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Tissue Culture for Crops Project
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STATEMENT OF GOALS

The goal of the Project is to help expand and accelerate the application of tissue culture for LDC crop improvement. The goals can be accomplished by strengthening LDC's capabilities for tissue culture and by linking and coordinating worldwide research. Specific areas of the Project include laboratory research aimed at continuing "pathbreaking" research: 1) evaluating progeny of plants selected for their stress tolerance, 2) providing training for persons from LDC's interested in applying tissue culture; and 3) establishing and expanding a network of scientists who are interested in the Project and who will benefit from the associations.

METHODOLOGIES FOR TISSUE CULTURE, SELECTION, AND PLANT REGENERATION

Tissue Culture

Rice. Long-term Culture. Embryogenic callus derived from leaves of Mahsuri has been maintained since February 1982. Regeneration capacity has remained high, but is dependent on careful selection of E callus to be transferred to regeneration media. In June 1984, IR-36 mature embryos were plated on various media to determine the optimal media for E callus growth and regeneration. The experiment is to determine length of time in culture on a single media, 0.5 mg/l 2,4-D and .3 mg/l KIN.

Callus Maintenance. Pokkali, IR-36, G-159, Calrose, RD-23, RD-25, IR-6, IR-54, IR-60 and IR-62 were all treated for E callus formation, optimal growth, and regeneration. These experiments dealt with growth regulator concentration (Table 1).

Table 1. Optimal media for E callus formation.

Cultivar	Intiation			Maintenance		Regeneration				
	2,4-D	KIN	TRP	2,4-D	KIN	IAA	BA	TIBA	NAA	CW
Calrose	1.0	0.2	100	2.0	0.3			0.5		
G-159	1.0	0.3		1.0	0.3	0.5	0.4			
IR-6	1.0	0.2		1.0	0.2	0.5	0.5			
IR-36	1.0	0.5	50	0.5	0.3	0.5	0.2			
IR-54	0.5	0.5		0.5	0.5	0.5	0.2			
IR-60	1.0	0.2		1.0	0.2	0.5	0.2			
IR-62	1.0	0.2		1.0	0.2	0.5	0.2			
Pokkali	0.5	0.2	50	0.5	0.4		0.5			
RD23	1.0	0.3		1.0	0.3		1.6		0.5	10%
RD25	1.0	0.4		1.0	0.2	0.5	0.3			

Media Conditioning. Preliminary results indicate that E callus produces compound with in-house regeneration that was tested by placing a piece of E callus on the regeneration media for one to two weeks then removing the first piece of callus and replacing it with a second piece of E callus. Regeneration was enhanced for both Pokkali and G-159 when jars were used, but not when vials were used.

Wheat. In both mature and immature embryos, two distinctive callus types were produced during the first culture passage. One type, derived from the root region, was distinguished by its friable morphology and was called NE callus. The other type, derived from the shoot region, was characterized by its smooth-compact (SC) appearance. The scutellum of mature embryos did not produce callus regardless of orientation on the medium. In cultures established from immature embryos, a third type of callus consisting of embryoids formed from scutellar tissue has been recognized as E. Embryo-like structures, indistinguishable from those established from immature scutella, were observed to form from the SC callus established from seeds and mature embryos and, infrequently, from immature embryos. The SC callus was capable of proliferating

embryoids for only a few passages.

The frequency and quantity of E callus initially produced per culture vessel was comparable between mature and immature explant sources. Embryogenic callus established from immature embryos is usually visible at the end of the initiation passage whereas that produced from mature embryos is often cryptic. However, with the selective subculture of the SC callus, embryoids established from mature explants are quite evident by the end of the second subculture.

Mature embryos dissected from imbibed seeds were preferred explants to whole seeds because the initial amount of NE callus produced was lower, and the amount of SC callus was higher than that produced from seeds. Thus, subsequent transfers involved less dissection to remove NE callus, and embryo-like structures were more evident.

Regardless of explant source used, it was essential that all E callus was carefully separated from other callus types to maintain E callus over several passages. Embryogenic callus seemed not to grow as fast as NE callus, and hence could by overgrown by it. Although E callus proliferated and was maintained for more than 20 passages, its growth fluctuated over time. This fluctuation was apparently due to technical problems, such as high temperatures which tended to decrease E callus growth, the inadvertent transfer of other callus types along with the E callus, and the length of time between transfers.

Non-embryogenic callus was not found to be regenerable whereas SC callus was moderately regenerable. In subsequent studies, it was determined that embryoids developed from the SC callus; it was concluded that embryoids were really responsible for the previously

observed regeneration from this SC callus. Embryogenic callus established from all explant sources readily regenerated into whole plants. Several entire plants were often formed from a small amount (e.g., 0.2 g) of E callus because several embryoids could by present in a given amount of E callus. No obvious differences in regeneration of E callus established from immature or mature embryos were observed. The majority of regenerates obtained from both short- and long-term cultures were phenotypically normal, reached maturity, and produced seed.

