PD-AAA-614 PN-22210

CLASSIFICATION

PROJECT EVALUATION SUMMARY (PES) – PART I

Report Control Symbol U-447

1. PROJECT TITLE	2. PROJECT NUMBER	3. MISSION/AID/W OFFICE
Tissue Culture for Food Production	936-4055	S&T/AGR/RNR

4. EVALUATION NUMBER (Enter the number maintained by the reporting unit e.g., Country or AID/W Administrative Code, Fiscal Year, Serial No. beginning with No. 1 each FY) 83-3 3-30-83

☒ REGULAR EVALUATION ☐ SPECIAL EVALUATION

5. KEY PROJECT IMPLEMENTATION DATES	6. ESTIMATED PROJECT FUNDING	7. PERIOD COVERED BY EVALUATION
A. First PRO-AG or Equivalent FY 80	A. Total $ 930,000	From (month/yr.) September, 1980
B. Final Obligation Expected FY 84	B. U.S. $ 930,000	To (month/yr.) March, 1982
C. Final Input Delivery FY ____		Date of Evaluation Review March 29-30, 1982

8. ACTION DECISIONS APPROVED BY MISSION OR AID/W OFFICE DIRECTOR

A. List decisions and/or unresolved issues; cite those items needing further study. (NOTE: Mission decisions which anticipate AID/W or regional office action should specify type of document, e.g., airgram, SPAR, PIO, which will present detailed request.)	B. NAME OF OFFICER RESPONSIBLE FOR ACTION	C. DATE ACTION TO BE COMPLETED
1. Extend the project and its scope of work through September 30, 1985 at a total cost of $430,000.	J.L. Walker B.J. Beckett S&T/AGR	3/20/83
2. Add $200,000 for FY 1983.	J.L. Walker B.J. Beckett S&T/AGR	3/30/83
3. Consider expansion of the present scope of work to include promising biotechnology activities, international training, technical assistance and international networking.	J.L. Walker T.S. Gill S&T/AGR	4/15/83

9. INVENTORY OF DOCUMENTS TO BE REVISED PER ABOVE DECISIONS

- ☐ Project Paper
- ☐ Financial Plan
- ☐ Logical Framework
- ☐ Project Agreement
- ☐ Implementation Plan and CPI Network
- ☒ PIO/T
- ☐ PIO/C
- ☐ PIO/P
- ☒ Other (Specify) Action Memo
- ☒ Other (Specify) PAF

10. ALTERNATIVE DECISIONS ON FUTURE OF PROJECT

- A. ☒ Continue Project Without Change for two-year extension
- B. ☐ Change Project Design and/or ☐ Change Implementation Plan
- C. ☐ Discontinue Project

11. PROJECT OFFICER AND HOST COUNTRY OR OTHER RANKING PARTICIPANTS AS APPROPRIATE (Names and Titles)

S&T/AGR/R.L. Walker — S&T/AGR/M.D. Mozyrski
S&T/AGR/T.S. Gill — S&T/AGR/B.J. Beckett
S&T/AGR/J. Frederick — S&T/DS/[illegible]

12. Mission/AID/W Office Director Approval

Signature

Typed Name S&T/A, J. S. Robins

Date 3/30/83

AID 1330-16 (3-78)

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Team Evaluation - Part II

Entity: S&T/AGR/RNR

Project Title: Tissue Culture for Food Production

Project Number: 936-4055

13. Summary:

About 50,000,000 hectares planted to wheat, rice and millet in LDCs are affected by salt stress; 40,000,000 hectares by aluminum toxicity and 50,000,000 hectares by drought. More than 270,000,000 people who live in these areas are affected by these problems which reduce crop yields markedly. The traditional approach toward resolving such problems has been through manipulating the environment in which crops are grown in these areas by physical means such as irrigation and drainage and by chemical approaches including the incorporation of gypsum to increase soil permeability and of liming materials to reduce aluminum saturation. These practices are so costly that they can be applied to only very limited parts of the affected areas. This constraint, in turn, means that only few of the producers in these stressed areas benefit from the implementation of such technology. In the absence of these methods for ameliorating such adverse conditions, farmers have sought crops that have a native ability to produce under these stressed environments. Thus, sorghum, millet, barley and cassava varieties along with pigeon peas have been crops of choice for some of the areas stressed by drought while some varieties of sorghum, millet, barley and rice have been grown in salt-stressed areas under various climatic conditions. Production of food crops in the vast areas of soils of the tropics and sub-tropics characterized by aluminum toxicities, in the absence of costly chemical treatment to reduce such conditions, has been restricted to a few food crops such as cassava, sorghum and cowpeas. The yields of all of these crops under these unameliorated conditions of salt, drought and aluminum stress are rarely above bare subsistence levels.

However, due to these soil stresses for the vast majority of these areas and their populations the most viable option has been livestock grazing and browsing. But, from all reports, the security that this has offered is rapidly deteriorating due to overgrazing and deforestation.

To date, efforts to improve food production in these marginal soil areas -- in the absence of irrigation, drainage and liming -- have necessarily concentrated upon laborious, time-consuming and expensive screening of the germplasm pool to identify both stress-tolerant varieties that were more reliable producers and new and underutilized crops that appeared promising. This work has been successful on a limited scale and should be continued. The world-wide estimated present yearly cost of such work is $200 million; $400,000 for each new or improved variety released to growers.1/

1/ From USDA/SEA Current Research Information System (CRIS) data base.

