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Agricultural Biotechnology/Plant Tissue Culture Project

Major Design Elements, Work Plans and Schedules

(December, 1985)

936-4137

I. DEVELOPMENT GOAL (Higher-Level Objective)

A. Background

Among the problems limiting food production, supply, and quality in the developing countries (DC's), unfavorable environmental conditions play an important role. Chief among these are the following:

1. Drought and excess salts affect around 40% and 25% respectively, of the world's arable land;

2. Soil acidity (which brings aluminum, manganese, and other toxic metals into the soil solution) affects about 25% of the world's arable land.

3. Weather fluctuations, including extreme temperatures, account for 60-80% of the variability in crop production.

These environments must be modified (irrigation, fertilization, liming etc.), if the vast majority of crop cultivars are to produce effective yields within them.

Improving the stress tolerance of crops important to the DC's, along with other technological advances in crop management, plant protection, storage, etc., will improve yields and availabilities in a cost-effective fashion, particularly on marginal and small farms.

B. Development Goal:

The Project seeks to accelerate the development of food crop varieties with higher yields under conditions of environmental stress. It is intended that such cultivars can be grown on marginal land by small farmers in developing countries.

C. Development Hypothesis or Justification

1. There are limits on what can be done to modify the environment to increase and sustain crop yields. For example, irrigation requires fresh water and 80% of readily available water is already used for agriculture. Irrigation systems, regardless of careful management, cannot eliminate salt build-up. Soil liming to reduce acidity is neither practical logistically nor financially possible in many areas. The high costs of mechanized agriculture, fertilizers, etc., severely constrain environmental modifications in the least developed countries and, as energy cost rises, increasingly in the more developed countries.

2. It makes sense, under these conditions, to approach these problems (see Background) by working to modify the plant to suit available environments. If, for example, the drought tolerance can be increased, then (a) land currently too dry for agriculture can be brought into cultivation; (b) the extent of coverage by available irrigation water can be extended; (c) salt build-up due to irrigation will be slowed because irrigation is reduced with respect to amount and frequency and (d) resources currently devoted to environmental modification and, in some cases, food importation, can be redirected.

3. Tissue culture research offers plant breeders a mechanism for speeding up the development of crop cultivars that are resistant to stresses such as those mentioned. This technology produces rapid selection mechanisms using only a limited amount of space and number of people.

4. However, use of emerging biotechnology focused on tissue culture methodologies is currently impeded by a number of problems, some typical to agriculture in tropical and sub-tropical areas, and others unique to plant biotechnology. These include:

a. Lack of fully developed and tested technologies and methodologies, especially for regenerating stress-tolerant crops important in DC's;

b. Failure by plant breeders, agricultural leaders and financing institutions to recognize potential cost-effective value of successful Plant Tissue Culture (PTC) technology applications in solving the problems of DC crop production;

c. Insufficient exchange of information;

d. Lack of problem-solving capabilities in many DC's including lack of trained people and facilities and insufficient funds.

II. PROJECT OBJECTIVES

A. Objectives

1. To facilitate the establishment and strengthening of capabilities in selected developing countries to produce stress-tolerant crops;

2. To expand the knowledge base of biotechnology;

3. To increase the rate of plant biotechnology transfer and its use by developing countries.

B. Project Approach or Hypothesis

The project objectives are interrelated and represent an approach which is a combination of research and development, training, technology transfer, and application. The focus is upon those problems which impede the development and transfer of biotechnological knowl-

edge and capabilities and their use by developing countries in solving agricultural production problems. It employs the networking concept as a device to expand the knowledge base, increase DC capacity and facilitate transfer and utilization. The project also provides the means to expand the involvement and increase the capabilities of CSU in several areas of plant biotechnology and assist interested DC's develop their own capabilities and programs. The results (outputs) of project activities, along with other events beyond the scope of the project, e.g. increased national and multi-lateral support, are expected to result in independently verifiable achievement of the project objectives by 1990.

C. Project Management

The AID Cooperative Agreement with Colorado State University (CSU) is to (1. continue and initiate new tissue culture research, (2) expand the knowledge base to new crops and environmental stresses, and (3) establish, coordinate, and provide leadership and support for an international tissue culture research network. CSU will have little or no direct control over most of the network participants. It will rely largely on the prospects of mutual benefits, persuasion, and network support activities to exercise its leadership role and produce the outputs and sub-outputs on schedule and as necessary to successfully achieve the project objectives by 1990.

The cooperative agreement mechanism has been chosen to provide CSU with the flexibility necessary to use innovative and indirect resources. These resources include special-purpose work groups, field visitations, award of new sub-grants, collaborative progress reviews, etc. CSU is expected to plan and obtain a reasonable amount of "management control" and to seek assistance from AID regarding matters outside its immediate control. Special measures will also be required to assure effective collaboration between CSU and AID and with active network participants.

Among these measures will be the early establishment (August, 1985) of a Management Review Group (MRG) composed of CSU, AID, and others familiar with the administration of multi-national agricultural research programs supplemented, as necessary, with research, and international networking specialists. The MRG will advise and monitor the project on matters concerning administration, networking and research. The MRG will consult with the CSU Project Director on the management implications of recommendations that it makes, and in reaching appropriate decisions. The group will pay special attention to keeping the project on track in accordance with the terms of the Cooperative Agreement between CSU and AID. As the project develops, it may prove necessary to establish additional advisory groups such as a Network Review Group and a Research Advisory Group funded outside the scope of this study. However, mobilization of such advisory groups is quite costly and presently not viewed as necessary given the level of project activity. Thus, the MRG will assume these functions and when necessary provide for their orderly transfer to a Research or Network Review Group as the program proceeds towards end-of-project-status indicators (EOPS).

During the first six months of the agreement, along with the recruitment and training of new staff and expansion of facilities, considerable effort has been given to (a) establishing objective, verifiable EOPS, and (b) clarifying the expected results (outputs and sub-outputs) of project activities to provide the framework for "management by objectives". Detailed work plans, schedules, and milestones have been developed for each output or sub-output for subsequent reporting, monitoring, and evaluation. The detailed specification of each output also provides the performance indicators of output production and explicit statements of the critical assumptions, i.e., events outside project management control but crucial to producing the output. The first work plan and subsequent annual revisions will be presented to the MRG for their endorsement and suggestions. These output statements will also provide the basis for annual reporting to AID on progress and problems in producing each output or sub-output.

Finally, AID, in collaboration with CSU, will conduct an in-depth evaluation of the project during the third and fifth years of the grant or as may be mutually agreed upon. The final evaluation of Project 936-4055 (Tissue Culture for Food Production) which is the pilot project to the present Cooperative Agreement will take place during the last half of CY 1986. The MRG will be the evaluation team and the evaluation will take place in connection with the first MRG meeting for the Biotechnology Project (936-4937).

D. End-of-Project-Status Indicators

End-of-project status indicators (EOPS) refer to the conditions which will exist at the successful completion of the project. They are signs that something happened as a result of project activities and are objectively verifiable. They can be useful during project operations for monitoring progress at the project objective level, which, when the project environment or state-of-the-art is changing rapidly, can be important to reassure ourselves that the project approach or hypothesis remains valid. In addition, EOPS indicators help us to determine when the project should end. They are also used in terminal or post-project evaluations to assess project effectiveness.

In short, we are looking for indicators, quantifiable and direct when possible, but at least recognizable and reasonable, which measure desirable changes in 1) increased capabilities in developing countries to use plant tissue culture techniques and produce stress-tolerant crops, 2) an expanded knowledge base covering more crops and their environmental stresses, and 3) an increasing rate of biotechnology transfer and use in the developing world. As these objectives are inter-related, so will be their EOPS. The following list may be refined and/or expanded as output definition proceeds.

1. Acceptance of use of tissue culture methods and germplasm by network collaborators as measured by field tests, published papers and reports, and trainees and graduates working in developing countries' tissue culture activities.

2. Acceptance and use of tissue culture methods by others (non-network) as measured by Science Citation Index references to published papers, invitations to national and international meetings, and requests for germplasm.

3. Increased financing of tissue culture activities by national and regional developing country sources.

4. Requests to CSU and to bi-lateral and multi-lateral agencies for technical assistance in establishing or strengthening tissue culture programs in developing countries as measured by USAID field missions PIO/T's, IARI's, FAO, etc.

5. Requests by developing countries for assistance in applying tissue culture methods to new crops and/or new environmental stress factors or initiation of new programs with or without network assistance.

III. PROJECT OUTPUTS/RESULTS

A. Introduction

Statements of planned project results are outlined below. Details of specific outputs (including activities/tasks required to complete each project objective), are described in section IV, together with a list of suggested milestones and dates to be used for monitoring purposes. Research objectives that are to be addressed by Network collaborators are indicated by "major activity" and/or specific "task" in section IV. The following outlines are intended to provide a reasonable basis for completing project objectives, but should be reviewed annually and modified as dictated by project exigencies.