Sorghum. Proliferating callus seemed to arise from the embryo axis. The callus quantity initially produced was small, usually less than 0.1 g total callus at the end of the first passage. Thus, all callus was subcultured onto fresh media. At the end of the second passage, callus types were more identifiable. Morphologically, NE callus was either a friable, greyish callus or, sometimes, a yellowish nodular callus. Embryogenic callus was characterized by its compact nodules, which were often cleft and always creamy white in color. Embryogenic callus established from mature embryo explants was morphologically identical to that which developed from immature embryos.

The cellular origin of E callus from mature sorghum embryos was not determined histologically in this study. When immature embryos were used, embryo-like structures arose directly from scutellar tissue without an intervening callus stage. The results did not indicate, however, that mature scutella tissue proliferated, but some embryoids may have arisen from these tissues. At later passages and in salt-stress experiments, embryoids arose from proliferating callus presumably established from the embryo axis.

The percentage of cultures established from mature embryos that gave rise to E callus at the end of the first passage was low. It was estimated, by inspecting several hundred cultures, that 30% or less of the initial cultures formed E callus, and what E callus was formed was a very small fraction of the total callus mass. At the end of the second passage, E callus production increased so that at subsequent subcultures the culture vessels were each inoculated with about 0.2 g of E callus. At the end of subsequent passages, it was visually estimated that 50% of the callus present in each culture was E callus, with the rest being the fast growing and friable NE callus. As with wheat, E callus was maintained for at least several months provided that NE callus was removed and discarded.

Only the E callus was capable of regeneration into whole plants. Virtually all E callus pieces per culture vessel produced at least one plant, although occasionally some E callus required another passage on regeneration medium before developing into plants. Regeneration could be maintained through several passages. Embryogenic callus from the cultivar Hegari was more regenerable for longer periods than callus from RTx-430 or RTx-7000. A second subculture of all regenerates on IBA-containing medium was found to be necessary; otherwise, regenerates could not be successfully transferred to a glass-house environment. Regenerates were phenotypically normal, grew to maturity, and produced seeds in amounts comparable to that produced by seed-sown plants.

Corn. The long-term tissue culture and regeneration of corn callus has been a challenge for many investigators. Part of the difficulty with corn culture is due to the inability of mature explants to yield

regenerable callus. Immature embryos will yield E callus, but long-term maintenance, and thus regeneration, is not readily achieved.

As part of a separate in-house program funded by Pioneer Hi-Bred of Johnston, Iowa, tissue culture parameters of corn callus culture have been investigated. Research has been conducted with mature embryos, immature embryos, and immature leaf sections of hybrid cultivars. Results will be applied to the tissue culture of three cultivars of corn—Muneng, Across, and Ciat—supplied by CIMMYT.

Unlike research with other cereals, experiments with mature embryos of corn have produced mostly negative results. Mature embryos, when cultured on LS media containing 2-10 mg/l 2,4-D, produced masses of roots; if callus was produced, it was nonembryogenic. Experiments with 20 mg/l 2,4-D resulted in fewer roots and more compact callus, but this compact callus was not embryogenic or regenerable. It was observed that 10 mg/l 2,4-D plus 20-100 mM proline produced results comparable to those obtained with 20 mg/l 2,4-D, but no regeneration was observed. Since the quality of callus obtained from mature embryos was poor, no further experimentation with this explant is planned.

Immature embryos are suitable explants for the production of E callus; however, the embryo must be cultured with scutellum facing away from the medium. Two types of E callus can be derived from immature embryos. Type I callus is a translucent, convoluted, and compact callus similar to the E callus found in rice, millet and sorghum. Type II callus is very friable, almost thread-like with well-defined somatic embryos on suspensor-like structures. Type I callus is produced from immature embryos cultured on LS media plus 1 mg/l 2,4-D and 12% sucrose. Type II callus is formed from immature

embryos cultured on LS media plus 1 mg/l 2,4-D, 1 mM L-asparagine, and 2% sucrose. Maintenance media for both callus types consists of LS media plus 2 mg/l 2,4-D and 4% sucrose.

Soybean. Research with soybean was initiated to define the conditions for plant regeneration. Prize cultivar of soybean has been used in the studies. A total of 33 different initiation/maintenance media have been tested. It was observed that callus produced on media containing 2,4-D was creamy in color and friable. Callus initiated on media containing IAA was greenish and more compact. Media containing NH_4Cl as a nitrogen source produced more vigorously growing callus, compared with media containing NH_4NO_3. Proline had no apparent effect on callus growth.

After careful observation with a dissecting microscope, two types of embryo-like structures were found in several cultures: smooth-shiny or rough embryoids. Of all the media tested, only those media containing 10 mg/l 2,4-D produced smooth-shiny embryoids. All other media produced rough embryoids.