The advent of biotechnology in the area of tissue culture adds a whole new dimension to the work going on to identify food crops for these stressed areas that will be reliable, higher yielding and more nutritious sources of human nutrition and which concomittantly have the potential for wide-scale adoption throughout such zones at much lower costs than those presented by the presently available alternatives - - - principally irrigation, drainage and liming.

The work supported by this project has been underway for three years. It attacks the problems of the generation and identification of food crops through plant tissue culture techniques that are tolerant to three of the major soil stresses that presently negatively affect yields: drought, salinity and aluminum toxicity, the latter a general characteristic of extremely acid soils. The average yearly costs of this project have been about $170,000.

Through efforts in this project so far, the technique for selecting plant cell tissue; rapidly and inexpensively multiplying it; successively selecting from it cells that multiply and that can subsequently be grown into entire plants which can reproduce sexually through ensuing generations has been proven for wheat, oats, rice and millet. It has also been established that wheat and rice cells, following the same procedures with the addition of increasingly high concentrations of salt, not only multiply into equally salt-tolerant plants but that this characteristic persists from one generation to another. Studies of the proper procedure to follow to select plant cells tolerant of aluminum and drought stress have been successfully initiated. A significant finding has been the identification of indoleacetic acid as a stimulator of embryogenic tissue development in the early stages of laboratory tissue culture. This has greatly assured success at the critical initial stage in plant tissue culture. The methodology for reducing the cost and for greatly increasing the capacity for handling tissue culture samples has also been established so that the technique can be implemented in other institutions serving the world between the tropics.

Progress reported in the Team Evaluation is highly satisfactory with outputs ahead of schedule for all phases of the research on the three crops under study: wheat, rice and millet.

The review team strongly recommends that the project be extended for at least two years beyond the present three-year contract which terminates on August 14, 1983. This would carry the project through 1985 to complete its originally planned five-year life-of-project, FY 1980-1984.

Research needs to be continued to solve the problems arising as tissue culture technology is developed and tested under laboratory conditions and as it expands into successive areas that culminate with stress-tolerant cultivars being available for use in farmers' fields in salt- aluminum- and drought-stressed areas in the LDCs. This will entail the refinement of tissue culture

techniques for serial screening of wheat, rice and millet for drought and aluminum tolerance as well as for saline conditions. And, it will also include reproducing whole, sexually mature plants from the stress-tolerant cell cultures and carrying these plants through successive generations of sexual reproduction to verify both their viability and the persistance of the stress-tolerant characteristics. This will require additional growth chambers or expanded growth room facilities and an automatic tissue-culture-medium dispensing system.

Outreach will be strengthened by reinforcing the growing linkages with national and international agricultural research centers which test the stress-tolerant varieties at no cost to A.I.D. But, the project will not be diluted to respond to the increasing demands for technical assistance and information under the continuation of funding for research contemplated for the two-year extension recommended by the Project Evaluation Team.

Such an expansion into biotechnology outreach and research will, as the Team Evaluation recommends, occur in the context of the establishment of training sessions in hands-on research and technical assistance; and, scientific information exchange on tissue culture technology. An expansion would also include work on legumes.

14. Evaluation Methodology: See Team Evaluation report, page 1.
15. External Factors: No major changes, see Team Evaluation report, pages 1 and 2.
16. Inputs: See Team Evaluation report, page 2.
17. Outputs: See Team Evaluation report, pages 2 and 3.
18. Purpose: See Project Design Summary Logical Framework.
19. Goal/Subgoal: See Project Design Summary Logical Framework.
20. Beneficiaries: See Project Statement, pages 4, 5 and 6.
21. Unplanned Effects: See Team Evaluation report, page 3.
22. Lessons Learned: See Team Evaluation report, pages 3 and 4.
23. Special Comments and Remarks:

Increasing demands for hands-on training, providing technical assistance to LDCs and the exchange of scientific information on the biotechnology of tissue culture, was recommended by the Evaluation Team as part of an enhanced effort that would also include work on legumes.

Attachments:

A. Report of Team Evaluation of Tissue Culture Project (936-4055), March 29-30, 1982
B. Scope of Work - Team Project Evaluation
C. FY 84 ABS Activity Data Sheet
D. Project Statement
E. Project Design Summary Logical Framework

Drafted by S&T/AGR, JWalker:cmg:1/25/83:WANG 0077g

Project Evaluation
US-AID Project

"A Utilization of Tissue Culture to Increase Food Production"

Project Number: 936-4055
Contract Number: AID/DSAN-C-0273
Name of Contract at Colorado State University:
Department of Botany and Plant Pathology
Fort Collins, Colorado 80523

Date of Evaluation: March 29-30, 1982

I. Evaluation Methodology

The purpose of the evaluation was to investigate the project progress compared with the original proposal and [redacted] that would assist the project in achieving its original goals and objectives.