B. Summary Statements of Major Outputs

1. Research and Development

a. Output 1. Methodologies developed for improved mutant selection and plant regeneration.

Output 1a. Techniques for obtaining high-frequency, long-term plant regeneration, including embryogenic suspensions, of selected species of cereals (rice, wheat, millet, corn, sorghum) and legumes (soybean, cowpea, common bean).

Output 1b. Techniques for selecting tissue cultures tolerant to environmental stresses.

Output 1c. Techniques for using tissue culture to promote gene exchange in wide crosses of wheat and/or corn.

Output 1d. Techniques for selecting tissue cultures more efficient at utilizing nitrate and phosphate.

Output 1e. Techniques for obtaining high-frequency plant regeneration from selected cereal and legume protoplasts.

Output 1f. Techniques for cloning nitrate reductase and introducing cloned nitrate reductase into protoplasts.

b. Output 2. Verification of stress tolerance selection techniques through greenhouse and field testing.

Output 2a. Greenhouse test results showing whether or not tissue culture selection techniques can give rise to stress-tolerant and fertilizer-efficient plants.

Output 2b. Field test results for four crop species showing whether and to what extent greenhouse tolerant plants show tolerance in the field.

Output 2c. Seeds with increased field tolerance for one or more stresses for distribution to plant breeders.

2. International Plant Biotechnology Network (IPBNet)

Enhanced capabilities in at least 10 developing countries in plant biotechnology focusing on tissue culture techniques to produce improved cultivars for local production of crops. This will be accomplished by establishing:

a. Output 3. Network core creation and support including but not limited to services to network participants including information exchange, literature and data base, technical assistance, and conference preparation.

b. Output 4. A Plant Biotechnology Training Center at CSU with a capacity to train 10 scientist/technicians per year in plant tissue culture techniques.

c. Output 5. At least six equal partner DC Collaborators involved in IPBNet as centers of tissue culture expertise and in information and personnel exchange, cooperative research, and field testing with CSU and other network members. Equal partner collaborators will be established through formal agreements as outlined in Output 3.

d. Output 6. Facilitation of information and personnel exchange.

e. Output 7. Facilitation of cooperative research.

f. Output 8. Facilitation of field testing of TCCP crops.

g. Output 9. Collaboration established with at least three IARC's and/or CRSP's, regional and crop-specific, for a) supply of germplasm b) field testing of stress-tolerant project germplasm, c) exchange of information on stresses affecting production, and, d) facilitating cooperative programs with

network members (no project funds).

h. Output 10. At least three developing country tissue culture labs strengthened to perform initial aspects of project tissue culture research, including field testing (sub-grants).

i. Output 11. Agreements with at least three U.S. universities or one CRSP actively involved in research and technical assistance in sub-tropical and tropical soils, to cooperate in (a) field testing TCCP products with developing country partners, (b) information exchange, and/or (c) research relevant to achieving TCCP objectives (sub-grants).

C. Statement of Critical Assumptions

1. The experimental approach used to develop regeneration methods for rice, wheat and millet will work, or can be modified to work, for regeneration of sorghum, corn, legumes and wide-cross embryos of wheat and non-wheat grass genera.

2. Techniques used to select for heritable salt-tolerance in other crops can be successfully modified to select rice, wheat, millets, sorghum, corn, soybean, cowpea and common bean, for tolerance to other stresses (aluminum, drought, heat, low nitrogen, etc.).

3. IPBNet collaborators with research capabilities and interests complimentary to overall TCCP objectives, and who are willing to address specific project outputs (through sub-grants), will be identified in a timely manner.

4. IPBNet collaborators will openly and freely communicate with the TCCP Project Director, Network Director and other Network members; and individual collaborators will pursue their research tasks in a timely and reasonable manner.

5. Project collaborators will provide the TCCP with sufficient germplasm to complete project activities, and collaborators responsible for field testing will accept TCCP germplasm, perform field tests, and report results in good faith and in a timely manner.

6. Successful completion of Output 1f assumes considerable progress in the field of plant molecular biology over the next five years regarding developments in the areas of identifying, cloning and movement of specific plant genes, and specifically, the development of suitable hybridization probes for nitrate reductase.

IV. Work Plan And Milestones

A. Research and Development (See Table 1)

1. Output 1. Methodologies developed for improved mutant selection and plant regeneration

Output 1a. Techniques for obtaining high-frequency, long-term plant regeneration from tissue cultures of selected cereal (rice, wheat, millet, corn, sorghum), and legume (soybean, cowpea, common bean) species.

Results to date: Predecessors to the Tissue Culture for Crops Project at Colorado State University concentrated on developing tissue culture methodologies for selecting NaCl, drought, and aluminum (Al^{3+}) tolerant rice, wheat, millet and oat plants since 1980. It had been shown by the CSU tissue culture lab previous to 1980 (using tobacco as a model) that long-term cell selection was prerequisite to stable whole plant tolerance in regenerated plants. However, plant regeneration from cereal tissue culture prior to 1980 had typically been sporadic and of short duration. Therefore, the first goal was to obtain methods for high-frequency, long-term plant regeneration for selected cereals. Progress toward this objective has been rapid and is nearly complete. For wheat, rice, pearl millet, proso millet, and oats, callus cultures have been obtained from mature and immature embryos. Embryogenic callus with high-frequency, long-term regeneration has been selected for each crop for callus derived from mature and immature embryos.

Major Activity. Develop methodologies and procedures for initiation, maintenance and regeneration of embryogenic callus cultures.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Determine appropriate explant sources for initiating tissue cultures of: a) rice (IR36, Pokkali, Mahsuri, G-159, Calrose 76), wheat, millet[a]		completed	
b) rice (IR6, IR54, IR60, IR62), corn, sorghum[b]		10-85	10-86
* c) common bean, cowpea, soybean[c]		10-85	10-86

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b cereals for which tissue cultures were initiated in 1984
c legumes for which tissue cultures were initiated in 1984

* work to be done in part by Network collaborators

(2) Determine		10-85	10-86

wt./vol. ratio for callus innoculum and basal medium for:			
a) rice (IR36, Pokkali, Mahsuri G-159, Calrose 76), wheat, millet		completed	
b) rice (IR6, IR54, IR60, IR62), corn, sorghum		9-85	9-86
* c) common bean, cowpea, soybean		9-85	9-86
(3) Determine hormonal conditions for initiation and long-term maintenance of embryogenic callus for:	x		
a) rice (IR36, Pokkali, Mahsuri, G-159, Calrose 76), wheat, millet		completed	
b) rice (IR6, IR54, IR60. IR62), sorghum, corn		9-85	9-88
* c) cowpea, common bean, soybean		9-85	9-90
(4) Determine hormonal conditions for long-term, high-frequency regeneration of:			
a) rice (IR36, Pokkali, Mahsuri, G-159, Calrose 76), wheat, millet		completed	
b) rice (IR6, IR54, IR60, IR62), sorghum, corn		9-85	9-88
* c) cowpea, common bean, soybean	x	9-85	9-90

Major Activity. Develop methodologies and procedures for initiation, maintenance and regeneration of embryogenic suspensions.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Determine hormonal requirements necessary for the development of embryogenic suspensions for wheat and rice.		9-85	3-90
(2) Determine	x	9-85	3-90

conditions necessary for regeneration of plants from embryogenic suspensions of wheat and rice cells.

Output 1b. Techniques for selecting tissue cultures tolerant to environmental stresses and regeneration of plants from stress-tolerant cell lines.

Results to date: Callus has been selected for tolerance to 3000, 6000 and 9000 ppm NaCl for rice, wheat, pearl millet, proso millet and oats. For rice, over 3500 plants have been regenerated from NaCl-stressed cultures. Greenhouse testing of progeny of IR36 and Calrose 76 plants regenerated from salt tolerant cultures shows seedling tolerance in some lines compared to controls (see Output 2a). For wheat and pearl millet over 200 plants have been regenerated from NaCl-stressed cultures, and for oats over 350 plants have been regenerated from NaCl-stressed cultures. The establishment of techniques used for the selection of drought and Al^{3+} tolerant cell lines has been somewhat more difficult. Media did not gel adequately when either Al^{3+} or polyethelene glycol (used in drought tolerance studies) was incorporated. Techniques have, however, been worked out and cell selections for Al^{3+} and drought tolerance are currently underway. Rice callus has been selected for growth on aluminum levels as high as 112 ppm for more than five months. Selection studies for drought tolerance in rice using polyethelene glycol are approximately three months old. Approximately six plants have been regenerated from Al^{3+}-selected cultures.

