced by reducing the pH of the media. Plants coming out of this type of *in vitro* selection method may be better adapted to acidic soils with high levels of exchangeable aluminum.

References

1. Massey, H.F. and Barnhisel, R.I. 1972. "Copper, Nickel, and Zinc released from acid coal mine spoil materials of eastern Kentucky." *Soil Science*. 113:207-12.

2. Lepp, N.W. (editor). 1981. *Effects of Heavy Metal Pollution on Plants; Volume 2, Metals in the Environment*. Applied Science Publishers. London and New Jersey. p.64-69.

3. Hanson, M.R. (Staples, R.H. and Toenniessen, G.H., editors). *Salinity Tolerance in Plants*. "Cell Culture and Recombinant DNA Methods for Understanding and Improving Salt Tolerance." John Wiley and Sons. New York. pp. 335-360.

Table 1. Effect of 0.1% Benlate on Seed Contamination Rate for IR-36

Procedure Initiated	Total # Seeds Initiated	Total # Seeds Contaminated	% Seeds
no Benlate	250	66	26.4
0.1 % Benlate	306	0	0.0

Table 2. Summary of mean embryogenic (E) callus weights for the first three passages.

Passage	Mean E Callus Weight (g)
3	0.08162 A*
4	0.06477 A
2	0.03693 B

*Means followed by the same letter are not significantly different at the 0.05 level according to Duncan's Multiple Range Test.

Table 3. Summary of mean embryogenic (E) callus weights for the media treatments during the three passages.

Initial pH	$AlCl_3$ ppm	Mean E Callus Weight (g)
5.5	0	0.0805
5.5	100	0.0631
4.2	0	0.0570
4.2	100	0.0438

Articles Requested

IPBNet members are strongly encouraged to submit information on a new technology and/or new research data obtained by using tissue culture or other biotechnologies, including micropropagation, plant regeneration, or cell and molecular genetics, for publication in the Newsletter.

Submit a title, a short summary (a maximum of two double-spaced, typewritten pages), and your name and address to the TCCP Editor. The information will be published in the next issue of the Newsletter.

Post Docs Welcome

The TCCP is accepting applications for post doctoral positions in plant biotechnology, including tissue culture and protoplast technologies, using crop plants. Post docs will be considered for the period of August 1, 1989, to July 31, 1990. Funding for these positions must come from the applicants or their sponsors. Please consult the IPBNet Directory for possible sources of support.

Submit a letter of interest, research plan, and *curriculum vitae* to:

Ms. Julie L.F. Ketchum
Operations Director
Tissue Culture for Crops Project
Colorado State University
Fort Collins, Colorado 80523 USA

The International Plant Biotechnology Network

Tissue Culture for Crops Project
Newsletter #11 May 1990

Fourth IPBNet Conference Set for 1991

Registrations are being accepted for the upcoming IPBNet Conference, entitled "Biotechnology for Tropical Crop Improvement in Latin America." The Fourth IPBNet Conference will be held at the Aurola Holiday Inn in San Jose, Costa Rica, January, 14-18, 1991. The Conference will be held in English and Spanish via simultaneous translation. Proceedings, however, will be printed in English only.

The Conference will begin with Keynote Speaker Dr. Gunter Kahl of the University of Frankfurt. Dr. Kahl is a geneticist and has conducted extensive research on transformation techniques, especially in yams. On Wednesday, Conference participants will attend a field trip to CATIE in Turrialba. Friday is reserved for poster presentations and roundtable discussions. Three afternoons have been reserved for oral presentations by participants. There will be 30, 20-minute oral presentations accepted; early submission of abstracts is suggested.

Accommodations are available at the Aurola Holiday Inn at a cost per day of $89.51 single and $95.17 double, including tax. A block of rooms is being held for Conference participants until November 1. A Hotel Reservation Form is included in this newsletter. Please note that hotel reservations and payments should be made directly to the hotel.

Registration is $125 per person and includes Conference materials, proceedings, coffee breaks, lunches, the dinner banquet, and the field trip to CATIE. Payments should be made in U.S. Dollars.

The deadline for abstract submission and registration is October 1, 1990. A late charge of $25 is due with registrations after October 1. All abstracts must be submitted in English. Refunds will not be made after December 1, 1990.

Conference Program

Registration: Sunday, January 13, 1991, 5 to 7 p.m.

Monday, January 14, 1991.

Session I: Transformation Techniques
Keynote Address: Dr. Gunter Kahl - Engineering Fungus Tolerance into Dioscorea (yam) Plants.
Plenary Speaker: Dr. Roger Beachy - Constructing Genes for Virus Resistance in Tropical Plants.
Oral presentations by participants.

Tuesday, January 15, 1991.