The review panel members included James R. Welsh, Director-Montana Agricultural Experiment Station, Montana State University, Bozeman, Montana--Team Leader, and Dr. A. M. Kazi (Plant Breeder), CIMMYT, Mexico 6DF. The review took place at Colorado State University in the Botany and Plant Pathology Department. Discussion was held with the project administrator, Dr. Murray Nabors and his staff. The team conducted an on-site visit to the laboratory facilities.

In attendance to provide AID information was Dr. T. S. Gill, Senior Program Manager, Office of Agriculture, Bureau for Science and Technology, US-AID, Washington. Discussions were also held with Botany and Plant Pathology Department Head Dr. Gary McIntyre and Colorado State University Experiment Station Director Dr. John Patrick Jordan.

II. External Matters

Relationship With International Centers--At the present time, the project has established linkages with the International Centers on an informal basis. The review team recommends that these linkages be reinforced and formalized to clearly identify the responsibilities and activities associated with various aspects of the project. It appears that the International Centers can assist in testing the genetic materials resulting from this project and supply an information feedback mechanism to aid in the development of the scientific information aspects of the work.

Information and Technical Assistance Training Requests--The project is receiving an increasing number of requests for information and technical assistance. This is the result of the recently published newsletter and interaction by the project director in the international arena. As these requests continue to increase, additional pressure will be applied for the utilization of personnel time and resources. The review team recognizes the ultimate value of extending this information to the international community. However, the team is also concerned that the project as originally written and funded not be diluted or redirected to respond to these demands in the current framework of resources allocated to the project.

The productivity and popularity of this project has resulted in additional participation in meetings, conferences and the development of communication mechanisms such as newsletters and publications. The review committee views this as a positive aspect of the project which should be continued.

III. Project Input

Project Management--It is the review team's opinion that the project leader is doing an appropriate job of resource management. The project is making maximum use of space available but is, to some extent, limited by equipment and other facilities which will be mentioned in a subsequent section of the report.

The project leader has utilized the resources of an outside consultant to assist in improving management efficieny. The project encompasses a wide range of diversified personnel including permanent employees, part-time student help, post doctoral fellows, graduate students, and international visiting scientists. The diversity of personnel is entirely appropriate to this project. They appear to be contributing adequately to the original project objectives. They also bring in excellent innovations to the research effort. The growth of the project has resulted in the taxing of the facilities and personnel to the maximum. The review team suggests that some reinforcement be provided in document preparation and communications.

University Administration Policies--The project appears to receive a good level of support from the University administration. It will be continually evaluated in priority listings with other projects in the University and the Experiment Station. The project has generated a high level of publicity and has provided visability for the University in this subject matter area. The project lends itself to the traditional role of contribution in the international area by Colorado State University.

IV. Project Output

The project has been in operation approximately 1.5 years. It is the review team's impression that the choice of crops selected for the research was appropriate to international concerns. The project is ahead of schedule for all phases of the research for all crops. The project has achieved appropriate levels of output for the regeneration of all crops, for the screening procedures, and for special studies which have addressed unique issues in this research. For example, regeneration has been accomplished for some

varieties of wheat, oats, rice, and millet. Millet is of particular importance since earlier opinions indicated that this could not be achieved easily. Regeneration now appears to be routine for these species. The screening techniques for salt tolerance in wheat and rice have been completed. Procedures are now being initiated to address aluminum toxicity and drought tolerance. An example of productive special studies include the identification of indole acetic acid (IAA) as a valuable input for embryogenic tissue development has contributed significantly to the success of the project. The inclusion of engineering expertise has resulted in the improvement of efficiency in mechanical apparatus utilization in media preparation and other mechanical aspects of the project. Approximately 100,000 vials of tissue culture samples are handled by this laboratory annually. The fact that the project is ahead of schedule in some areas has resulted in additional problem areas which will be addressed in our recommendations.

V. Recommendations and Comments

1. Based on the review information and project achievements to date, the review committee [redacted] extension of the project for at least two years beyond the [redacted] contract. The project appears to be meeting or exceeding its original time table and information output. The project objectives as originally described are technologically achievable and should have significant impact in the international community of food production. The successful development of regeneration methodology has resulted in larger than anticipated volumes of material which must be propagated and tested to insure the success of the project. This has resulted in the identification of additional needs for the project. Addtional growth chambers are required to address the special propagation needs of several species. Either three chambers or some modified growth room is essential.

2. A half-time secretary and an additional laboratory technician are recommended. An automatic medium dispensing system is recommended to improve efficiency in utilization of personnel time. During the recommended project extension period, salary considerations should be addressed. Additional competition on the part of industry combined with inflation rates have resulted in the requirement to adjust technical salaries appropriately.

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3. [redacted]--Numerous inquiries are received regularly by the project for assistance in establishment of tissue culture laboratories and for background scientific information on tissue culture technology. Also, requests are received for training in tissue culture methodology. The requests represent a wide-range of expertise from post doctoral fellows to technicians in laboratories. These requests are received from all economic levels of the world. This broad spectrum of requests indicates the wide interest in this methodology and should be responded to in an appropriate manner. It is the review team's opinion that these requests will increase rapidly as the project achieves additional visability and success. The project does not currently have in its objectives the development of a training component. However, the review team feels strongly that this issue should be carefully addressed in the near future. [redacted] The review team recommends that the project leader and AID carefully consider the interaction of this program with current national programs and needs in order to complement one another rather than duplicating efforts. [redacted] designed cannot address these requests without additional resources. [redacted] the utilization of this project's accomplishments, some mechanism should be developed to enhance the information and training aspect of this work.