Cotyledonary segments obtained from different sizes of immature embryos (3-10 mm) were obtained from greenhouse-grown plants. The cultures produced shiny embryoids on media containing 10 mg/l 2,4-D, and in media with 2,4-D in combination with 0.5-1.0 uM ABA. The greatest number of embryoids were produced from cotyledons 6 mm long. When 2,4-D was used in combination with 1 uM ABA, normal development of shiny embryoids occurred. The number of embryoids produced was lower than what generally was produced on media with 10 mg/l 2,4-D plus 0.5 uM ABA. In a separate set of experiments where meristems and buds were used as explants, the embryoids produced were all of the smooth-shiny type.

Smooth-shiny embryoids derived from callus from cotyledonary segments cultured for one to two weeks in the dark on regeneration medium, produced four different shapes of shoots and a few shoots with roots (plantlets). Plantlets were also obtained from shiny embryoids cultured on regeneration media. Shoots from shiny embryoids were also obtained when cultured on media containing ABA plus either GA_3 or zeatin. These shoots were then cultured on B5 medium which contained lesser amounts of salts than LS, supplemented with 0.5 uM IBA for rooting. Rooted plantlets were transferred to three-inch pots containing vermiculite and grown in a growth chamber. To date, over 145 shiny embryoids have regenerated shoots. From these, about 21 were rooted and formed plantl

developmental capacity than shiny embryoids. Morphologically, they appeared as green and very compact regions within softer types of callus. Rough embryoids could be obtained from all explant sources. There was an apparent inverse relationship between callus initiation and length of hypocotyl segments obtained from seedlings; moreover, low concentrations of 2,4-D produced fewer rough embryoids which required a longer time for their development into discrete structures. In contrast, at the higher concentrations of 2,4-D, a greater number of embryoids were produced and less time was required for their development.

The rough embryoids failed to produce shoots on the media used for the smooth-shiny types. After testing several growth regulators, it was observed that when rough embryoids, obtained from cotyledonary segments, were transferred to media containing 5 mg/l 2,4-D, a secondary mass of callus was produced. After six, four-week passages, twelve shiny

embryoids were produced from this callus. In subsequent studies, about 40% of the callus derived from rough embryoids gave rise to the smooth-shiny type.

TCCP is among the first laboratories in the world to successfully regenerate soybean plants from tissue culture. The technology is now available for use in the production of agriculturally useful variants.

Germplasm of dry bean and cowpeas have been obtained and preliminary experiments have been initiated.

Stress Selection

Rice. Salt. Experiments designed to produce plants from various NaCl stress levels contained NaCl in all media including the regeneration media. In G-159, NaCl levels up to 9 g/l allowed plant regeneration. IR-36 is more sensitive to NaCl *in vitro* and plants were produced only up to 6 g/l NaCl. A summary of the plants regenerated is in Table 2.

Table 2. Number of plants regenerated from NaCl selected callus.

	Total Plants Regenerated	
NaCl (g/l)	IR-36	G159
0	184	373
3	301	456
6	122	589
9	-	103

A second type of NaCl selection was started in May, 1985. In this experiment G-159 callus was started on 0 and 9 g/l and IR-36 was initiated on 0 and 6 g/l. Beginning at the third passage and continuing through the experiment, the callus on NaCl was divided and part was placed on media with a higher concentration of salt with the remaining callus maintained on the same concentration. This experiment was in progress during the move to the new building and many cultures were lost.

Aluminum. Experiments were conducted to determine the selection levels required for G-159, IR-36 and Pokkali. The number of plants regenerated is summarized in Table 3. From the total number of plants regenerated, G-159 showed the most tolerance.

Table 4. Number of Plants Regenerated from $AlCl_3$-selected callus.

Total Number of Rice Plants Regenerated from Selection Experiment on Aluminum.

$AlCl_3$ ppm	Cultivar IR-36	G-159	Pokkali
0	314	75	150
50	74	-	26
100	187	3	9
200	26	129	-
250	19	22	54
500	18	11	3
1000	-	10	-

PEG. Preliminary experiments were conducted on G-159 and Pokkali to determine selection levels of PEG 4000. G-159 produced 23 plants from 50 g/l and Pokkali produced 82 plants from 10 g/l, indicating that Pokkali may be more sensitive to PEG *in vitro*.

Sorghum. Screening for salt tolerance. Embryogenic callus produced on basal medium was successfully cultured on media containing NaCl. Callus which was stressed on 1.2% (12 g/l) NaCl decreased in fresh weight over time, with trace amounts of callus remaining at the end of the third, four-week passage. Callus cultured on 0.9% NaCl also lost fresh weight, but was still present after four passages. Callus stressed first on 0.6% NaCl then moved to media containing 0.9% NaCl, initially lost fresh weight, but recovered after two passages.