Session II: Biotechnology at International Centers.
Plenary Speakers: Dr. Victor Villalobos - Agricultural Research and Training at CATIE. Dr. William Roca - Biotechnology Research at CIAT. Dr. John Dodds - Biotechnology for Potato Improvement at CIP.
Oral presentations by participants.

Wednesday, January 16, 1991.

Field trip to CATIE and tour of facilities.

Thursday, January 17, 1991.

Session III: Utilization of Biotechnology.
Plenary Speakers: Dr. Joel Cohen - Biosafety Considerations. Dr. M.R. Sondahl - Biotechnology of Tropical Fruit Trees: Coffee, Cacao, and Oil Palm. Dr. Lyndsey Withers - *In Vitro* Germplasm Conservation (tentative).
Oral presentations by participants.
Dinner Banquet.

Friday, January 18, 1991.

Session IV: Poster Presentations and Roundtable Discussions.
Poster Presentations.
Roundtable Discussions
1) Germplasm Preservation and Exchange.
2) National Policies for Biotechnology.
3) Regional Training Programs: Needs and Opportunities.

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Colorado State University

Field Tests Performed on Rice, Wheat, and Sorghum

Whole plant testing for verification of improved stress tolerance of *in vitro* selected lines continued at the TCCP this year. During 1989, 16 collaborating scientists assisted TCCP in conducting field and greenhouse investigations on regenerated material in 10 international sites and seven domestic sites. International testing sites included the Philippines, Mexico, India, Pakistan, and Colombia.

Published by the Tissue Culture for Crops Project (TCCP)
Department of Biology
Colorado State University
Fort Collins, Colorado 80523, USA

Julie L.F. Ketchum, Editor, Operations Director
Murray W. Nabors, Project Director
Oluf L. Gamborg, Associate Director
Sunitha Siriwardana, Training Coordinator
Reagan M. Waskom, Field Research Coordinator
Jolanta L. Menert, Greenhouse Manager
E. Lee Olton, Office Manager
Daniel R. Miller, Research Associate, Whole Plant Testing
Deborah M. Neubaum, Research Associate, Tissue Culture (*Phaseolus*)
Carol S. Ostrom, Research Associate, Tissue Culture (Wheat and Sorghum)
Fariha S.F. Sayied, Research Associate, Tissue Culture (Sorghum and Wheat)

Technical Staff: Scott Ahern, Scott Bailey, Barbara Bucknam, Jennifer Clark, Laurie Clauss, Dawn Dow, Jan Emming, Bob Flynn, Hal Gibson, Lynn Herrmann, Julie Hewitt, Bob Kelly, Kris Lienert, Marcus Murray, Dana Nichols, Nitschka ter Kuile, David Timm, Julie Vanderkolk, Scott Warnke

Graduate Students: Raymond Ketchum, Douglas McMurray, Amani Mnayer, Poungpet Poonsapaya, Sathish Puthigae, Gaith Sasi, Nanan Widiyanto.

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Dr. Joel I. Cohen, Biotechnology Specialist, Agriculture Production Division

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Centro Internacional de Agricultura Tropical (CIAT), Colombia
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Faisalabad Agricultural University, Pakistan
Institut Agronomique et Veterinaire (IAV) Hassan II, Morocco
Kala Sha Kaku Research Institute, Pakistan
Nuclear Institute for Agriculture and Biology (NIAB), Pakistan
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Universidad Autonoma de Baja California Sur, Mexico
University of Arizona, USA
University of Georgia, USA
University of the Philippines at Los Baños (UPLB), Philippines
Visayas State College of Agriculture (ViSCA), Philippines

For more information, contact:
Tissue Culture for Crops Project
Colorado State University
Fort Collins, Colorado 80523, USA
Phone: 303/491-6996
Telex: 3711418 TISCLT
FAX: 303/491-2293 (Attn. TCCP)

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 *in vitro* selected lines to non-selected regenerates for whole plant stress tolerance, 2) compare specific stressing agents, stress levels, and culture times for efficiency in producing tolerant lines, and 3) compare individual regenerated lines to non-regenerated checks for superior tolerance or agronomic traits.

Rice Field Trials In The Philippines

Fifty-nine R_2 and R_4 rice lines derived from IR-36, Mahsuri, and Pokkali were field tested for salinity tolerance at Pampanga, Philippines. Significant differences were not detected between Mahsuri- and Pokkali-regenerated lines for the variables measured. Maximum salinity levels measured were approximately E.C. 6.1 dS/m, which may not have been high enough to detect differences in these two cultivars. Among the IR- 36 regenerated lines tested, nine lines showed significantly improved vegetative salinity rating, reproductive salinity rating, seed yield per plot, or percent stand as compared to the non-regenerated check. Eight of these nine lines were regenerated from *in vitro* NaCl selected cultures. One line was significantly better for all variables and two additional lines were significantly improved for three of the four variables. These lines will be re-tested under both greenhouse and field saline stress conditions to confirm their improved saline tolerance.