4. International Linkages--The review team [redacted] that this project maintain close communications with International Centers both to achieve the goals of this project and to enhance the development of additional research for handling current problems and identifying future research areas. The review team recommends that the project leader utilize cooperative expertise wherever available both nationally and internationally.

9

Signed:

James R. Welsh
Director
Montana Ag Experiment Sta.

A. M. Kazi
Plant Breeder
CIMMYT

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January 28, 1982

TO: S&T/FA, Dr. J. S. Robins

FROM: S&T/AGR/RNR, Tejpal S. Gill

SUBJECT: Scope of Work for Team Evaluation - "Tissue Culture" Research Project With Colorado State University.

Your approval is required for the attached Scope of Work to conduct a team evaluation of the "Tissue Culture" research project with Colorado State University, during the month of March, 1982. The project was initiated in September, 1980 and is presently funded until September. 1983.

Approved _____

Disapproved _____

Date _____

Clearance:
S&T/AGR, D.R. Fiester Date 28 I 82

cc: S&T/PO, B. Masters
S&T/AGR, M. Mozynski

Attachment:
Subject Scope of Work

TEAM PROJECT EVALUATION

Scope of Work

A. Project Title: "Utilization of Tissue Culture to Increase Food Production."

Project No: 936-4055

Contract No: AID/DSAN-C-0273

B. Name of Contractor: Colorado State University
Department of Botany and Plant Pathology
Fort Collins, Colorado 80523

C. Purpose and Rationale for Team Evaluation:

The project was initiated during September, 1980 and is funded until September, 1983. Most of the studies under this project are being conducted in the University laboratories at Fort Collins. The contractor is supposed to make collaborative arrangements with 2 - 3 international crop research centers to evaluate environmentally tolerant crops in the field.

It is a five-year project, but the Research Advisory Committee (RAC) endorsed it only for three years. AID agreed with RAC that further extension of the project (beyond 3 years) should be subject to an on-site review and a satisfactory progress during the first fifteen months. RAC recommended that the Office of Agriculture arrange for an evaluation of the project as soon as possible after this initial operational period.

The team will visit Fort Collins and evaluate the project work. It will make a report to S&T/AGR on:

a) whether project is making a good progress and is on schedule;

b) whether project needs extension to achieve its objectives;

c) utilization of the project output for the benefits of LDCs;

d) possible improvement in project management.

D. Composition of Team: The evaluation team will consist of the following:

1) Dr. James Welsh (plant breeder)
Dean of Agriculture
College of Agriculture
Montana State University
Bozeman, Montana 59717

2) Dr. A. M. Kazi (plant breeder)
CIMMYT
Mexico 5, D. F.

3) A member of RAC
(subject to availability of funds from S&T/PO)

E. Other participants:

1) AID/ST/AGR, Project Monitor, Dr. T. S. Gill;

2) Colorado State University, Principal Investigator, Dr. M. Nabors.

F. Dates and Place of Evaluation:

March 29 and 30, 1982, Colorado State University, Fort Collins, Colorado.

H. Problems and Issues to be Addressed: The team will consider project-specific items as those listed below. It will also consider, as far as possible, additional issues that may be raised by AID, Colorado State University and the team itself.

1) Project design; adequacy, field testing.

2) Personnel; adequacy, balance.

3) Management of project operations.

4) Achievement towards targets of the project.

5) Reporting and information dissemination.

6) Training component needs.

7) Budgeting; adequacy, relevancy, adjustments.

8) Projected utilization of the results and impact on AID target group.

9) Team recommendations for changes in project design or implementation.

10) Summary of the evaluation (one page).

I. List of documents for the Team:

1) Project paper including the matrix.

2) Annual report (first).

3) News letter (first).

4) Brochure on the project.

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J. Agenda for the Evaluation (Deadlines):

1) February 19, 1982. All preliminaries for the evaluation completed, including distribution of the documents to the team members.

2) March 15, 1982. All travel arrangements for the team members completed.

3) March 31, 1982. Team evaluation completed.

4) April 15, 1982. Team chairperson (Dr. J. Welsh) submits a written report about the evaluation to the attention of the Director, S&T/AGR.

PROGRAM: CENTRALLY FUNDED

ACTIVITY DATA SHEET

PROJECT MANAGER: J. L. Walker

TITLE: Tissue Culture for Improved Food Crop Varieties	FUNDS: Agriculture, Rural Development and Nutrition	PROPOSED OBLIGATION (In thousands of dollars): FY 84 225	LIFE OF PROJECT: 1150
NUMBER: 936-8055; GRANT ☒ LOAN ☐; NEW ☐ CONTINUING ☒	PRIOR REFERENCE: FY 1983 Annex V, Centrally Funded Programs, p. 25	INITIAL OBLIGATION: FY 80	ESTIMATED FINAL OBLIGATION: FY 85
			ESTIMATED COMPLETION DATE OF PROJECT: FY 86

Purpose: To test the use of tissue culture as a cost effective technique to improve stress tolerant characteristics to wheat, rice millet.