The callus stress response associated with successively higher NaCl concentrations could be visualized by the extent of phenolics secreted into

the medium. Rarely did callus growing on salt-containing media not secrete any phenolics. It has been suggested that the phenolics produced by the calli indicate stresses associated with the dedifferentiated state of tissue; it appeared in the present study that salinity exacerbated these stresses. It was not observed that the production of phenolics interfered with callus growth or differentiation on medium lacking or containing low NaCl amounts; however, the callus stressed on higher concentrations (0.6% or more) of NaCl eventually became intensely pigmented with the black phenolics and it was difficult to determine if the callus was still viable. Once transferred to regeneration media, some of the stressed calli produced regions of embryoid clusters. These embryoid regions would subsequently regenerate into plants.

To test whether or not E callus could be successfully grown in liquid cultures, liquid media (35 ml in 250 Erlenmeyer flasks) were inoculated with 0.5 gm of E callus per culture. Every two to three days, medium was poured off and fresh medium added. At each media change interval, some callus was collected and plated onto solid regeneration medium. The callus recovered from liquid cultures was frequently completely pigmented black, but within a few days formed regions of embryogenic clusters and regenerated. The greatest number of plants were recovered after the first few days in liquid culture; however, plants were recovered from callus cultured for up to 12 days in liquid media.

Having demonstrated that E callus could be cultured in liquid media, and retain its regeneration capacity, experiments were initiated with liquid saline cultures. Generally, callus recovered from liquid saline cultures was completely pigmented black. When plated directly on solid regeneration media, some calli produced E regions which regenerated into

plants (5 plants from ca. 0.4 gm E callus). If the callus was planted first on solid saline media, and then cultured on regeneration media, less E callus was recovered, and hence regeneration was poor (1 plant from ca. 0.15 mg callus). In one experiment, however, callus recovered from liquid saline media was cultured on media containing 0.9% NaCl, then placed on 1.0% NaCl, upon transfer to regeneration media, 24 plants from 0.3 gm callus were recovered.

All plants recovered from salt-stressed cultures were subcultured on media supplemented with 0.3 mg/l IBA to ensure vigorous root formation. When roots are fully established, plants will be established in the greenhouse for seed production and progeny screening for enhanced salt resistance.

Embryogenic callus has been cultured on media containing hydroxyproline (HYP) as a means of selecting proline over-producing variants. Of the three concentrations tested (2, 5, and 10 mM), 10 mM HYP resulted in maximal reduction in callus growth. Excess callus from control cultures are routinely subcultured on LS media plus 10 mM HYP for one to four months. Surviving callus was placed on regeneration media.

Nitrate Efficiency. Enhancing the fertilizer efficiency of crops would reduce one of the major costs of farming. *In vitro* selections for nitrate efficiency could be accomplished in three ways: 1) growing cells in a reduced amount of nitrate. Cells capable of growing on minimal nitrate may be more efficient in utilizing available nitrate; 2) growing cells in the presence of chlorate, a competitive substrate. Nitrate reductase will chemically reduce the chlorate to chlorite, which is lethal to cells; however, variant cells with altered nitrate reductase—having increased specificity for the substrate nitrate—will not be

affected by the chlorate, and thus will preferentially grow; 3) using chlorate with step-wise increases in concentration as a means for selecting for gene amplification of nitrate reductase.

In initial chlorate experiments conducted with IR-36, it was determined that 50 mg/l chlorate selectively inhibited callus growth to about a tenth of control growth. Surviving cultures from chlorate screens were subsequently cultured on media containing one-half, one-quarter, and one-tenth concentrations of LS nitrate; however, none of these chlorate-selected calli grew as well on the nitrate reduced media as the controls. Because selection time and pressure were low for this experiment, additional chlorate selections are planned to generate chlorate-resistant calli, and screen calli on minimal nitrate media.

Results from rice cultures were used to determine the selective level for sorghum. For rice cultures, 50 mg/l chlorate was the most selective, and preliminary experiments with sorghum parallel this result. Excess callus from control cultures will be plated on media with chlorate for one to four months. Surviving callus will be regenerated and whole plants screened for nitrate efficiency.

Protoplasts/Suspensions.

Research on the isolation and culture of monocot protoplasts has been initiated. Several cereals, rice (Oryza sativa), wheat (Triticum aestivum), and millet (Panicum mileaceum), as well as a halophytic grass, (Distichlis spicata), have been studied. The underlying philosophy of the protoplast studies is to increase the yield of protoplasts obtained from enzymatic digestion of embryogenic or meristematic cells. The objectives included steps to minimize the severity of the isolation treatments such as enzyme concentration, time and temperature of incubation, as well as

attempts to maintain the sterility during isolation andculture. The overall goal is to develop isolation and culture procedures which would results in plant regeneration. The technologies of protoplasts could then be used in the production of useful mutants or in gene transfer research.