Major Activity. Develop methodologies and procedures for selection of tissue cultures tolerant to NaCl; perform selections and regenerate plants from NaCl-tolerant cell lines.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Determine selection media with appropriate levels of NaCl to give 1%, 10% and 20% of normal callus initiation and growth, for:			
a) rice, wheat, millet		9-85	9-86
* b) sorghum, corn		9-86	9-87
* c) legumes		9-87	9-88
(2) Determine regeneration rates for callus tolerant to NaCl, modify regeneration media as necessary for long-term regeneration of:			

a) rice, wheat, millet		completed	
* b) sorghum, corn		9-86	9-88
* c) legumes		9-87	9-90
(3) Inititiate callus on medium without NaCl and expose non-selected callus to appropriate NaCl levels at each cell tissue culture passage as a control for NaCl-tolerance cell selections, for:			
a) rice, wheat, millet		9-85	9-88
* b) sorghum, corn		9-86	9-89
* c) legumes		9-87	9-90
(4) Select cell lines tolerant to NaCl as compared to control at a constant NaCl level, for:	x		
a) rice, wheat, millet		9-85	9-88
* b) sorghum, corn		9-86	9-89
* c) legumes		9-87	9-90
(5) Select cell lines for tolerance to NaCl as compared to control with step-wise increases in NaCl concentration over successive passages, for:			
a) rice, wheat, millet		9-85	9-88
* b) sorghum, corn		9-86	9-89
* c) legumes		9-87	9-90
(6) Check NaCl-selected cell lines for stability by removing and then replacing NaCl stress. Compare tolerance in callus selected at a constant NaCl level with callus selected in a step-wise manner, for:			
a) rice, wheat, millet	x	9-86	9-88
* b) sorghum, corn		9-87	9-89
* c) legumes		9-88	9-90

(7) Regenerate plants from NaCl-tolerant callus cultures, for:	x		
a) rice, wheat, millet		9-85	9-88
b) sorghum		9-86	9-89
* c) legumes		9-87	9-90
(8) Develop an *in vitro* NaCl-tolerance testfor progeny of NaCl-selected cell lines, for:			
a) rice, wheat, millet		9-85	9-88
b) sorghum		9-88	9-89
* c)legumes		9-89	9-90
(9) Determine optimal selection pressure (level of NaCl and length of selection period) for routine production of NaCl-tolerant plants (based on *in vitro* progeny test and/or greenhouse tests).	x	9-85	9-90

Major Activity. Develop methodologies and procedures for selection of tissue cultures tolerant to "drought"; perform selections and regenerate plants from "drought"-tolerant callus:

Activity/Tasks	Selected Milestones	Target Dates Start	End
(1) Determine selection media with appropriate levels of polyethelene glycol (PEG) to give 1%, 10% and 20% of normal callus growth, for:			
* a) rice, wheat, millet		9-85	9-88
b) sorghum		9-86	9-89
** c) other crops		9-86	9-89

** work to be done by Network collaborators; crop species to be determined in consultation with individual collaborators

(2) Determine regeneration rates for callus tolerant to PEG, modify regeneration media as necessary for long-term regeneration of:		
* a) rice, wheat, millet	9-85	9-88
b) sorghum	9-86	9-89
** c) other crops	9-86	9-89
(3) Inititate callus on medium without PEG and expose non-selected callus to appropriate PEG levels at each tissue culture passage as a control for PEG-tolerance cell selections, for;		
* a) rice wheat, millet	9-85	9-88
b) sorghum	9-86	9-89
** c) other crops	9-86	9-89
(4) Select cell lines for tolerance to PEG at a constant PEG level for:		
* a) rice, wheat, millet	9-85	9-88
b) sorghum	9-86	9-89
** c) other crops	9-86	9-89
(5) Select cell lines for tolerance to PEG with step-wise increases in PEG concentration over successive passages, for:		
* a) rice, wheat, millet	6-86	9-88
b) sorghum	9-86	9-88
** c) other crops	9-86	9-88
(6) Check PEG-selected cell lines for stability of tolerance by removing and then replacing PEG stress. Compare tolerance in callus selected at a constant PEG level with callus selected in a step-wise manner, for:		
* a) rice, wheat, millet	9-87	9-88
b) sorghum	9-87	9-89

** c) other crops		9-87	9-89
(7) Regenerate plants from callus tolerant to PEG, for:	x		
* a) rice, wheat, millet		9-85	9-88
b) sorghum		9-86	9-89
** c) other crops		9-86	9-89
(8) Develop an *in vitro* PEG-tolerance test for progeny of PEG-selected celllines, for:			
* a) rice, wheat, millet		9-87	9-88
b) sorghum		9-88	9-89
** c) other crops		9-88	9-89
(9) Test callus tolerant to NaCl for PEG tolerance.		9-85	9-90
(10) Determine optimal selection pressure (level of PEG and length of selection period) for routine production of drought-tolerant plants (based on *in vitro* program test and greenhouse tests).	x	9-85	9-90

Major Activity. Develop methodologies and procedures for selection of tissue cultures tolerant to Al^{+3}; perform selections and regenerate aluminum-tolerant plants.

Activity/Tasks	Selected Milestones	Target Dates Start	End
(1) Determine selection media with appropriate levels of $AlCl_3$ to give 1%, 10% and 20% of normal callus growth for:			
a) rice, wheat, * millet		9-85	9-88
b) sorghum		9-86	9-89
* c) legumes		9-86	9-89
(2) Determine regeneration rates for callus tolerant to $AlCl_3$, modify regeneration media as necessary for long-term regeneration of:			

a) rice, wheat, * millet	9-85	9-88
b) sorghum	9-86	9-89
* c) legumes	9-87	9-90
(3) Initiate callus on medium without $AlCl_3$ and expose non-selected callus to appropriate $AlCl_3$ levels at each tissue culture passage as a control for $AlCl_3$ tolerant cell selections, for:		
a) rice, wheat, * millet	9-85	9-88
b) sorghum	9-86	9-89
* c) legumes	9-87	9-90
(4) Select cell lines tolerant to $AlCl_3$ at a constant level, for:		
a) rice, wheat, * millet	9-85	9-88
b) sorghum	9-86	9-89
* c) legumes	9-86	9-89
(5) Select cell lines for tolerance to $AlCl_3$ with step-wise increases in $AlCl_3$ concentration over successive passages, for:		
a) rice, wheat, * millet	6-86	9-88
b) sorghum	9-87	9-89
* c) legumes	9-88	9-90
(6) Check $AlCl_3$-selected cell x lines for stability by removing and then replacing $AlCl_3$ stress. Compare tolerance in callus selected at a constant $AlCl_3$ level with callus selected in a step-wise manner, for:		
a) rice, wheat, * millet	9-86	9-88
b) sorghum	9-87	9-89
* c) legumes	9-88	9-90
(7) Regenerate plants from x		

$AlCl_3$-tolerant callus cultures, for:			
a) rice, wheat, * millet		9-85	9-88
b) sorghum		9-86	9-89
* c) legumes		9-87	9-90
(8) Develop an <u>in vitro</u> $AlCl_3$-tolerance test for progeny of $AlCl_3$-selected cell lines, for:			
a) rice, wheat, * millet		9-87	9-88
b) sorghum		9-88	9-89
* c) legumes		9-89	9-90
(9) Determine optimal selection pressure (level of $AlCl_3$ and length of selection period) for routine production of aluminum-tolerant plants (based on <u>in vitro</u> progeny test and greenhouse tests).	x	9-85	9-90

<u>Major Activity.</u> Develop methodologies and procedures for selection of wheat tissue cultures tolerant to heat; perform selections, and regenerate plants from heat-tolerant callus.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Expose wheat callus to high temperatures and select callus with normal growth (as compared to controls growing at optimal temperatures).		6-86	6-89
(2) Check stability of callus growth for callus tolerant to heat by removing and then replacing heat stress over a period of one to several months, select callus with stable tolerance to heat.		12-86	6-89
(3) Regenerate plants from heat tolerant wheat callus.		12-86	6-89
(4) Develop an <u>in vitro</u> heat-tolerance test for generative progeny of heat-selected cell lines.		6-88	6-89

(5) Determine optimal selection pressure (temperature and length of selection period) for routine production of heat tolerant plants (based on *in vitro* progeny tests and greenhouse tests).	x	6-87	9-90

Major Activity Develop methodologies and procedures for selection of rice tissue cultures tolerant to cold.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Expose rice callus to critical low temperatures reducing growth to 1%, 10% and 20% of normal.		3-89	9-90
(2) Determine regeneration rates for cold-tolerant callus.		6-89	9-90
(3) Initiate and grow callus at normal temperatures and expose to critical low temperatures at each tissue culture passage, as a control for cold-tolerance cell selections.		3-89	9-90
(4) Select cell lines tolerant to cold as compared to control.		6-89	9-90
(5) Check cold-selected lines for stability of tolerance by removing and then replacing temperature stress. Compare controls to cold-selected lines.		9-89	9-90

Output 1c. Techniques for using tissue culture to promote gene exchange in wide crosses of wheat and/or corn.