Background and Progress to Date: This project is based on a successful conclusion of an earlier A.I.D. funded small research study by Colorado State University. That study demonstrated that by exposing plant cells (tobacco) to increasing levels of salt (Na Cl), one can select cells that will tolerate much higher levels of salt than the original cells. These cells are regenerated into whole plants which retain their salt tolerance from one generation to another. In the tropical and sub-tropical world, over two billion hectares experience stresses such as mineral deficiencies or toxicities, low and high temperature and drought conditions. Current approaches to alleviate the situation are to change or amend the soil to fit the plant needs or in a few instances fit the plant to the environment. However, certain conditions either cannot economically be corrected with the current technology or a viable system is not available to deliver a suitable technology to the small farmer in the LDCs. These problems can be addressed by the possible development of stress tolerant varieties by means of plant tissue culture techniques. The project will study wheat, rice and millet crops for tolerance to salt, aluminum toxicity and drought.

Host Country and Other Donors: The project is linked with international and national agricultural research centers with regards to gene plasm and utilization of the technique. These centers will test the stress tolerant crop varieties at no cost.

Beneficiaries: The overall thrust of the program is to advance knowledge for crop production on marginal lands. It holds great promise for increased benefits for the poor farmer, especially women, who have traditionally played a major role in such areas.

FY 84 Program: To continue work started during 1981-82 and obtain desired tolerance levels for salt and aluminum toxicity in rice and salt tolerant cell lines of millet. Start greenhouse testing of salt tolerant wheat and rice varieties. Regenerate other wheat and rice lines tolerant to salt and aluminum toxicity.

Major Outputs and A.I.D. Units:

	All Years Units
Wheat varieties tolerant to 3 stresses	6
Rice varieties tolerant to 2 stresses	4
Millet varieties tolerant to 1 stress	2
Trained scientists	5

A.I.D. Financed Inputs in $000s:

	FY 84
Personnel (30 person months)	$145
Logistics	40
Equipment and Supplies	40
TOTAL	$225

U.S. FINANCING (In thousands of dollars)

	Obligations	Expenditures	Unliquidated	Funding Period	Principal Contractors or Agencies
Through September 30 1981	500	168	332		
Estimated Fiscal Year 1982	–	172		10/1/81 to 7/30/82	Colorado State University
Estimated through September 30 1982	500	340	160		
Proposed FY 1983	200	160		10/1/82 to 9/30/83	
Estimated through Fiscal Year 1983	700	500	200		
Proposed FY 1984	225	Future Year Obligations	Estimated Total Cos.	10/1/83 to 9/30/84	
		225	1150		

PROJECT STATEMENT

Utilization of Tissue Culture to Increase Food Production

(Project # 936-4055)

Office of Agriculture
Bureau for Science and Technology
Agency for International Development
Washington, D.C. 20523

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Appendix A. Project Design Summary - Logical Framework

Appendix B Tech. Paper: Salt Tolerant Tomato from Cultured Cells - Earlier small research project. AID/ta-c-1113)

PROJECT STATEMENT

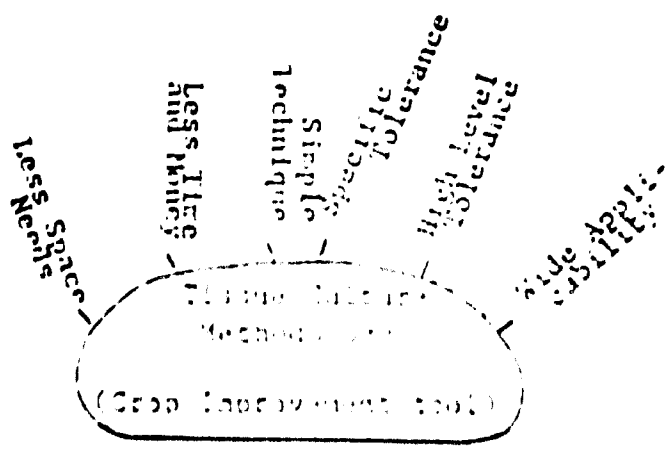
I. Project Summary

A. Statistical

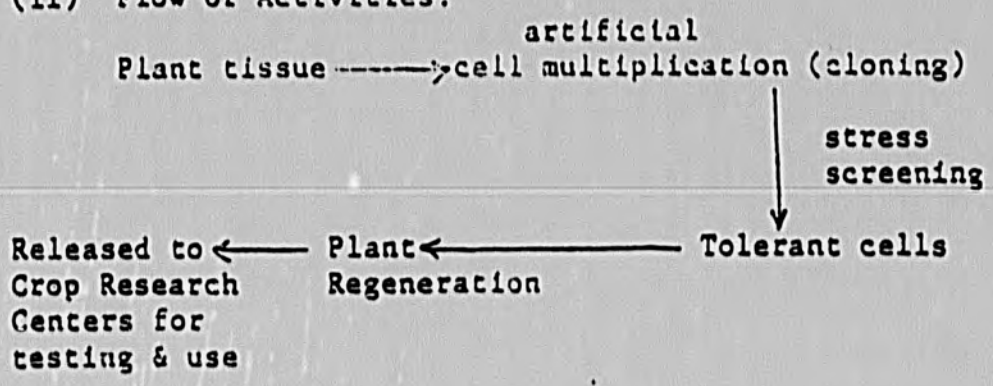
Project Title:	Utilization of Tissue Culture to Increase Food Production
New or Extension:	New (Initiated Sept. '80)
Contractor:	Colorado State University Department of Botany and Plant Pathology Fort Collins, Colorado 80523
Principal Investigators:	Dr. Murray W. Nabors
Duration:	About Five Years
Initial Three Years Funding:	First Year: $200,000 Second Year: 150,000 Third Year: 150,000 TOTAL : $500,000
Project Manager:	Dr. T. S. Gill, S&T/AGR/RNR