Greenhouse Evaluation Of Rice

The most vigorous 30 regenerated rice lines from previous greenhouse salt evaluations were selected for re-testing under saline conditions in the greenhouse. These lines were evaluated for salt tolerance using a hydroponic solution containing 3 g/L NaCl. After 48 days of salt stress, E.C. levels had reached 17 dS/m and the plants were evaluated for height, tiller number, and shoot dry weight. Among the 30 R_2 lines tested, six 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. All six of the improved lines were from *in vitro* selected cultures at 3 g/l NaCl. Two of the R_2 lines were significantly better for all

three variables and warrant further testing.

Greenhouse Evaluation Of Spring Wheat

Fifty-seven R_1 spring wheat lines derived from Pavon were evaluated for salt tolerance in a greenhouse environment. 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. 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 to verify these results and validate the greenhouse test procedure.

Field Evaluation Of Spring Wheat

Field evaluation of spring wheat regenerates from *in vitro* 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. Of these, eighty regenerated lines from NaCl-selected cultures are currently in the field at La Paz, Mexico, under saline conditions. Preliminary data indicate that several of the lines have improved tolerance as compared to the non-regenerated parent. In addition, $AlCl_3$-selected regenerates are being screened at CIMMYT for improved tolerance to acid soil conditions. The initial screen of this material revealed that cultivars Glennson and Pavon had been improved via *in vitro* selection with $AlCl_3$. Confirmation screens are currently in progress.

Insect Tolerance in Sorghum

Data from field and laboratory studies conducted from 1986 through 1989 indicate that a tissue culture regenerated sorghum line has statistically verified resistance to fall armyworm (*Spodoptera frugipera*) feeding. Two selections from this particular 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 ofantibiosis and non-preference mechanisms. The results of this study indicate that somaclonal variation from tissue culture can be employed to generate new sources of genetic diversity for use in crop improvement programs. The two selections identified from this research are slated for sorghum germplasm release in 1990.

Evaluation of Sorghum For Drought Tolerance

Evaluation of drought tolerance was conducted on regenerated material at Tucson, Arizona, in 1989. This experiment 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 selected cultures which out yielded the check.

Evaluation Of Acid Soil Tolerance In Sorghum

Four R_5 lines which survived two previous seasons of acid soil stress were re-tested at Griffin, Georgia, 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 and further selection. In 1990, seed from Puerto Rico will be strip-planted and yield tested on the Griffin low pH field. Distinct phenotypes will be determined at this time. Germplasm release will be dependent on the 1990 results.

Recent field testing results at the TCCP 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. Field testing in spring wheat has indicated that selection for complex traits such as salt tolerance is better accomplished in R_4 or later generations. After three generations of selfing and selection for uniformity, lines should be more homozygous at loci affecting stress tolerance and selection for these traits should be more productive. This approach necessitates a strong linkage with a conventional plant breeding program. 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.

Graduate of Training Program to Obtain Degree

Mr. Simon Ndirangu, who graduated from the TCCP Tissue Culture Training Program in 1986, will be completing his B.S. in Horticulture this December.

Before coming to Colorado State, Mr. Ndirangu had been employed as a Senior Research Assistant at the Tea Research Foundation of Kenya, where he performed research in tissue culture and cytogenetics of tea, a major cash crop in Kenya. Wanting to improve his education and his ability to contribute to the research at the Foundation, Mr. Ndirangu enrolled at Colorado State soon after completing training at the TCCP.

While attending the Training Program, Mr. Ndirangu researched micropropagation of tea using shoot and nodal cuttings, meristems, and axillary buds, in combination with various media. Tea is a very slow-growing crop, so finding a method of rapid propagation is important. Mr. Ndirangu was able to obtain multiple shoots from nodal cuttings of tea.

Mr. Ndirangu has been able to use the skills he obtained at the TCCP to help finance his education. He works with Dr. Ken Knutson, of the Colorado State Horticulture Department, on a research project to maximize the yield of potato seed tubers. Tissue Culture is used to multiply the potato plants, which are then transferred to a greenhouse where research is performed to determine the optimal depth of planting and spacing between plants.

After graduation from Colorado State, Mr. Ndirangu hopes to return to work at the Tea Research Foundation in Kericho, Kenya.

Sorghum Tissue Culture Continues at TCCP

Plant regeneration from cultured cells is possible in most major cereals, but the methods are not generally applicable. In grain sorghum, the plant regeneration efficiency is excellent in some varieties and difficult if not impossible in others. In our experience, the cultivar M35-1 regenerates very efficiently at about 282 plants per gram of embryogenic callus. The RTx430 cultivar, on the other hand, has been very difficult to induce to produce embryogenic callus and regenerate plants. In some cultivars, the results have improved by adding 1% sorbitol to the regular medium, but that was not true for all cultivars. The M35-1 responded favorably.