Studies have been completed to define enzyme concentrations, and combinations as well as sources and purity of commercial preparation. In addition, extensive studies have also been conducted on optimum temperature and time of incubation, agitation speed, lighting conditions, osmotica, purification protocol, culture media, hormones, culture density, culture conditions, and optimum time to add fresh media.

Protoplast isolation has been achieved using NE suspensions of the salt marsh grass, Distichlis spicata. Investigations into the role of cell cycle stage, and the rate of cell division on regenerability of isolated protoplasts are in progress. Additionally, the effect of the application of ABA and GA_3 to explant tissues and their influence in the culture of protoplasts is under study.

Future goals of protoplast research include the fusion of cereals with species with high salt tolerance.

EVALUATION OF REGENERATED PLANTS.

Wheat. Wheat testing has been initiated with Chris and Pavon 76. Seed from regenerated Chris plants was sent to CIMMYT to increase stock. Analysis of root tips revealed abnormal chromosomes in 18% of the increased plants regardless of whether they were from stressed cultures or controls. Hydroponic testing of plants from control and 3 g/l salt stressed cultures did not show any increased salt tolerance in the whole plants. Cultures have been obtained for callus tolerant to 9 g/l NaCl.

Plants regenerated from the higher level will be used in future testing.

Oats. 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.

Using hydroponic testing methods, oat seedlings were placed in solutions containing 0.0, 4.0, 8.0, 12.0 or 16.0 g/l 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 survived. Field-grown seeds clearly did not commonly possess increased levels of tolerance. R_1 plants derived from non-selected cell cultures possess more tolerance than plants from field-grown seeds, probably because cell culture increases mutation rates, leading to the development of somaclonal variants with increased salinity tolerance expressed in plants.

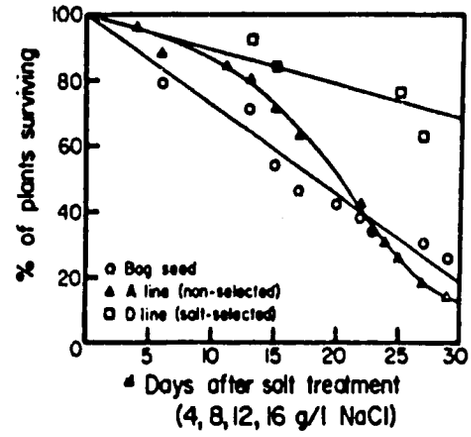

Figure 1

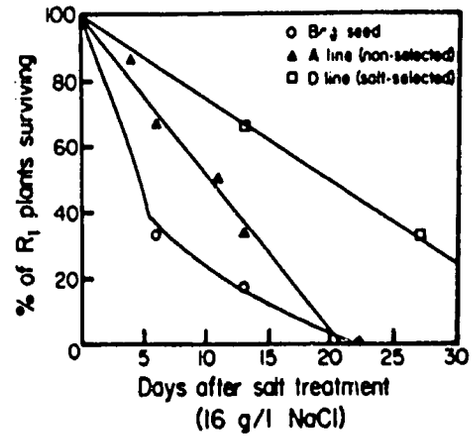

Figure 2

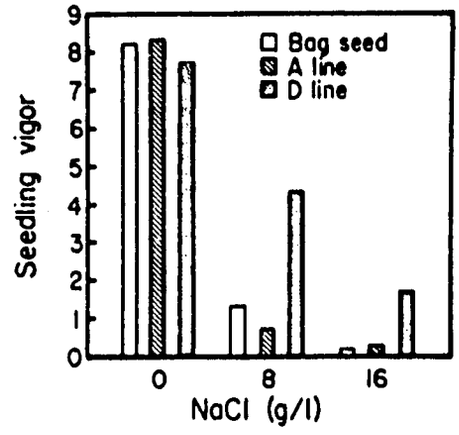

Figure 3

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TRAINING.

The first year of the Cooperative Agreement was devoted almost completely to planning and organization of the Training Program. In March 1985, training brochures and applications were printed and mailed to over 500 persons. Trips were made to Asia and Latin America to publicize the Program. The Course was also announced in the IPBNet Newsletter in June.

The Office of International Training Programs at CSU was enlisted to procure housing, process visas, and present arrival orientation to the trainees. This office also provides trouble-shooting and counseling for foreign students and trainees.

In July, the process of planning Course lectures and labs began. Dr. Harrison Hughes of CSU's Department of Horticulture was enlisted to assist in Course preparation and later instruction. At the first of August, newly-hired Training Coordinator, Sunitha Siriwardana, began preparing a Training Manual and ordering stock for the new training lab.

The first session of the Training Program began September 2, 1985. As shown in Table 4, eight applicants were accepted; three were unable to attend and were deferred to the spring 1986 session.