B. Graphic

(1) Possible Advantages:



(ii) Flow of Activities:



(iii) Out-puts: Scope of work

- o Wheat variety(s) tolerant to salt concentration, aluminum toxicity, drought conditions

- o Rice variety(s) tolerant to salt concentration and aluminum toxicity.

- o Millet variety(s) tolerant to salt concentration

C. Descriptive:

This proposal pursues A.I.D. objectives to expand the technology on management of tropical soils to increase food production. It has one principal objective: to test the use of plant tissue culture as an efficient technique to obtain varieties of food crop plants that have specific tolerance to enviornmental stresses. In the tropical and subtropical world, over two billion hectares experience stresses such as mineral deficiencies or toxicities, low and high temperature, drought conditions, etc.

Current approach to alleviate the situation is to change or amend the soil to fit the plant needs or in a few instances fit the plant to the enviornment. However, certain soil conditions either cannot economically be corrected with the current technology or a viable system is not available to deliver an adapted technology to the small farmer in LDC.

Estimate of annual expenditure in the tropics on crop improvement and soil management studies, conservatively speaking, is over $120 million. Generally these studies concentrate on producing pest resistant and high yielding varieties that are bred under adequate soil fertility and moisture conditions. Very limited attempts have been made to develop varieties that can stand adverse soil situations. On the other hand studies in this area

suggest existance of slight to significant amount of tolerance among varieties for soil stress conditions. Search for such tolerance via worldwide collection of domesticated and wild relatives, of various crop species, however, would be time consuming and an expensive proposition and may not even meet the needs.

It can be visualized that the problem of worldwide soil stress situations is large but so is the potential benefit if suitable solutions could be found. It is to this challenge that this project addresses. It is a new approach, an innovative and cost effective technique to speed the process of plant adaptation to adverse environments.

The approach briefly stated is as follows: a group of cells are removed from a plant and cultured aseptically on either soild or liquid medium containing relatively high level of an auxin as well as standardized concentration of ions and vitamins. This encourages proliferation of the cells (clones/callus). The callus is then turned into a cell suspension. A 100 mls. of the suspension in a flask may contain 10 million cells. Selection is applied at this time. For example, by increasing salt (sodium chloride) level in the suspension culture, the salt tolerant cells are selected because their growth and cell division is favored. Such selection procedures can rapidly produce the desired level of tolerance among the cells. These cells are regenerated into whole plants. The tolerance thus established is carried genetically from one generation to another. From here on standard procedures of plant breeding are followed as necessary.

Specifically the project will develop laboratory methodology for developing soil stress tolerance (against high sodium and aluminum, and drought) among the following crops: rice, wheat, and millet. Not all stresses against all crops will be studied.

The project will be intimately linked with the international and national agricultural research centers with regards to germ plasm and utilization of the technique.

The project is expected to last five years and will trigger the utilization of this tool, worldwide, for the purpose of crop improvement and food production. Simplicity of the technique should help resolve local crop adaptation problems.

II. Project Proposal

A. Goal: To improve the efficiency of food production in the developing nations through increased utilization of land resources, especially by growing crop varieties more suited to the prevailing environments.

B. Purpose: To test the use of tissue culture as a cost effective technique to add stress tolerance characteristics to wheat, rice and millet.

C. Objectives:

1. To utilize tissue culture procedures to obtain Na+ tolerant, Al+++ tolerant, and drought tolerant alleles cell lines of useful cultivars of three food crop plants-wheat, rice, and millet.

2. Regenerate stress-tolerant plants from the cell lines and test these plants in green house and the field to determine stress limits and inheritance pattern.

3. Help the utilization of these stress tolerant plants in the national and the international crop improvement programs.

D. Benefits: Feeding the world's population is a much discussed problem with identifiable components. These include:

1. increasing production on currently arable land
2. bringing non-arable land into production
3. dealing with problems of distribution and storage

The first two problems can be approached either by environmental modification (fertilization, irrigation, use of pesticides, mechanization, etc.) or by plant modification (new plant varieties with increased yields on unmodified enviornments). The first group of solutions has worked well in already developed nations but is energy-intensive and, therefore, frequently of limited utility in developing nations. In addition, the increasingly acute world energy shortage means that an agriculture based on enviornmental modification is becoming more costly for developed nations as well.