A major objective of the tissue culture work at TCCP is the selection for cell lines with increased resistance to soil stress and drought. It is relatively easy to select for aluminum tolerance in tissue culture. In the past, the selections *in vitro* by the TCCP and other laboratories were made at the normal pH for tissue culture, which ranges from 5.5 to 5.8. Since the objective is to obtain plants with acid soil/aluminum tolerance, it would perhaps be more appropriate if the selection was made at a pH closer to that which prevails in acid soils. Our results to date have shown that callus cultures of sorghum grow well within the pH of 3.8 to 4.4. It is a well known fact that plant tissue culture media are poorly buffered. When calli are grown on agar media for a period of even one week, the pH has changed. The most frequent trend is for an increase in pH. It is thus necessary to include a buffer in the medium in order to stabilize the pH at a desired value. The preliminary data with sorghum indicate that it may be possible to stabilize the pH in the range of about 4.0 to 4.3 for selection for aluminum tolerance and that the callus grows satisfactorily at that pH.

Scientist from Indonesia Visits TCCP

Dr. Sugiono Moeljopawiro was a visiting scientist at TCCP for professional and technical training from November 15, 1989, to February 17, 1990. He came as a result of the visit by Dr. Oluf Gamborg to Indonesia in October. Dr. Gamborg had been invited as advisor to assist in establishing the agricultural biotechnology laboratory and program at the Agency for Agricultural Research and Development (AARD) in Bogor, Indonesia (see related article). Dr. Sugiono has been chosen as a leader in the biotechnology program at the Central Research Institute for Food (Field) Crops (CRIFC). During his visit to TCCP, Dr. Sugiono obtained first-hand experience in the tissue culture of several crop species. In addition to gaining information and experience on programs and technologies in research, Dr. Sugiono also became acquainted with the system of management and the Training Program at TCCP. While in the U.S., Dr. Sugiono also visited other laboratories and institutions that are prominent in other areas of biotechnology not represented at TCCP.

The visit by Dr. Sugiono was sponsored by Winrock International and USAID. Dr. Sugiono has received funding from the Rockefeller Foundation for research in biotechnology applied to rice. Rice is the most important food crop in Indonesia and will be the principal crop for research at the CRIFC biotechnology program.

Progress is Made in Bean Regeneration

Regeneration from tissue cultures of *Phaseolus* species has remained very difficult. We have reported on regeneration from suspension cultures of tepary bean (*P. acutifolius*) (Kumar *et al.*, 1988, Plant Cell Reports 7:322- 325). A more convenient procedure would be regeneration by a more direct and efficient route. Preliminary data indicate that primary leaf tissues of aseptically germinated seeds of tepary bean can be cultured to give rise to a callus from which plants can be regenerated. After testing a great variety of explant sources, *vulgaris* and *acutifolius* lines, basic media and hormone regimes, the primary leaf tissues and the inclusion of picloram as the auxin may be major factors. The objective from the beginning was to devise a procedure which would be efficient and could be used to produce transgenic plants. The conditions are being tested to determine if the method can be used for several genetic lines of *Phaseolus* species and to test the regenerated plants for uniformity.

The research is a collaboration between CIAT in Cali, Colombia with Dr. W. Roca and Paul Chavarriaga, and TCCP (O.L. Gamborg). In the past few months, Dr. Ahmed E. Abou-Salha from Egypt has also participated in the research. Dr. Abou- Salha was sponsored by the Peace Fellowship Program of the Cultural and Educational Bureau of the Arab Republic of Egypt.

TCCP and Affiliates Train Scientists from Around the World

Colorado State University, U.S.A.

The TCCP has trained a number of scientists from Indonesia, Pakistan, the Philippines, and Saudi Arabia in four independent short courses since December 1988. Two groups of scientists from Pakistan were trained on *in vitro* and greenhouse selection strategies applied to cereal crops. All seven trainees in these two groups were sponsored by the USAID through the University of Idaho Irrigation Systems Management Project.

Recent graduate Mr. Osmundo Orense of the Philippines, funded by Deutsche Gesellschaft Technische Zusammenarbiet (GTZ), gained theoretical and practical skills on application of tissue culture techniques for crop improvement. Mr. Orense returned to the Philippines in April to continue research on rapid propagation of elite cultivars of coconut at the Philippine Coconut Authority (PCA). The project is a joint effort of the PCA and GTZ.