Problems Encountered. The major obstacle to overcome was the lack of funding from sources other than the TCCP. This was partly due to the fact that the Course was new and not yet recognized by many agencies. Increased publicity is needed to make the Training Program and the TCCP known as a leader in the field of tissue culture. The TCCP should assist applicants in obtaining funding by writing letters of support to the various agencies where candidates seek sponsorship.

Another problem resulted from the amount of time required to process

visas for trainees. The TCCP staff, ignorant of the intricate procedures involved, did not allow enough time, thus, some trainees were delayed in arriving. Applications need to be revised to include the needed visa information, and the application deadline should be moved-up.

Results and Conclusions. The Training Program fulfills a need heretofore unanswered (so far as we know). Courses of shorter duration and narrower scope exist, but no one else provides a comprehensive tissue culture course. The TCCP Training Program was designed to meet the needs of researchers in developing countries who have little or no experience in tissue culture. A feedback questionnaire is being designed which will be sent to graduates of the Program to determine the consequences of their training. The primary goal is to ensure that knowledge gained here is used and/or passed on to others in their home countries.

Table 4. Information on Applicants

Fall 1985 Training Session Participants

Name	Country	Funding Source(s)
Ms. Iteu Hidayat	Indonesia	Transportation & Stipend - AID/Jakarta Fees - TCCP
Mr. Francisco Gutierrez	Mexico	Transportation & Stipend-National Council of Science & Technology of Mexico (CONACYT) Fees - TCCP
Dr. Flordeliza Javier	Philippines	Transportation & Stipend - AID/Manila Fees - TCCP
Mr. Rachmat Kartapradja	Indonesia	Transportation & Stipend - AID/Jakarta Fees - TCCP
Mr. Ben Musyimi	Kenya	Transportation - University of Nairobi Stipend & Fees - TCCP

Accepted for Fall 1985 - Unable to attend

Name	Country	Funding	Crop(s) of Interest
Mr. Renato Avenido	Philippines	Transportation - PCARRD Stipend & Fees - TCCP	Potato and Citrus
Ms. Merlina Dionzon	Philippines	Transportation - PCARRD Stipend & Fees - TCCP	Wheat and Corn
Mr. Simon Ndirangu	Kenya	Transportation - XX Stipend & Fees - TCCP	Tea

INTERNATIONAL PLANT BIOTECHNOLOGY NETWORK (IPBNet)

Network Activities. The Network serves to strengthen the research and technology capabilities within the TCCP and in laboratories of participating scientists in developing countries.

Formation of the International Plant Biotechnology Network (IPBNet) began formally in January 1985. Surveys were designed and sent to 360 contacts in 38 different countries. Contacts included IARC's, University-related science laboratories, USAID Missions, embassies, and individual scientists currently on the TCCP mailing list. To date, 152 responses have been received. Survey questions were aimed at ascertaining whether or not plant tissue culture research was being pursued at the various locations, and whether or not training in the areas was wanted and needed. As a result of the survey, 353 additional contacts in agricultural research and 147 possible training candidates were added to the mailing list.

In addition to publicity through surveys, personal contacts were made through visits by Mr. Tom Dykes, and Ms. Susan Ferguson, Network Coordinators, to several countries each in Latin America, Asia, and Africa. The contacts have resulted in arrivals of trainees and graduate students and in exchange of technical information. In the fall of 1985, the TCCP was invited to participate in the US-Pakistan Biosaline Research Workshop in Karachi and the UNIDO Conference on Agricultural Biotechnology in Delhi, India. Dr. Oluf Gamborg attended and presented papers on the Project's research. During the meeting in Karachi, plans were made for a collaborative effort between the Nuclear Institute for Agriculture and Biology (NIAB) and the TCCP to produce hybrids of rice and kallar grass by protoplast fusion. Details of these trips and

the resulting outcomes are contained in trip reports which have already been sent to AID.

The TCCP staff began planning the First Annual Conference of the International Plant Biotechnology Network in June 1985. The Conference will be held October 21 through 25 at the University Park Holiday Inn in Fort Collins. The Holiday Inn was chosen as the site because of its proximity to the CSU campus, its convention facilities, and its staff's experience in organizing meetings.

Dr. Trevor A. Thorpe, University of Calgary, will give the Keynote Address on the "Application of Tissue Culture Technologies to Agriculture and Forestry." Dr. E.C. Cocking, University of Nottingham, will give a presentation on protoplast and cell fusion, and Dr. Karen Hughes, University of Tennessee, will present a paper on mutation selection and in tissue culture work. In addition, various participants have been asked to present their research.

A unique component of the Conference will be its Roundtable Discussions. Drs. Abraham Krikorian and Ludwig Muller, Oluf Gamborg, Irvin Asher, D.S. Brar and Mujeeb-Kazi have been asked to moderate discussions on tropical crops, cell genetic manipulation, grant writing, and IARC's, respectively. In addition to these discussions, planning sessions have been scheduled to obtain feedback on the Conference, Training Program, Newsletter and other IPBNet programs and services. About 100 participants are expected for the Conference.