Results to date: An agreement has been made with Dr. Mujeeb-Kazi of CIMMYT to employ tissue culture as a potential means of increasing the rate at which useful genes from "alien" (non-wheat) grasses are incorporated into wheat--after wide-crosses have been made. Actual tissue culture of wide-cross embryos is scheduled to begin in March of 1985.

Major Activity. Develop methodologies and procedures for initiation, maintenance and regeneration of embryogenic callus from wide-cross embryos.

Activity/Tasks	Selected Milestones	Target Dates Start	End
(1) Initiate tissue cultures from wide-cross embryos.		3-86	9-88
(2) Determine hormonal conditions appropriate for maintenance of embryogenic callus from wide-cross embryos.	x	3-86	3-87
(3) Determine hormonal conditions appropriate for high-frequency regeneration from tissue cultures of wide-cross embryos.	x	3-86	9-88

Major Activity. Develop methods for determining rate of chromosome exchange in tissue cultures of wide-cross embryos.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Select wheat cultivars that are amenable to wide crossing.		completed	
(2) Perform wide-crosses between alien genera and wheat.		3-86	9-88
(3) Initiate tissue cultures of wide cross embryos and regenerate plants.	x	3-86	9-88
(4) Examine chromosomes of regenerated plants with C and N banding techniques to determine the rate of chromosome exchange.		3-86	9-88

* Major Activity. Backcross hybrid plants to obtain stable, diploid wheat.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Backcross plants regenerated from cell cultures of wide cross progeny to wheat.		3-87	9-90
(2) Continue backcrosses		3-87	9-90

until stable plants with normal wheat/corn ploidy are obtained.			
(3) Examine plants cytogenetically to determine alien chromosome translocation rates.	x	3-87	9-90

* Major Activity. Perform in vitro tests of wheat, and backcrossed wide-cross progeny to determine rate of useful alien gene transfer into wheat genome—determine if cellular based increases in stress tolerance have been obtained.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Initiate tissue cultures of stable wheat plants containing translocated DNA from alien grass species.	x	9-87	9-90
(2) Initiate tissue cultures of parental type wheat (as control).		9-87	9-90
(3) Expose tissue cultures to specific stress agents (eg. NaCl or fungal toxins).		9-87	9-90
(4) Monitor growth of stressed cell cultures—compare controls to backcrossed wide-cross progeny.		9-87	9-90
(5) Determine if any increase in tolerance to the stress in question is observed in cell cultures of backcrossed wide-cross progeny.		9-88	9-90

Output 1d. Techniques for selecting tissue cultures more efficient at reducing nitrate and regeneration of plants from cell lines with altered nitrate-reductase activity.

Results to date: Preliminary investigations have begun to select tissue cultures efficient at reducing nitrate by using low nitrate levels in selection media. Lowered nitrate levels in basal media have been shown to affect strongly, rates of callus growth. Currently, these callus cultures are being monitored monthly to check for callus with tolerance to low nitrate.

Major Activity. Develop methods and procedures for determining NR

activity.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Review literature concerning assays for determination of *in vitro* and *in vivo* NR activity.		9-85	3-86
(2) Test various NR assays for consistency and accuracy, both *in vitro* and *in vivo*.		9-85	9-86
(3) Determine appropriate assays for NR activity in both cell cultures and whole plants.	x	9-85	9-86
(4) Perform assays on selected cell lines as described below.	x	9-85	9-88

Major Activity. Develop methods and procedures for selection of nitrate reductase (NR) variant tissue cultures using rice; perform selections and regenerate plants from NR variant tissue cultures.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Determine appropriate levels of chlorate added to growth medium to give 1%, 10% and 20% of normal callus growth.	x	9-85	9-86
(2) Determine appropriate levels of nitrate in growth media (as the sole nitrogen source) to give 1%, 10% and 20% of normal callus growth.		9-85	9-86
(3) Determine appropriate levels of tungstate in growth medium to give 1%, 10% and 20% of normal callus growth.		6-86	6-87
(4) Select callus with high or low growth rates on selection media with chlorate, low nitrate, or tungstate, grow callus on medium without stress as a control.	x	9-85	9-88
(5) Perform assays of NR		9-86	9-88

activity (see above major activity) in cell lines with high and low growth rates. Compare to controls.			
(6) Select cell lines with high, and normal NR activity and regenerate plants.	x	9-86	9-88
(7) Determine optimal selection pressure (type of inhibitor, concentration of inhibitor and length of selection period) for routine production of cell lines with altered nitrate reductase activity (based on greenhouse tests—see below).	x	9-86	9-90

Output 1e. Techniques for obtaining high-frequency plant regeneration from selected cereal and legume protoplasts.

Results to date: Appropriate enzyme concentrations, as well as favorable osmotic conditions have been determined for protoplast isolation from immature rice and wheat embryos, rice and wheat callus, wheat suspensions, and callus and suspensions of other grasses.

Major Activity. Develop methods and procedures for isolation of protoplasts from various donor tissues.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Determine appropriate enzyme levels and combinations, appropriate medium conditions, appropriate incubation periods and incubation temperatures for high protoplast yields, using various donor tissues of selected cereals.		9-85	9-87
(2) Determine adequate culture medium requirements to maintain viable protoplasts.	x	9-85	9-87
(3) Determine minimum innoculation density of protoplasts to promote division.		9-85	9-87

Major Activity. Develop methods for embryogenic callus production from

isolated protoplasts.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Determine which donor tissues produce viable protoplasts that tend to give rise to embryogenic callus cultures.		9-85	9-88
(2) Determine hormonal requirements for enhancing embryogenic callus production from protoplasts.	x	9-85	9-88
(3) Determine effect of pre-conditioning donor tissues, on production of viable embryogenic callus from protoplasts.		9-85	9-88
(4) Determine effect of pre-conditioning medium on cell division and embryogenic callus production.		9-85	9-88

Major Activity. Develop methods and procedures for plant regeneration from protoplast-derived callus.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Determine medium conditions including hormone concentrations for regenerating plants from protoplast-derived callus.	x	9-86	9-90
(2) Determine appropriate conditions for transplanting protoplast-derived plants to soil for growth to maturity.	x	9-86	9-90

Output 1f. Techniques for cloning nitrate reductase and introducing cloned nitrate reductase into protoplasts.

Result to date. None

* Major Activity Identify, clone and transfer the rice nitrate reductase gene.

Activity/Task	Selected	Target Dates

Milestones	Start	End
(1) Produce a genomic library of rice DNA in an appropriate lambda phage.	9-86	9-89
(2) Prepare a cDNA library using cloned double stranded DNA's of plant mRNA's.	9-86	9-89
(3) Screen the genomic library and the cDNA library using an appropriate hybridization probe.	9-86	9-89
(4) Isolate the hybridized DNA fragment and insert into an appropriate isolated plasmid (cloning vector).	9-86	9-89
(5) Reintroduce (hybridized) plasmid into bacteria to obtain a large number of cloned rice DNA fragments.	9-88	9-90
(6) Isolate hybrid plasmid DNA and excise original rice DNA fragment.	9-88	9-90
(7) Introduce cloned DNA fragment into isolated protoplasts using a suitable vector or by microinjection.	9-88	9-90

2. Output 2. Verification of stress tolerance selection techniques through greenhouse and field testing (testing to be done for selected plants of some species only, depending on in vitro results).

Output 2a. Greenhouse test results showing whether tissue culture techniques can give rise to stress-tolerant and fertilizer-efficient plants.

Results to Date: Seedling tests have been completed for two rice cultivars, IR-36 and Calrose 76. In these tests, seed was collected from plants that had been regenerated from both NaCl tolerant callus and callus that had never been selected for NaCl tolerance. The seeds were germinated and placed into beakers containing one-half strength Hoagland's solution and 0, 3, 6, 9, 12, or 15 g/l NaCl. After five days in the NaCl solution, plants were rated for vigor. The results indicated that rice callus selected for salt-tolerance in vitro, produced whole plants tolerant to salt during the seedling stage. The data further suggested that both the level of salt in the selection medium and the length of the selection period were important in obtaining salt-tolerant whole plants. In addition, earlier work with oats demonstrated that NaCl-tolerance traits selected in cell cultures also appear in regenerated plants, are stable in

the plants in the absence of stress, and can be inherited by subsequent generations.