After several years of effort to locate a sponsor, a scientist from Indonesia is able to attend the six-month training program at TCCP beginning in March 1990. Mr. Zurhan Mukhri, who is supported by the International Atomic Energy Agency, is on leave from his job at the Research Centre for Nuclear Techniques in Bandung. Upon completion of the Training Program, Mr. Mukhri will return to Indonesia to continue research on rice.

In order to accommodate growing interest in short-term or specialized training courses, the TCCP has scheduled the following courses for 1990:

Three-month courses
May 14 - Aug 10, 1990
Sept 3 - Nov 30, 1990

Six-month courses
Sept 3, 1990- Feb 15, 1991

Both training courses emphasize tissue culture techniques, with the major difference being the amount of time spent on the research project. For further information, contact Ms. Julie Ketchum at the TCCP.

CATIE, Costa Rica

The first CATIE/TCCP Training Course was held in the spring of 1987 in response to the need for instruction in Spanish. Several of the participants had applied to the Training Program at the TCCP but language and financial barriers prevented them from attending.

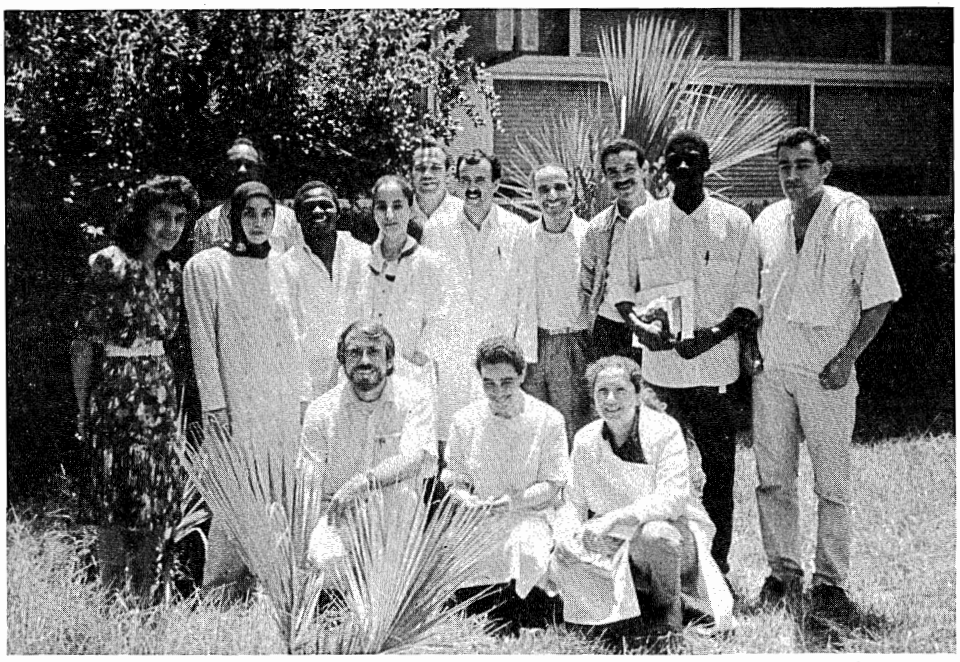
The third regional Training Course, held in the fall of 1989, again proved to be successful in transferring technology to the Latin American scientists. Fourteen participants representing Costa Rica, El Salvador, Guatemala, Nicaragua, Ecuador, Venezuela and Argentina completed the course on applications of tissue culture to tropical crops, ranging from ornamentals to fruits to medicinal plants. The CATIE instructing staff, headed by Dr. Victor Villalobos who is the coordinator for the course, utilized their diverse expertise in training the participants, whose backgrounds ranged from the B.S. to the Ph.D. level. The TCCP Training Coordinator, Ms. Sunitha Siriwardana, traveled to CATIE in September 1989 to assist in basic instruction in sterile technique, cell and tissue culture, and *in vitro* stress selection.

The next course is scheduled to be held in September 1990. For more information, contact:
Dr. Victor Villalobos
Curso Teorico - Practico
Programa Mejoramiento de Cultivos Tropicales
Apartado 15
Turrialba, Costa Rica

IAV Hassan II, Morocco

In 1989, regional courses were expanded to Agadir, Morocco, where the first all-French training course was jointly sponsored by IAV Hassan II and the TCCP and funded in part by FAO and USAID. Nine participants representing four African Francophone countries — Morocco, Rwanda, Guinea (Bissau), and Guinea (Conakry) — attended the course from May 15 - June 23, 1989. They received training on *in vitro* propagation techniques applied to cereal crops, potato, and selected tropical and temperate crops.

The next course will be held May 7 - June 15, 1990. For more information, contact:
Dr. Mohamed Aaouine
IAV Hassan II - complexe
Horticole d'Agadir
B.P. 121 Ait Melloul
Agadir, Morocco



Participants and instructors of 1989 International Tissue Culture Training Course at IAV Hassan II in Morocco

Training Course Offered in Hawaii

A non-degree, three-month training course in plant tissue and cell culture of tropical and horticultural crops is being offered at the University of Hawaii at Manoa. The course will provide academic and practical training, as well as guidance in a specific tissue culture project.