Outreach over the past nine months has resulted in strengthening and formalization of existing collaborations, and initiation of new ones. Memoranda of Agreement have been established with CIMMYT, Chulalongkorn University, ICRISAT, IRRI, and PCARRD (Table 5). Memoranda of Agreement

with IAV Hassan II, Morocco, and CATIE, Costa Rica, are in progress.

Table 5. Current Memoranda of Agreement.

Institution	Country	Activity
CIMMYT	Mexico	Tissue culture and widecrosses of wheat with wild relatives
Chulalongkorn U.	Thailand	Collaborative rice research, graduate student exchange
ICRISAT	India	Germplasm exchange, stress tolerance research in millet and sorghum
IRRI	Philippines	Rice field testing
PCARRD	Philippines	Coordination of Philippine research

Another important function of IPBNet is information dissemination. Research news is provided by the IPBNet Newsletter, published twice yearly by the TCCP. Newsletter No. 4, which focused on the development of IPBNet collaborations, was published in June 1985. Newsletter No. 5, scheduled for publication in January 1986, will highlight the IPBNet Conference and update the TCCP Training Program. The TCCP also provides scientific information by maintaining a computerized literature file which now includes over 10,000 refereed journal citations. Lists of specific crop-related publications are available to IPBNet members upon request.

Conclusions and Recommendations. Interest in the Network has been great, as evidenced by survey responses, and growth of IPBNet is expected to hasten as its services and benefits are publicized. It is becoming evident that more effort must be put into expanding and maintaining the

literature file and a half-time person has been hired to fill this role. Funding also becomes increasingly important as IPBNet members seek to come to Fort Collins for technical training, graduate training, post-doctoral research, or technical assistance. It is clear that the TCCP cannot alone support travel, training, and conference expenses, and an effort will be made to assist researchers in obtaining such funding elsewhere. As the TCCP and IPBNet become more recognized, it will be easier to solicit money from other sources. The TCCP is currently designing a publicity brochure for broader distribution to the agricultural community. Surveys continue to be received and distributed, and the importance of "networking" grows apparent as more persons vie for fewer resources.

MANAGEMENT AND OPERATIONS

At the inception of the Cooperative Agreement it was recognized that, while under the previous smaller AID Contract it had been possible for one person to operate the Project and oversee the research, changes in staffing patterns, operational procedures, and organizational policies would be necessary to efficiently run a project of this magnitude. Thus, in January, 1985, Mr. Kirby De Mott, Mr. Tom Dykes, and Ms. Julie Fischer were hired to take responsibility for specific sections of the Project in Research, Networking and Operations, respectively.

Along with the Project Director, the three newly-employed directors worked as a team to incorporate the new research and networking components into the existing program. Much time was spent on preparation of IPBNet surveys and analysis of data received, preparation and distribution of publicity brochures for the Conference and Training Program, and design and implementation of internal operational policies

such as hiring, evaluation, and budgetary planning systems.

In August, Dr. Oluf Gamborg was hired as Associate Director to assist in building the Network and overseeing the research, and Ms. Sunitha Siriwardana was hired as Training Coordinator. Subsequently, a Training Manual was prepared and lessons and research demonstrations outlined for the upcoming Training Program. Ms. Susan Ferguson, who had been hired in June, then took over as Network Coordinator to replace Tom Dykes who left to attend medical school. Dr. Christy MacKinnon, former graduate student of Dr. Nabors, was also hired to co-direct the research with Mr. De Mott.

FACILITIES

With a management and research team in place, on August 12 the Project moved into its new $700,000 (funded by CSU) specially-designed 638 sq meter research, training and office complex adjacent to its existing 765 sq meter 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, two large laboratories (main and training), quiet study and office areas, 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.

REVISED BUDGET: SEPTEMBER 1984 - SEPTEMBER 1985

TOTAL INPUTS

	OPERATION	NETWORK	TRAINING	RICE	WHEAT	LEGUMES	MILLET	CORN	SORGHUM**	PROTO/SUS	WIDECROSS	OATS	TOTAL
PERSONNEL	39000	31964	9444	44526	37096	20281	28073	17877	52177	13610	7804	19608	321460
TRAVEL	0	11737	0	2435	2435	0	2435	0	1307	0	0	2435	22784
MAT & SUP	5381	4036	1345	8272	6926	2690	5581	8941	10326	1345	1345	4237	60426
OPERATING EXP	2149	1611	537	6116	5579	1074	5042	7326	205	537	537	4506	35221
PRINT/COPY	973	730	243	979	736	437	492	487	0	243	243	250	5863
CONSULTING	10207	0	0	0	0	0	0	0	1850	0	0	0	12057
TRAINING FEES	0	0	3977	0	0	0	0	0	0	0	0	0	3977
TUITION	0	0	0	0	3240	0	0	0	0	1958	0	0	5198
SUBGRANTS	0	0	0	0	0	0	0	0	0	0	0	0	0
EQUIPMENT	22171	0	5543	18074	12531	11085	6989	11085	3154	22171	5543	1447	119793
INDIR COSTS*	22348	19430	6032	24183	20476	9519	16150	8586	25556	6105	3853	10106	172344
TOTAL	102229	69508	27121	104585	89019	45136	64762	54303	94575	45970	19325	42589	759123