* Major Activity. Develop methods for and complete evaluation of NaCl tolerance of whole plants in greenhouse tests.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Identify normal, highly fertile phenotypes and/or potentially valuable somaclonal variants among plants regenerated from NaCl-tolerant tissue cultures (regenerated plant = R_0, first generation after regenerated plant = R_1, etc.), Perform cytological analysis on selected regenerates to determine if any chromosomal aberrations are present, for;	x		
(a) wheat, rice		9-85	9-90
(b) sorghum		9-87	9-90
(c) legumes		9-89	9-90
(2) Collect R_1 seed from regenerated plants (identified in activity 1) and file according to original level of NaCl in the *in vitro* selection medium and length of selection period, for:			
(a) wheat, rice		9-85	9-90
(b) sorghum		9-87	9-90
* (c) legumes		9-89	9-90
(3) Test parental-type plants (plants of the original cultivar genotype that have never passed through a tissue culture cycle) in hydroponic systems to determine critical concentrations of NaCl in hydroponic solutions that i) cause plant death, ii) allow some vegetative growth but do not permit seed set, and iii)			

allow seed set but lower seed yield, for:		
a) wheat, rice	9-85	9-86
b) sorghum	9-86	9-87
* c) legumes	9-88	9-89
(4) Germinate seed of NaCl-selected lines and seed from plants never selected through tissue culture for NaCl-tolerance, place seedlings intoreplicated hydroponic systems with critical levels of NaCl as defined in (3), for:		
(a) wheat, rice	9-85	9-90
(b) sorghum	9-87	9-90
* (c) legumes	9-89	9-90
(5) Monitor plants in hydroponic systems and record seedling vigor, height, and number of leaves, for:		
(a)wheat, rice	9-85	9-90
(b) sorghum	9-87	9-88
* (c) legumes	9-89	9-90
(6) For plants not reaching maturity in hydroponic systems record data to include, percentage of plants surviving, days to plant death (if plant does not survive), and plant height, for:		
(a) wheat, rice	9-85	9-90
(b) sorghum	9-87	9-90
* (c) legumes	9-89	9-90
(7) Collect data from plants that reach maturity in hydroponic systems to include percentage of plants maturing, days to maturity, plant height, number of seeds and seed weight, for:		
(a) wheat, rice	9-85	9-90

(b) sorghum		9-87	9-90
* (c) legumes		9-89	9-90
(8) Increase seed for plants showing tolerance in R_1 generation for continued greenhouse tests (tasks 9, 10, 11) and field tests of future generations (output 2b), for:			
(a) wheat, rice		9-85	9-90
(b) sorghum, corn		9-87	9-90
* (c) legumes		9-89	9-90
(9) Collect seed from tolerant plants and repeat tasks 4-7 for R_2 and R_3 plant generations to verify the stability of NaCl-tolerance and to determine the genetic basis of NaCl-tolerance, for:	x		
(a) wheat, rice		9-85	9-90
(b) sorghum		9-87	9-90
* (c) legumes		9-89	9-90
(10) Begin crosses between R_3 generation plants with NaCl-tolerance and parental-type plants to help determine the genetic basis of NaCl-tolerance and to transfer the NaCl-tolerance to the parental genotype, (repeat tasks 4-7 for the resulting progeny to determine if the tolerance was transfered), for:	x		
(a) wheat, rice		9-86	9-90
(b) sorghum		9-88	9-90
* (c) legumes		9-89	9-90
(11) Begin crosses between different NaCl-tolerant genotypes to determine allelic relationships of NaCl-tolerance among these genotypes, for:			

(a) wheat, rice		9-86	9-90
(b) sorghum		9-87	9-90
* (c) legumes		9-89	9-90
(12) Of plants that show stable NaCl-tolerance, determine if a correlation exists between degree of tolerance in whole plants and *in vitro* selection pressure.	x	9-85	9-90
(13) For legumes showing stable tolerance to NaCl, determine the effects of stress on rhizobium/legume interactions by comparing % yield increases of NaCl-tolerant plants, with and without nodules, grown under critical levels of NaCl stress.			

* Major Activity. Develop methods for and complete evaluation of drought tolerance of whole plants in greenhouse tests.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Identify normal, highly fertile phenotypes and/or potentially valuable somaclonal variants among plants regenerated from polyethelene glycol (PEG)-tolerant tissues culture, for:	x		
* (a) rice, wheat, millet		6-87	9-90
(b) sorghum		9-87	9-90
** (c) other crops			
(2) Collect R_1 seed from regenerated plants (identified in activity 1) and file according to original level of PEG in the *in vitro* selection medium and length of selection period, for:			
* (a) rice, wheat, millet		6-87	9-90
(b) sorghum		9-87	9-90

** (c) other crops		
(3) Test parental-type plants in pots containing soil and various levels of water to determine critical water potentials that (i) do not support plant growth, (ii) allow some vegetative growth but do not permit seed set and (iii) allow seed set but lower seed yield, for:		
* (a) rice, wheat, millet	6-86	6-87
(b) sorghum	9-86	9-87
** (c) other crops		
(4) Establish seedlings from regenerated plants of drought-tolerant cell lines, and from seed not selected through tissue culture for drought tolerance (as controls), in pots containing soil, for:		
* (a) rice, wheat, millet	6-87	6-90
(b) sorghum	9-87	9-90
** (c) other crops		
(5) Subject potted plants (established in activity 4) to critical soil water potentials by withholding water to various extents (as determined in activity 3) to determine effects of low soil water potential on plants derived from PEG-tolerant cell cultures as compared to effects on control plants; record seedling vigor, height, and number of leaves, for:		
* (a) rice, wheat, millet	6-87	9-90
(b) sorghum	9-87	9-90
** (c) other crops		
(6) For plants not reaching maturity, record data to include, percentage of plants surviving, days to		

plant death (if plant does not survive), and plant height, for:			
* (a) rice, wheat, millet		6-87	9-90
(b) sorghum		9-87	9-90
** (c) other crops			
(7) Collect data from mature plants exposed to various soil water potentials, include percentage of plants maturing, days to maturity, plant height, number of seeds, and seed weight, for:			
* (a) rice, wheat, millet		6-87	9-90
(b) sorghum		9-87	9-90
** (c) other crops			
(8) Increase seed for plants showing tolerance in R_1 generation for continued greenhouse tests (tasks 9, 10, 11) and field tests of future generations (Output 2b), for:			
* (a) rice, wheat, millet		6-87	9-90
(b) sorghum		9-87	9-90
** (c) other crops			
(9) Collect seed from tolerant plants and repeat tasks 4-7 for R_2 and R_3 plant generations, to verify the stability of drought-tolerance, and to determine the genetic basis for the drought-tolerance, for:	x		
* (a) rice, wheat, millet		12-87	9-90
(b) sorghum		3-88	9-90
** (c) other crops			
(10) Begin crosses between R_3 generation plants with drought tolerance and parental plants to help determine the genetic basis of drought tolerance and to transfer the drought	x		

tolerance to the parental genotype, (repeat tasks 4-7 for the resulting progeny to determine if the tolerance was transfered), for:			
* (a) rice, wheat, millet		6-88	9-90
(b) sorghum		9-88	9-90
** (c) other crops			
(11) Begin crosses between different drought-tolerant genotypes to determine allelic relationships of drought-tolerance among these genotypes; for:			
* (a) rice, wheat, millet		6-87	9-90
(b) sorghum		9-87	9-90
** (c) other crops			
(12) Of plants that show stable drought-tolerance, determine if a correlation exists between the degree of tolerance in whole plants and the *in vitro* selection pressure.	x	9-85	9-90
(13) Perform tasks 4-7 on plants exhibiting stable NaCl tolerance to determine the degree to which NaCl-tolerant plants are also drought tolerant.	x	9-86	9-90

Major Activity. Develop methods for and complete evaluation of heat tolerance of wheat plants in greenhouse tests.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Identify normal, highly fertile phenotypes and/or potentially valuable somaclonal variants among plants regenerated from heat tolerant tissue cultures of wheat.	x	6-87	9-90
(2) Collect R_1 seed from regenerated plants (identified in activity 1) and file according to the original		6-87	9-90

temperature in the *in vitro* selection system and length of selection period.			
(3) In temperature controlled growth chambers, test parental-type plants to determine critical (high) temperatures that (i) allow vegetative growth but do not permit seed set and (ii) allow seed set but lower seed yield, for standard wheat cultivars.		6-86	6-87
(4) Establish wheat seedlings from regenerated plants of heat-tolerant cell lines and from seed not selected through tissue culture for heat tolerance (as controls), in pots containing soil.		6-87	9-90
(5) Subject potted plants to critical temperatures (as determined in 3) by placing them in temperature controlled growth chambers.	x	6-87	9-90
(6) Monitor plants in growth chambers and record seedling vigor, height and number of leaves.		6-87	9-90
(7) For plants not reaching maturity in growth chamber tests, record data to include, percentage of plants surviving, days to plant death (if plant does not survive), and plant height.		6-87	9-90
(8) Collect data from plants that set seed to include, percentage of plants maturing, days to maturity, plant height, number of seeds and seed weight.		6-87	9-90
(9) Increase seed for plants showing tolerance in the R_1 generation for continued greenhouse tests (tasks 9, 10, 11) and field tests of future generations (Output2b).		6-87	9-90

(10) Collect seed fromheat-tolerant plants and repeat tasks 4-6 for the R_2 and R_3 plant generations to verify the stability of heat-tolerance and to determine the genetic basis of heat-tolerance.	x	12-87	9-90
(11) Begin crosses between R_3 generation plants with heat tolerance and standard wheat cultivars to help determine the genetic basis of heat-tolerance and to transfer heat-tolerance to the parental genotype, (repeat 4-7 for the resulting progeny to determine if the tolerance was transfered).		6-88	9-90
(12) Begin crosses between different heat-tolerant genotypes to determine allelic relationships of heat-tolerance among these genotypes.		6-87	9-90
(13) Of plants that show stable heat-tolerance, determine if a correlation exists between the degree of tolerance expressed in whole plants and the original <u>in vitro</u> selection pressure.	x	6-87	9-90

* <u>Major Activity.</u> Develop methods for and complete evaluation of aluminum tolerance of whole plants in greenhouse tests.