For more information, contact:
Harold J. McArthur
Coordinator for International Programs
College of Tropical Agriculture
University of Hawaii/Manoa
3050 Maile Way, Rm. 214
Honolulu, Hawaii 96822 U.S.A.
Fax: (808) 948-6442

Development of Plant Regeneration and Selection Techniques for Stress-Tolerant Corn and Rice in the Philippines

Nguyen T. Thanh-Tuyen
Tissue Culture Laboratory
Visayas State College of Agriculture
ViSCA, Leyte 6521-A, Philippines

Rice

Data on seed production and agronomic characters of R_0 plants of IR43 and UPLRi5, regenerated from the experiment on specific gravity of the seeds, indicate large variations in total weight of filled seeds at 14% moisture content (min. 4.3g, max. 56.3g for UPLRi5 and min. 2.1g, max. 29g for IR43) and number of productive tillers (min. 5, max. 16 for UPLRi5 and min. 5.75, max. 15.3 for IR43) produced per plant among the regenerants derived from one mass of callus.

The regenerants with promising performance will be field tested in acidic soil in Matalom, Leyte.

The third trial of the field test for the performance of R_1 plants (cv. IR43), derived form the experiments conducted in 1988 in acidic soil in Matalom, has been completed. Three checks, i.e. the best entries of the Acid Upland Observation Trial (AUOT 15) and Acid Upland Yield Trial (AUYT 136) and IR 43 seeds, were included for comparison with seed progeny of our tissue culture materials. Drought spells occurred twice at seedling and grain filling stages during the growing season.

In terms of pest incidence, the tissue culture materials had the average score of 1-2 versus the score of 4-5 for the checks (with score 1 being the lowest and 9 the highest incidence).

Visual assessment of drought resistance indicated that (1) at seedling stage, both the test materials and the checks were moderately damaged; and (2) at grain filling stage, the check AUYT 136 was heavily damaged and no filled seeds were obtained at all.

Quantitative data on the performance of the entries are being computed.

The promising lines identified in the second trial in Matalom are being multiplied under both stressed (i.e. Matalom) and non-stressed conditions (i.e. IRRI and ViSCA). They will be included in IRRI Acid Upland Observation Trial in different locations for dry season, 1990.

Corn

A preliminary experiment on *in vitro* selection for P-deficiency tolerance has been set-up with the use of immature embryos of IPB 2 and SMARC 1283. The cultures were subjected to 4 treatments corresponding to 4 levels (i.e. 0, ¼, ½ and full strength) of PO_4 content of N_6-based medium. Four batches of cultures were given the treatments at second, third, fourth and fifth passages. Each batch was exposed to the treatments for two passages before being transferred to regeneration medium.

Plant regeneration has been observed in all treatments, including zero PO_4, starting from the second passage. However, it is noted that, for SMARC 1283, the regenerative potential reckoned as number of green shoots produced per culture declined on medium containing reduced levels of PO_4. The experiment is still on-going.

Another experiment will be conducted in 1990 with prolonged treatments of zero or a reduced level of PO_4 in callus initiation medium to impose stress and trigger the selective survival mechanism of the cultured cells.

Somaclonal Variation for Disease Resistance in *Populus Deltoides* (Eastern Cottonwood)

Dr. C.S. Prakash
School of Agriculture and Home Economics
Milbank Hall
Tuskegee University
Tuskegee, AL 36088, USA
Telephone: (205) 727-8023

Leaf segments of *P. deltoides* clone K417 were used to regenerate somaclones via callus phase on a woody plant medium. Leaf disks of forty such somaclones were inoculated independently with two races of leaf rust, *Melampsora medusae*. The reaction of most of the somaclones was similar to that of the parent. However, there were a few somaclones which were either more resistant or more susceptible, compared to the parent. Somaclonal variation for leaf rust disease reaction was also race-specific in nature, as significant interaction between the somaclone and the pathogen race was observed for two traits, latent period and mean uredinial number. Somaclones, in general, developed the disease a little slowly but complete resistance was not observed in any individual. It is concluded that somaclonal variation may be useful to increase the partial resistance in eastern cottonwood to leaf rust. Current research effort, funded by USDA, is focused on molecular mechanisms of somaclonal variation in eastern cottonwood.