OTHER FUNDS - DIRECT SUPPORT

AID CONTR #0273	269		2185	37600	39515	37559						37589	154717
GRI									94575				94575
PIONEER HIBRED								12503					12503
USDA												5000	5000
TOTAL													266795

OTHER FUNDS - INDIRECT SUPPORT

LIBYAN GOV'T					12000								12000
TOTAL													12000

*INDIR COSTS = 38.9%, not calculated on TUITION, EQUIPMENT, PIONEER or USDA

**SORGHUM program totally funded by the Gas Research Institute

TOTAL INPUTS based on AID Coop Agreement(#4137) funds of $492328 plus total DIRECT SUPPORT

FINANCIAL REPORT.

Expenditures during the first year of the five-year cooperative agreement were considerably less than anticipated for three reasons: 1) The IPBNet Conference was not held until October, 1985, in grant year two; 2) The first session of the Training Program was not held until August, 1985; and 3) Subgrant recipients were not yet identified or funded. As a result, approximately $300,000 was carried-over from the grant-year-one budget to the grant-year-two budget.

See the Revised Budget for details of expenditures by line item and crop.

APPENDIX I

Publications and Reports

Dykes, T.A. and M.W. Nabors. 1985. Tissue Culture of Rice and its Application in Selecting for Stress Tolerance. International Rice Genetics Symposium IRRI, Philippines, (May).

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).

Gamborg, O.L. Cells, Protoplasts and Plant Regeneration in Culture. In: Manual of Industrial Microbiology and Biotechnology. A.L. Demain andN.A. Solomon (eds.) Amer. Soc. Microbiology, Washington, D.C., In press.

Gamborg, O.L. and M.W. Nabors. Somatic Hybridization—Constraints and Prospects. International Workshop on Biotechnology in Agriculture, New Delhi, India, In press.

Raghava Ram, N.V. and M.W. Nabors. 1985. Plant Regeneration from Tissue Cultures of Pokkali Rice is Promoted by Optimizing Callus to Medium Ratio Ratio and by a Medium-Conditioning Factor Produced by Embryogenic Callus, Plant Cell Tissue Organ Culture 4:241-248.

The International Plant Biotechnology Network, Tissue Culture for Crops Project Newsletter #4. 1985. J.L. Fischer (ed.) Colorado State University, Fort Collins, (June).

APPENDIX II

Staff List

Administrative Staff

Name	Title
Murray Nabors	Project Director
Oluf Gamborg	Associate Director
Julie Fischer	Operations Director
Kirby De Mott	Research Coordinator
Christy MacKinnon	Research Coordinator
Susan Ferguson	Network Coordinator
Sunitha Siriwardana	Training Coordinator

Full-time Technical Staff

Deborah Kelly	Lab Manager, Rice Group
Mary Waters	Greenhouse Manager
Barbara Ashton	Wheat Group
Kirby De Mott	Rice Group
Fariha Faizi	Soybean Group
Touba Ghazi	Souybean Group
Glenys Gunderson	Sorghum Group
Glen Hildreth	Sorghum Group
Kerri Wright	Rice Group

Part-time Technical/Support Staff

Carolyn Angleton	Greenhouse Group
Gwendolyn Bane	Greenhouse Group
Tammie Fjellstrom	Greenhouse Group
Lori Frederickson	Office Group

Graduate Students

Robert Fjellstrom	Oat Group
Raymond Ketchum	Protoplast Group
Douglas McMurray	Legume Group
Akbar Mohmand	Wheat Group
Poungpet Poonsapaya	Rice Group
Gaith Sasi	Wheat Group
Sathish Puthigae	Rice Group

Student Support Staff

Julie Cotton	Robert Oswald
Theresa De Mersseman	Gregory Pott
Nancy Ekren	Scott Price
Paula Glasgow	Stephanie Schaal
David Hewitt	Peter Schuerman
Jeannette Hut	Erin Scott
Koji Kawamura	Debra Singleton
Larry Klima	Nitschka ter Kuile
Eric Leftwich	David Timm
Bryan Lobmeyer	Susan Todd
Deborah Neubaum	Callie Vanderbilt
Laurie Osborne	