Activites/Tasks	Selected Milestones	Target Dates Start	End
(1) Identify normal, highly fertile phenotypes and/or potentially valuable somaclonal variants among plants regenerated from $AlCl_3$-tolerant tissue cultures, for:	x		
(a) wheat, rice, * millet		9-86	9-90
(b) sorghum		9-87	9-90
* (c) legumes		9-88	9-90

(2) Collect R_1 seed from regenerated plants and file according to original level of $AlCl_3$ in the <u>in vitro</u> selection medium and length of selection period, for:		
(a) wheat, rice, * millet	9-86	9-90
(b) sorghum	9-87	9-90
* (c) legumes	9-88	9-90
(3) Test parental-type plants in hydroponic systems to determine critical concentrations of $AlCl_3$ in hydroponic solutions that i) cause plant death, ii) allow some vegetative growth but do not permit seed set, and iii) allow seed set but lower seed yield, for:		
(a) wheat, rice, *millet	6-86	6-87
(b) sorghum	9-86	9-87
* (c) legumes	9-87	9-88
(4) Germinate seed of $AlCl_3$-selected lines and seed from plants that were never selected through tissue culture for aluminum-tolerance; place seedlings into replicated hydroponic systems with critical levels of $AlCl_3$ as defined in (3), for:		
(a) wheat, rice, * millet	9-86	9-90
(b) sorghum	9-87	9-90
* (c) legumes	9-88	9-90
(5) Monitor seedlings in hydroponic experiments and record seedling vigor, height, and number of leaves, for:		
(a) wheat, rice, * millet	9-86	9-90
(b) sorghum	9-87	9-90
* (c) legumes	9-88	9-90
(6) For plants not reaching		

maturity in hydroponic systems, record data to include, percentage of plants survivng, days to plant death (if plant does not survive), and plant height, for:			
(a) wheat, rice, * millet		9-86	9-90
(b) sorghum		9-87	9-90
* (c) legumes		9-88	9-90
(7) Collect data from plants that mature in hydroponic systems to include, percentage of plants maturing, days to maturity, plant height, number of seeds and seed weight, for:			
(a) wheat, rice, * millet		9-86	9-90
(b) sorghum		9-87	9-90
* (c) legumes		9-88	9-90
(8) Increase seed for plants showing tolerance in R_1 generation for continued greenhouse tests (tasks 9, 10, 11) and field tests of future generations (Output 2b), for:			
(a) wheat, rice, * millet		9-86	9-90
(b) sorghum		9-87	9-90
* (c) legumes		9-88	9-90
(9) Collect seed from tolerant plants and repeat tasks 4-7 for R_2 and R_3 plant generations to verify the stability of aluminum-tolerance and to determine the genetic basis of aluminum-tolerance, for:	x		
(a) wheat, rice, * millet		3-87	9-90
(b) sorghum		3-88	9-90
* (c) legumes		3-90	9-90
(10) Begin crosses between R_3 generation plants with aluminum-tolerance and parental-type plants to help determine the genetic basis of	x		

the aluminum-tolerance and to transfer aluminum tolerance to the parental-genotype (repeat tasks 4-7 for resulting progeny to determine if tolerance was transferred) for:		
(a) wheat, rice, * millet	9-87	9-90
(b) sorghum	9-88	9-90
* (c) legumes	9-89	9-90
(11) Begin crosses between different aluminum-tolerant genotypes to determine allelic relationships of aluminum-tolerance among these genotypes, for:		
(a) wheat, rice, * millet	9-86	9-90
(b) sorghum	9-87	9-90
* (c) legumes	9-88	9-90
(12) Of plants that show stable aluminum-tolerance, determine if a correlation exists between degree of tolerance in whole plants and *in vitro* selection pressure.	9-86	9-90
(13) For legumes showing stable aluminum tolerance, determine effects of stress on rhizobium/legume interactions by comparing % yield increase of aluminum-tolerant plants, with and without nodules, grown under critical levels of aluminum stress.		

Major Activity. Develop methods for and complete evaluations of nitrate reductase efficiency in rice plants in greenhouse tests.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Identify normal, highly fertile phenotypes and/or potentially valuable somaclonal variants among rice plants regenerated from tissue cultures exhibiting nitrate reductase efficiency.		6-87	9-90

(2) Assay whole plants regenerated from rice tissue cultures with altered nitrate reductase activity for nitrate reductase activity.	x	6-87	9-90
(3) Collect R_1 from regenerated plants exhibiting altered nitrate reductase activity (as identified in activity 2) and file according to original level of chlorate, tungstate or nitrate in the <u>in vitro</u> selection medium, and length of selection the period.		6-87	9-90
(4) Determine critical, minimum concentrations of lowered nitrate in hydroponic solutions that i) cause plant death, ii) allow some vegetative growth but does not permit seed set, and iii) allows seed set but lowers seed yield for rice (using parental-type plants).		6-86	6-87
(5) Germinate rice seed derived from nitrate reductase-efficient lines and seed from plants that were never selected through tissue culture for nitrate reductase efficiency; place seedlings into replicated hydroponic systems containing critical levels of nitrate as defined in (4).		6-87	9-90
(6) Monitor rice seedlings in hydroponic experiments and record seedling vigor, height, and number of leaves.		6-87	9-90
(7) For plants not reaching maturity in hydroponic systems, record data to include, percentage of plants surviving, days to plant death (if plant does not survive), and plant height.		6-87	9-90
(8) Collect data from plants that mature in hydroponic		6-87	9-90

systems to include percentage of plants maturing, days to maturity, plant height, number of seeds and seed weight.			
(9) Increase seed of plants demonstrating nitrate reductase efficiency in R_1 generation for continued greenhouse tests (tasks 9, 10, 11) and field tests of future generations (Output 2b) for rice.		6-87	9-90
(10) Collect seed from plants with efficient nitrate reductase and repeat tasks 4-7 for R_2 and R_3 plant generations to verify the stability of nitrate-reductase efficiency and to determine the genetic basis of the altered nitrate reductase.	x	12-87	9-90
(11) Begin crosses between R_3 generation plants with nitrate-reductase efficiency and parental-type rice plants to help determine the genetic basis of altered nitrate reductase and to transfer efficient nitrate reductase to the parental genotype (repeat tasks 4-7 for the resulting progeny to determine if the desired trait was transfered).		6-88	9-90
(12) Of plants that show stable nitrate reductase efficiency determine if a correlation exists between degree of tolerance in rice plants, and *in vitro* selection pressure.		6-87	9-90

* Major Activity. Develop methods for evaluating rate of useful alien gene transfer through tissue culture of wide-cross progeny using wheat and alien genera.

Activities/Tasks	Selected	Target Dates

	Milestones	Start	End
(1) Collect R_1 seed from regenerates of tissue cultured wide-cross embryos and backcross to wheat until stable hybrid plants are obtained with 42 "wheat" chromosomes (see Output 1c).	x	3-87	9-88
(2) Subject backcross progeny to NaCl, drought, aluminum, (as outlined above) or pathogens to determine if desired genes from alien genera have been transferred to wide-cross hybrid.	x	3-87	3-89

Output 2b. Field test results of plants from four crop species showing whether and to what extent greenhouse tolerant plants show tolerance in the field.

Results to Date: Rice seed derived from NaCl-tolerant tissue cultures has been sent to Dr. Milton Rush at Louisiana State University and to Dr. Gurdev S. Khush at IRRI for preliminary field testing.

* Major Activity. Develop methods for and begin evaluation of NaCl tolerance in field trials.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Identify plant breeding programs with active interest in NaCl-tolerance of rice, wheat, millet and sorghum.		9-85	9-88
(2) Arrange for field tests of germplasm with demonstrated NaCl-tolerance in greenhouse tests, for:	x		
(a) rice		9-87	9-90
(b) wheat		3-88	9-90
(c) sorghum		9-88	9-90
(3) Monitor progress of field tests and collect data from various field stations.		9-87	9-90
(4) Correlate field test	x	9-87	9-90

results to greenhouse and *in vitro* results.