Because of rising energy costs and decreasing supplies the first two goals above can be best achieved by producing plant varieties with high yields on available soil and water conditions. Currently utilized crop varieties frequently do poorly in such conditions. Stress-resistant varieties would be area specific (e.g. for dry, saline soils; or wet, acidic, aluminum-containing soils) and would increase the capabilities of developing nations to feed themselves utilizing their own resources, without overdependence on imported, expensive agricultural practices and inputs.

Plant breeders have been quite successful over the years in producing new varieties of plants with specific improved traits. Many of these improved plants are themselves dependent on energy-intensive agricultural practices.

> Green revolution agriculture uses high energy crop production technology, . . . While one may not doubt the sincerity of the U.S. effort to share its agricultural technology so that the rest of the world can live and eat as it does, one must be realistic about the resources available to accomplish this mission. In the United States we are currently using an equivalent of 80 gallons of gasoline to produce an acre of corn. With fuel shortages and high prices to come, we wonder if many developing nations will be able to afford the technology of U.S. agriculture.1

> Recently breeders have placed increased emphasis of varieties which yield in available environments and are thus economical users of energy. The international plant improvement centers are now are now turning more of their over $64 million yearly budget away from energy-intensive green revolution crops and toward" . . . developing plant types and practices to benefit the small farmer, who must operate without the benefit of controlled irrigation or much help from fertilizers and pesticides."2

> It is conservatively estimated that plant breeding programs on major food crop plants currently cost $200 million per year ($50 million in the U.S.) and employ 5400 people.3

An estimated 480 new or improved varieties are released to growers each year at a development cost of over $400,000 per variety. Eight to ten years breeding research are required per variety.

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1 D. Pimentel, L.E. Hurd, A.C. Bellotti, M.J. Forster, I.N. Oka, O.D. Sholes, and R.J. Whitman. Food production and the energy crisis. Science 182: 443-449 (1973).

2 CGIAR, New York, 1976. U.N. Development Programme, One United Nations Plaza, New York, N.Y. 10017.

3 Revised and expanded data from Science and Education in Agriculture Current Research Information System (CRIS).

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It is suggested that the incorporation of tissue culture techniques into plant breeding programs for stress resistance can considerably economize and expedite the release of new or improved varieties to increase food production.

III. Project Design and Scope of Work

A. Experimental design

Various alleles for resistance to soil, water, and environmental stresses will be selected in cell cultures of varieties of food crop plants. Table 1 summarizes the plant species to be used and the specific stress tolerances to be selected. For each plant and stress condition the following procedure will be utilized:

a. Crop varieties and stress limits are selected by AID and IARCs personnel. Schedule of student and personnel exchanges between tissue culture labs at CSU, and International Centers is established.

b. A cell culture is established for each food crop variety.

c. Cell proliferation occurs until a large population (millions) of cultivated cells is obtained.

d. Cultured cells on solid medium or in liquid suspension are stressed to a level which inhibits growth of normal cells but favors the growth of stress tolerant cells. Stress conditions will be established as follows:

(1) Na^+ or Al^{+++} tolerance: Add NaCl or $AlCl_3$ to growth medium.

(2) Drought tolerance. Add NaCl or high molecular weight, osmotically active compounds to growth medium.

e. Plants are regenerated from cultured cells.

f. Stress tolerance and inheritance of stress tolerance are tested in greenhouse experiments.

g. Stress tolerant plants are field tested at International Agricultural Research Centers.

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B. Research methodologies and techniques.

The production of mutant plants by tissue culture techniques can be divided into four steps.

a. Production of callus or cell suspension cultures of plant cells.

b. Selection of desired mutants.

c. Regeneration of mutant plants from callus or suspension cultures.

d. Testing of regenerated plants in the greenhouse for phenotype persistance and inheritability of the mutant trait.

A callus is a mass of growing, dividing, but undifferentiated cells. The production of callus can be considered routine for most plants. Typically a small section of stem, root, or cotyledonary tissue is placed in sterile culture on a solid nutrient medium containing known concentrations of sugar, mineral nutrients, and plant growth hormones. The composition of the medium is adjusted to stimulate the proliferation of cells. This is usually accomplished by using high levels of an auxin which encourages cell division and growth, but inhibits the formation of shoots and roots. When a sufficient amount of callus is obtained, cell suspensions are initiated simply by placing callus tissue in a baffle-bottom Erlenmeyer flask containing liquid medium on a gyrotory shaker. The mechanical motion of the shaker disrupts the callus into small groups of cells. Cell suspensions are subcultured when cell density reaches a minimum value.

Mutant cells are selected by exposing cell cultures to conditions that slow or prevent the growth of normal cells. Specific stress tolerant mutants will be selected as follows:

a. Na^+ or Al^{+++} tolerance.

Mutants tolerant to these specific cations are selected by adding increasing concentrations or NaCl or $AlCl_3$ to the medium. Selection will occur in a stepwise fashion. At first low levels of a particular salt are added to a rapidly growing culture. The salt will tend to inhibit growth and division of normal, non-tolerant cells while allowing or perhaps encouraging growth and division of tolerant cells. A single culture will have millions of non-tolerant cells and perhaps only one or two individual tolerant cells. When the culture reaches maturity it is subdivided with some subdivisions placed on medium with higher salt concentrations and others on plant regeneration medium. This process continues until the cultures can no longer successfully adapt to high levels of the toxic ion. As a result of this technique we will obtain a series of cell lines resistant to higher and higher concentrations of the toxic ion. In this regard it should be noted that although a tolerance of

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3000 ppm may be desired, a variety with tolerance to 6000 ppm might be more useful in terms of maximizing productivity at the lower concentration.