In Vitro Plant Regeneration and Selection for Aluminum Tolerance in Peanuts

[1]Associate Professor Dr. Marziah Mahmood (Coordinator)
[1]Associate Professor Dr. Mohd. Arif Syed
[2]Mr. Ahmad Abdul Rahman
[1]Department of Biochemistry and Microbiology
[2]Department of Biology
Universiti Pertanian Malaysia
Serdang, Selangor, Malaysia

Our main objective is to develop aluminum-tolerant cells using cell suspension cultures, and to regenerate them into whole plants with the hope that they contain aluminum-tolerant traits. Plant cell suspension cultures were established using callus tissues initiated from leaf explants of 10-day-old seedlings germinated *in vitro*. The cell suspensions were grown and maintained in L-6 liquid medium containing 4.00 μM 2,4-D and 2.50 μM kinetin. In aluminum-toxicity studies, the cultures were grown in reduced strength of L-6 liquid maintenance medium with different levels of aluminum (0, 50, 100, 200, 400, 600 μM), supplied as $Al_2(SO_4)_3 \cdot 18H_2O$. In order to maximize the effects of aluminum, the initial pH of the medium was set at 4.0. Fresh medium was added every week. Cell growth was monitored after 4 weeks of culture by measuring the Packed Cell Volume (PCV). The results obtained showed that the PCV was significantly reduced in aluminum-treated cultures. The cell suspensions with added aluminum appeared brown and the degree of browning increased in intensity with the increase of aluminum applied. When observed under the microscope, the aluminum-treated cell clumps appeared smaller in size and number. Cell clumps obtained from aluminum-treated cell suspensions are being induced for embryogenesis and regeneration.

Cooperative Date Palm Research with Morocco

Dr. Harrison Hughes
Department of Horticulture
Colorado State University
Fort Collins, CO 80523

Research on *in vitro* hardening of date palm is an ongoing cooperative research project with Dr. Abdelouahhab Zaid at the tissue culture facility of the Central Station of Saharan Agronomy in Marrakech, Morocco and Dr. Harrison Hughes in the Department of Horticulture at Colorado State University. Dr. Zaid came to Colorado State as a Ph.D. student in the spring of 1987. During his initial two years here he began research into the use of a high osmoticum on *in vitro* hardening of date palm. He returned to Morocco to continue the work, but recently came back to Colorado State to complete his Ph.D. in January 1990. Now back in Morocco, he continues research into *in vitro* culture of date palm including further work with *in vitro* hardening.

This cooperative research has shown that a high osmoticum such as polyethylene glycol (PEG) may be used to reduce subsequent moisture loss from leaves of *in vitro* propagated plantlets moved *ex vitro*. Increased wax deposition on leaf surfaces of hardened plantlets (PEG-treated), as compared to non-hardened plantlets, was demonstrated by quantification of wax extracted and by scanning electron micrographs. *In vitro* hardening studies are continuing in Morocco with various molecular weight PEGs and with plant survival comparisons in greenhouse and field.

Studies on cryopreservation of date palm with Dr. Leigh Towill (USDA) and Dr. Hughes were initiated during Dr. Zaid's stay in Colorado. Preliminary results using various cryopreservatives and freezing to -196°C were promising. This work is continuing in Colorado.

Biotechnology for Agriculture in Indonesia

Oluf L. Gamborg*, Dr. Ibrahim Manwan**
* AARP-II, Winrock International, Box 62, Bogor, Indonesia
**Director, Central Research Institute for Food Crops, Bogor, Indonesia

The government of Indonesia has made the decision to establish plant biotechnology research with integration into conventional plant breeding and crop production operations. Major programs are being established at the Central Research Institute for Food Crops (CRIFC) in Bogor. The CRIFC is under the Agency for Agricultural Research and Development, Ministry of Agriculture. The Minister for Research and Technology has designated the facilities as the Center for Research on Agricultural Biotechnology. Current research is housed in a new building financed by the Government of Japan; however, a new building to house the facilities is being erected.

Dr. Oluf Gamborg, Associate Director of Tissue Culture for Crops Project at Colorado State University in Fort Collins, is in Bogor as a consultant to the AARP-II project and is to provide guidance, expertise and training for the establishment of laboratory facilities, operations, trained personnel, and research programs. Dr. Gamborg, and the activities in which he is involved, is sponsored by USAID through Winrock International and the Government of Indonesia. The specific type and the nature of the involvement of Dr. Gamborg is unique. It is transfer of technology, skills and information with the establishment of up-to-date research and development within food crop improvement programs.

The activities will consist of setting-up research programs in biotechnology in food crops which are of major importance to Indonesia including rice and other cereal crops, grain legumes such as soybean, kidney bean, peanut and mungbean, as well as cassava and sweet potato. A component within the research is the training of the technical and professional Indonesian personnel. They will acquire the information, procedures and practical approaches which are directly applicable to the crops.