* *Major Activity.* Begin field evaluations of aluminum-tolerance for two crops.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Identify plant breeding programs with active interest in Al3+-tolerance of rice and wheat.		9-85	9-88
(2) Arrange for field tests of germplasm with demonstrated Al3+ tolerance in greenhouse tests, for:			
(a) rice		9-87	9-90
(b) wheat		6-88	9-90
(3) Monitor progress of field tests and collect data from various field stations.		9-87	9-90
(4) Correlate field tests results to greenhouse and *in vitro* results.		9-87	9-90

**Major Activity.* Complete field evaluations of drought-tolerance for two crops.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Identify plant breeding programs with active interest in drought-tolerance of millet and sorghum.		9-85	9-88
(2) Arrange for field tests of germplasm with demonstrated drought-tolerance in greenhouse tests, for:	x		
(a) millet		6-88	9-90
(b) sorghum		9-88	9-90
(3) Monitor progress of field tests and collect data from various field stations.		9-87	9-90
(4) Correlate field tests	x	9-87	9-90

results to greenhouse and *in vitro* results.

Output 2c. Seeds with increased field tolerance for one or more stresses, for distribution to interested plant breeders.

Results to date: Not applicable.

*Major Activity. Produce seeds with demonstrated field tolerance for distribution to interested plant breeders.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Identify plant breeders worldwide with interest in germplasm with demonstrated field tolerance to various stresses.		9-85	9-90
(2) Increase seed stocks of crop species with demonstrated field-tolerance to one or more stresses.		9-89	9-90
(3) Distribute seed of stress-tolerant crop species to appropriate plant breeders.	x	3-90	9-90

B. International Plant Biotechnology Network (IPBNet) (See Table 2)

1. Output 3. Network core creation and support including but not limited to services to network participants, e.g., information exchange, literature and data base, technical assistance, and conference preparation.

The Tissue Culture for Crops Project will establish and coordinate the International Plant Biotechnology Network. The primary purpose of this network will be to establish working partnerships among researchers worldwide with interest in using plant biotechnology to accelerate the development of stress tolerant crops. The Network will also function to ensure and speed biotechnology transfer as it relates to the use of tissue culture to produce stress-tolerant cultivars and other genetically engineered improvements in useful crops species. Sub-groups of this network will focus on specific crops and stresses.

Results to date: An informal tri-lateral network has already been established among the TCCP, the International Rice Research Institute and Chulalongkorn University in Bangkok, Thailand. Preliminary negotiations aimed at formalizing this incipient network have begun through informal discussions and correspondence among the parties involved. An agreement has been made between the TCCP and AID to expand the network approach.

Network functions will include institution building, technology transfer and increasing the tissue culture knowledge base as it relates to producing stress-tolerant cultivars of specific crops. A computerized tissue culture literature and data base has already been designed and established by the TCCP. The computer files currently contain over 7,000 article references specifically related to plant physiology and tissue culture. A system for literature collection, classification, storage, retrieval and distribution has been developed. Additionally, three project newsletters have been written and distributed worldwide and direct technical assistance has been provided by the TCCP to tissue culture researchers in Thailand, the Phillipines and Paraguay.

Major Activity. Establishing and expanding IPBNet.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Develop a survey form and cover letter for survey of existing DC and IARC tissue culture laboratories.		12-85	1-86
(2) Send form to all AID missions, US embassies in AID countries, and to labs in the directory of The International Plant Tissue Culture Association.	x	12-85	1-86
(3) Compile results of survey, identify possible equal partner collaborators, general IPBNet members and sub-grant recipients.		1-86	4-86
(4) Target organizations/ institutions to be participants in IPBNet.	x	3-86	5-86
(5) Consult with MRG to assist with identification of countries and establishment of informal collaborative agreements.	x	1-86	3-86
(6) Consult with AID and NAC on who/how to approach targeted institutions for a) general membership,		3-86	5-86
b) equal partner collaboration			
c) sub-grant recipients.			

(7) Contact prospective IPBNet participants and describe purpose of IPBNet a) directly (site visits, mail, etc.		3-86	6-86
b) indirectly (newsletter, articles in professional magazines, etc.)			
(8) Establish memoranda of agreement with interested institutions to include: purpose of IPBNet, participant contribution to IPBNet, and network core/AID contribution.	x	1-86	8-86
(9) Establish annual reviews by MRG to supply recommendations for strengthening of network, suggestions for inclusion of new scientists, programs and institutions, and review of interface with AID Science Advisors Research Program and Biotechnology components of other Research Grants Programs.		6-87	9-90

Major Activity. Maintain and expand computer file pertinent to technical literature.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Continue existing computer filing system		9-85	9-90
(2) Expand computer system to include additional, upgraded equipment and make appropriate design changes.		1-86	4-86
(3) Establish procedures for input/output of literature and data by IPBNet members.	x	4-86	6-86

Major Activity. Establish and coordinate annual IPBNet conferences and workshops.

Activity/Task	Selected	Target Dates

	Milestones	Start	End
(1) Prepare for and convene 1st annual conference in Ft. Collins, as indicated below (a-f).		4-86	10-86
a) establish objectives for conference	x		
b) prepare an agenda for conference			
c) invite network members and other interested parties			
d) make necessary logistical arrangements for conference			
e) convene conference	x		
f) conduct feedback survey of conference; write follow-up report.	x		
(2) Prepare for and convene 2nd annual conference in Ft. Collins (repeat tasks a-f).	x	4-87	10-87
(3) Choose site for 3rd annual conference, after consulting IPBNet members and MRG.		10-87	10-87
(4) Prepare for and convene 3rd annual conference in cooperation with hosting IPBNet member (repeat tasks a-f).	x	4-88	10-88
(5) Choose site for 4th annual conference; consult with IPBNet members and MRG.		10-88	10-88
(6) Prepare for and convene 4th annual conference in cooperation with hosting IPBNet member (repeat tasks a-f).	x	4-89	10-89
(7) Choose site for 5th annual conference; consult with IPBNet members and MRG.		10-89	10-89

(8) Prepare for and convene 5th annual conference in cooperation with hosting IPBNet member (repeat tasks a-f).	x	3-90	9-90
(9) Organize crop-stress specific workshops as needed by IPBNet members for introducing new techniques and for problem-solving.		as needed	

Major Activity. Provide technical assistance to IPBNet members.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Provide appropriate technical assistance to IPBNet members concerning lab design and set-up, establishment of tissue cultures of specific crops, regeneration of whole plants from cell cultures of specific crops, and selection of stress-tolerant cell lines and regeneration for particular stresses of importance to specific crops.		as needed	

2. Output 4. A Plant Biotechnology Training Center at CSU with a capacity to train 10 scientist/ technicians per year in plant tissue culture techniques.

Results to date: Space has been secured at CSU and remodeling is underway to establish a 1200 square foot training center. Equipment for this center has been ordered.

Major Activity. Determination of and preparation for the kinds of training to be provided.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Organize basic training module for making medium and sterile technique.		10-85	12-85
(2) Organize basic training module for sterilizing plant materials and producing callus.		10-85	12-85

(3) Organize basic training module for regenerating plants.	10-85	12-85
(4) Organize basic training module for selection of stress-tolerant cultures.	10-85	12-85
(5) Organize basic training module for meristem propagation of plants.	10-85	12-85
(6) Organize basic training module for preparation of protoplasts.	10-85	12-85
(7) Organize basic training module for literature utilization.	10-85	12-85
(8) Organize basic training module for set-up and operation of a tissue culture laboratory.	10-85	12-85
(9) Organize literature modules for biotechnological techniques involving specific crops.	10-85	12-85

Major Activity. Development of a logistical framework for operation of the training laboratory.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Develop a basic six-month curriculum for a training course.	x	10-85	12-85
(2) Develop optional, additional training arrangements with Research Director.		as needed	
(3) Prepare a training brochure or addition to a Network Newsletter which outlines training program.	x	8-85	9-85
(4) Develop a method for nominating and selecting trainees.		10-85	11-85

(5) Arrange for housing, transportation, and other logistics.		as needed

Major Activity. Development of an evaluation procedure for the training program.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Develop an evaluation form for use by graduate trainees.		12-85	1-86
(2) Develop an interview procedure to obtain additional information from graduates.		12-85	1-86
(3) Develop a procedure for following-up on alumni on return to their home countries. Continue to supply information and assistance when needed to make sure training is utilized.	x	12-85	1-86
(4) Modify training procedure and logistics of training based on feedback data.		as needed	

3. Output 5. At least six equal partner DC collaborators involved in IPBNet as centers of tissue culture expertise and in information and personnel exchange, cooperative research, and field testing with CSU and other network members. (Equal partner collaborators will be established through formal agreements as outlined in output 3).

Results to Date: Two equal partner collaborators have been identified. Mrs. Montakan Vajrabhaya, Department of Botany, Chulalongkorn University, Bangkok, Thailand has an AID Science Advisor's grant to obtain salt-tolerant cultivars of Thai rice using tissue culture techniques. Our laboratory serves as an advisory group for her project. Information exchange is already underway. Mrs. Montaken has spent a month in our laboratory; we visit her laboratory for one week each year. Dr. Nguyen Thi Thanh-Tuyen, Visyas State College of Agriculture, VISCA, Leyte, Philippines is working on tissue culture of coconut palms to improve plant regeneration techniques. Information exchange with her laboratory is underway. A visit to Leyte was made in January, 1985. We have agreed to collaborate with her in obtaining NSF or Science Advisor's Office funding in the future.

Major Activity. Monitor, evaluate, and facilitate improved relationships

with equal partner cooperators.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Each equal partner collaborator will be visited at least once a year.		9-85	9-90
(2) Each collaborative agreement will be informally evaluated each year by the Network Director to determine progress of the DC lab and extent of scientific interaction.	x	9-85	9-90
(3) In conjunction with the MRG, changes in the nature of collaboration will be suggested.		9-85	9-90
(4) Particular attention should be paid to assisting labs in obtaining funding for their research activities (see output 7).		9-85	9-90

4. Output 6. Facilitation of information and personnel exchange.

Results to date: Information exchange among the TCCP and collaborators, between 1980 and present, has been facilitated by publication of project newsletters, reports, technical publications, and feedback information surveys. The project has also worked to identify personnel in DC's (at IRRI and Chulalongkorn University) that are interested in working for 6 months to a year at the TCCP in Ft. Collins.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Encourage (via memoranda of agreement) individual Network members to open lines of communication between themselves and other Network members.		9-85	9-90
(2) Publish a biannual newsletter, annual reports, and other project publications to include research results of all Network members. Encourage direct participation of all Network members.		9-85	9-90

(3) Distribute newsletters, annual reports, and other project publications to Network members and other interested parties.	x	9-85	9-90
(4) Develop and conduct feed-back surveys to help determine usefulness of project publications and how specific publications may be improved.		9-85	9-90
(5) Encourage individual Network members to circulate their own annual reports and other publications throughout the Network (via memoranda of agreement).		9-85	9-90
(6) Encourage direct participation of Network members in TCCP conferences, and workshops as well as in national and international meetings (via memoranda of agreement).		9-85	9-85
(7) Identify (in conjunction with individual DC project leaders) individuals in collaborating DC labs interested in working in other IPBNet labs. Encourage short to intermediate term exchanges of personnel between Network labs.	x	9-85	9-90

5. Output 7. Facilitation of cooperative research.

Results to date: The TCCP has been involved with the development of a Thai tissue culture project from its inception. The TCCP participated in the planning stages of the Thai project by helping to write a grant proposal submitted to the AID Science Advisors Office. Subsequently, TCCP has monitored progress of the Thai project in conjunction with AID/Thailand. TCCP personnel have made three trips to the Thailand since 1983, to insure that satisfactory progress was being made and to offer suggestions concerning technical aspects of the project.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Establish clearly-defined research agenda with Network members,	x	9-85	9-87

stressing cooperative effort towards achieving common goals.			
(2) Make contacts with funding agencies (other than AID) in cooperating countries, determine procedures for submission of applications for funding.		9-85	9-87
(3) Aid Network cooperators in the preparation of grant proposals to obtain independent funds for research.		9-85	9-90
(4) Meet with government administrators as well as research project leaders to discuss potential benefits of emerging plant biotechnologies.		9-85	9-90
(5) Meet periodically with equal-partner collaborators to discuss research progress.	x	9-85	9-90
(6) Arrange technical assistance for cooperators who request such assistance.	x	as needed	

6. Output 8. Facilitation of field testing of TCCP crops.

Results to date: Contracts have been established with plant breeders interested in testing TCCP rice germplasm. Field testing has begun for rice at IRRI and Louisiana State University.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Identify plant breeders in DC Network countries, or at US institutions with ties to DC's, interested in field testing TCCP germplasm. Encourage full participation of breeders in IPBNet planning conferences, workshops.	x	9-85	9-90
(2) Arrange for shipping of seed to various breeders, secure appropriate permits.		9-85	9-90
(3) Ship germplasm to		9-85	9-90

breeders and monitor field results via site visits.			
(4) Compile field test results from various locations, review results with each plant breeder involved in testing.		9-85	9-90

7. Output 9. Collaboration established with at least three IARC's, regional and crop-specific, for a) supply of germplasm, b) field testing of stress-tolerant project germplasm, c) exchange of information on stresses affecting production, and, d) facilitating cooperative programs with Network members (no Project funds).

Results to date: An informal cooperative agreement exists between the present project (AID/DSAN 396-4137) and CIMMYT for field testing of wheat, supply of wheat and corn germplasm, and cytological examination of regenerated plants. A similar agreement exists with IRRI for supply of germplasm and field testing of rice. Informal contacts have been made with ICRISAT and ICARDA.

Major Activity. Determine the desired extent of cooperation with each IARC.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Determine which IARC's have tissue culture capabilities, and which have interest.	x	9-85	6-86
(2) Determine which IARC's can serve as sources for seeds for the project.		9-85	6-86
(3) Determine which IARC's have the capabilities and interest to field test project germplasm.		9-85	6-86
(4) Determine which IARC's would be useful to the project and are interested in collaborative research and/or personnel exchange.		9-85	6-86
(5) Initiate contact with IARC's not currently involved.		1-86	6-86
(6) Formalize cooperative agreements with all IARC's to be involved, beginning with three IARCs.	x	1-86	10-86

(7) Consult with MRG to determine extent of IARC involvement in network and network meetings.	6-86	6-87
(8) Annually review and modify agreements. Involve MRG in review process. Review should include a written summary of activities performed.	6-86	6-87
(9) Determine the feasibility each year of adding another IARC.	2-86	6-87

8. Output 10. At least three DC labs strengthened to perform initial aspects of project tissue culture research, including field testing. (Subgrants.)

Results to Date: A method for selecting recipients for sub-grants has been developed. Institutions will be chosen for subgrants by the TCCP Project Director, Network Director, and the MRG. (The MRG will function as a peer review group.) Subgrant recipients will be chosen based on (a) quality of research proposal, (b) applicability of research proposal to TCCP objectives, and (c) ability of the institution requesting funds to carry out proposed research. Effort will be made to insure that sub-grantees represent diverse geographical locations. (Note: The MRG may not be fully organized by the time that the first few subagreements are made. In this case, proposals will be selected by the Project Director, Network Director, AID Project Monitor, and a person from the AID Science Advisor's Office.)

Major Activity. Match available instututions to research objectives (see Research and Development), establish research agreements.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Utilize DC tissue culture survey to determine which institutions are interested in receiving subgrants to do research (tissue culture or field testing), complimentary to overall TCCP goals.	x	4-86	5-86
(2) Determine research priorities relating to project goals.		2-86	6-86
(3) Match institutions to goals.		6-86	8-86

(4) Make initial contacts with targeted institutions.		6-86	8-86
(5) Visit targeted institutions.	x	6-86	6-87
(6) Solicit formal proposals from targeted institutions, determine which project objectives can best be completed by each DC collaborator.		6-86	6-87
(7) Award sub-grants and sign formal agreements for target institutions.	x	6-86	6-87
(8) Develop progress reporting system for subgrantees.	x	6-86	6-87
(9) Investigate methods by which subgrantees can obtain independent funding (and become equal partner collaborators) in the future.		2-86	6-87

9. Output 11. Agreements with at least three U.S. universities or one CRSP actively involved in research and technical assistance in sub-tropical and tropical soils, to cooperate in a) field testing TCCP products with developing country partners, b) information exchange, and/or c) research relevant to achieving TCCP objectives (sub-grants).

Results to date: Louisiana State University is presently field testing project rice germplasm to determine the numbers and types of somaclonal variants for growth habit changes and disease tolerance. Currently over 400 entries are in the field. Formal subagreements will be awarded as described above (Output 10).

Major Activity. Match available institutions to research priorities and initiate formal research agreements.

Activities/Tasks	Selected Milestones	Target Dates Start	End
(1) Determine available institutions from literature surveys and personal contacts in the US.	x	1-86	4-86
(2) Determine DC		1-86	4-86

collaborative involvement of targeted institutions.			
(3) Determine those research priorities relating to project goals best suited to collaborating institution.		1-86	4-86
(4) Match institutions to goals.	x	1-86	4-86
(5) Make initial contacts with targeted institutions.		1-86	4-86
(6) Solicit formal proposals from targeted institutions.		4-86	6-86
(7) Award sub-grants and sign formal agreements for target institutions.	x	6-86	8-86
(8) Develop progress reporting system for sub-grantees.		6-86	8-86
(9) Increase involvement of sub-grantees in Network activities.		6-86	9-90