A major emphasis in the training programs is to develop a strong technology base for plant regeneration from tissues, meristems, cells, anthers, embryos, and possibly protoplasts of crop plants. Plant regeneration is the fundamental, essential technology for any application in crop improvement and production (propagation). Also included will be the setting- up and testing of gene transfer methods such as the Agrobacterium procedures, as well as applying tissue culture and plant regeneration procedures in producing genetic variants for disease, insect, and stress resistance. The training will be three or six months long, and will include participation and assistance by personnel who are involved in the on-the-job training. The overall training will also include the set-up, operation, and management of plant biotechnology facilities for research and development applied to agricultural crops.

There is a very strong interest on the part of the Government of Indonesia in establishing collaborations in molecular biology with scientists and laboratories in the USA. The need is for collaboration that will integrate the Indonesian skills in plant tissue culture and gene transfer with molecular biology, to clone genes of particular interest and/or make available genes for virus resistance, insect resistance, and nutritional quality for their crops. There are also plans to set-up plant molecular biology facilities and operations, but it will be some time before they become functional.

One of the important features of the research program is the establishment of a planning and monitoring group for each crop type. The group includes, in addition to the P.I., a breeder, an entomologist, tissue culture personnel, and perhaps a pathologist. The group will formulate the plans and have regular reporting and planning sessions. There will also be an attempt to invite a representative from a relevant industry to join the group.

There are plans to have a workshop, which will coincide with the formal training course. The intent is to have invited speakers from Indonesia and a few from the USA, who will serve as resource persons to provide information as well as guidance in technical procedures.

Indonesia is a very progressive country. The people are industrious, enterprising, and have a keen interest in moving forward in basic and applied sciences. That is true also in all the biological sciences, not the least of which are those that relate to food, feed, and fuel crops. There is a similar strong interest in taking advantage of biotechnology for application to spice and medicinal crops as well as estate crops such as tea, oil palm, rubber, cocoa, coconut and coffee. For estate crops, there are individual laboratories which already have tissue culture operations. Dr. Gamborg is assisting in the programs of these laboratories.

Program in Science and Technology Cooperation (PSTC)

The U.S.A.I.D.'s Program in Science and Technology Cooperation (PSTC), initiated by a Congressional mandate in 1981, seeks to stimulate new and innovative scientific research on problems that confront developing countries. The Program is administered by the Office of the Science Advisor (AID/SCI), and provides highly competitive research grants which are subject to external scientific peer review.

The PSTC gives highest priority to submissions from scientists in those developing countries which receive USAID development assistance. Cooperation with U.S. scientists is strongly encouraged, but not required. Applicants may be affiliated with universities, non-profit or for-profit organizations, and/or government laboratories. The grants competition is also open to U.S. investigators and scientists from middle income countries such as Argentina, Brazil, Colombia, Malaysia, Mexico, Nigeria, and Venezuela, when the research is particularly relevant, unusually innovative and there is strong scientific collaboration (intellectual partnership) with developing country investigators. The PSTC does not support research in developed countries such as Australia, Canada, Germany, Japan, Sweden and United Kingdom, nor in China. Research involving Israel is funded by other programs, not by PSTC.

The PSTC seeks new research ideas in the natural sciences, such as biology and chemistry, and in engineering. By research, we mean the testing of a scientific hypothesis or the development of a new technology through organized observation in an experimental setting (laboratory or field). Innovative ideas that will eventually lead to solutions to serious developing country problems are accorded highest priority.

For more information, contact:
Agency for International Development (A.I.D.)
Office of the Science Advisor
Room 320 SA-18
Washington, D.C. 20523-1818
U.S.A.
Telephone: (703) 875-4444

TCCP Seeks Feedback from Scientific Community

The Tissue Culture for Crops Project (TCCP) came into existence in August 1980. During the past nine and one-half years, we have achieved the following objectives:

1) Developed long-term, high frequency plant regeneration methods for rice, wheat, sorghum, oats, and pearl and proso millet.

2) Developed efficient plant regeneration methods for moth bean, pigeonpea, soybean, and tepary bean.

3) Selected cell lines of some cereals tolerant to insects, high salinity, acidity, or drought.

4) Tested plants from tolerant cell lines in greenhouse and field. Release of improved germplasm is scheduled for 1990.

In addition, the TCCP has completed training of 38 persons in tissue culture and related technologies. Two regional training programs have been established in Costa Rica and Morocco.

The International Plant Biotechnology Network (IPBNet), which was established in 1985, has sponsored 3 international conferences, produced 11 newsletters (since 1982) and 2 directories, and involved itself in countless collaborations with researchers worldwide.

Funding for the TCCP and the IPBNet is scheduled to end in January 1991. In order to gauge the effectiveness of the Project's activities, we need your feedback. Please complete and return the survey, included with this newsletter, by August 1, 1990. Your assistance is greatly appreciated.

Publications Available from the TCCP

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