This selection process can be carried out in any one of three manners:

(1) Mutant cell lines can be selected in callus cultures by incorporating the salt into the solid medium. This selection method works well in practice although the exact concentration of salt in contact with cellus tissue is difficult to determine accurately.

(2) Mutant cell lines can be selected in suspension cultures which are subdivided into new culture medium when mature growth is obtained. This selection system works well, and one knows exactly the salt concentration to which the cells are exposed. However, frequent subdivision of the cultures requires time and effort, and opening the cultures allows the possibility of bacterial or fungal contamination.

(3) Mutant cell lines can be selected in continuous suspension cultures maintained in fermentors. In this selection system new medium with increasing levels of the selective agent is gradually added at a rate which allows a maximal growth rate to be maintained. A new medium is added, suspension is removed for plant regeneration. This selection method requires the most expensive equipment but is most satisfactory in that technician time is minimized, and the culture is never exposed to possible contamination.

Na^+ toxicity is frequently associated with calcium-deficient soils. It may thus be necessary for some environments to obtain plants which have a heightened efficiency of Ca^{++} uptake as well as or in place of Na^+ resistance. Such mutants would be selected by exposing cultures to an abnormally low level of Ca^{++}.

Al^{+++} toxicity is always associated in nature with soils of low pH. Even in the lab chelated Al^{+++} solutions may well lower cellular pH after Al^{+++} enters the plant. Thus it might be useful to select cell lines resistant to low pH for physiological comparisons to those resistant to Al^{+++}.

b. <u>Drought tolerance</u>.

Na^+-tolerant plants are often drought tolerant. This can be seen in our lines of Na^+-tolerant tobacco plants as well as in nature. Thus Na^+-resistant plants will also be tested for drought resistance. Drought-tolerant mutants will also be selected by inhibiting water uptake in cultured cells. This is accomplished by lowering the water potential of the culture medium with increasing ionic concentrations or by the addition of a non-penetrating osmoticum such as PEG 4000 or dextran. Only mutant cells with a lower than normal water potential will grow under these conditions. Selection of increasingly drought-tolerant mutants will be achieved in a stepwise manner, similar to the Na^+ and Al^{+++} mutant selection process. That such osmoticum-resistant cell lines result in drought-tolerant plants remains to be tested.

C. Scope of work.

a. First year of contract

(1) Work to meet research objectives.

(a) Communicate with plant breeding personnel in A.I.D. and at International and National Agricultural Research Centers (IARC'S and NARC'S) to obtain recommendations for stress tolerances to be obtained and varieties to be utilized. Obtain germplasm to be used in tissue culture stress selection.

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(b) Establish linkages with IARCs and developing nations to initiate student and personnel training in tissue culture laboratories.

(c) Establish and maintain cell lines (on solid and in liquid medium) on wheat, rice, and millet. Begin stress selection for Na^+, Al^{+++}, and drought tolerance.

(d) Compile a bibliography of useful tissue culture methodology for specific plants for use by plant breeders wishing to include tissue culture in their breeding strategies.

(e) Attend national and international tissue culture and plant breeding meetings.

(f) Initiate contact with soil scientists and agricultural engineers interested in soil and water management. Add these people to mailing list compiled

(2) Expected product.

(a) Initial contacts made with interested worldwide plant breeding personnel. Mailing list initiated. Personnel and student exchange plans initiated.

(b) Cultured, stress-resistant cell lines under production for rice, and millet.

(c) Na^+-tolerant wheat plants ready to begin greenhouse testing phase for one variety; Na^+-tolerant cell lines established for other varieties; Al^{+++}, drought tolerant cell lines established for wheat.

(d) Tissue culture bibliography for each crop plant available to interested breeders.

(e) National and international tissue culture and plant breeding meetings attended.

(f) Contacts initiated with soil scientists and agricultural engineers interested in soil and water management. Names added to mailing list.

b. Second year of contract

(1) Work to meet research objective

(a) Obtain desired tolerance levels in Na^+, and Al^{+++}, all lines of rice and Na^+ and drought-tolerant cell lines of millet. Determine maximum tolerances obtainable in tissue cultures.

(b) Begin greenhouse testing phase for one variety of Na+-tolerant wheat plants. These experiments will determine whole plant tolerance levels and tolerance inheritance patterns.

(c) Begin plant regeneration from other Na+-tolerant wheat lines. Begin plant regeneration from Al+++- and drought tolerant lines of wheat.

(d) Initiate short visits and personnel exchanges between International Centers and developing nations.

(e) Complete progress report to assist A.I.D. review process.

(2) Expected product.

(a) Na+-, and Al+++-, tolerant cell lines of rice, and Na+ tolerant cell lines of millet.

(b) Preliminary results from greenhouse testing of Na+-tolerant wheat showing limits of tolerance and pattern of inheritance through F1 generation for one variety.

(c) Complete plant regeneration studies of other Na+-tolerant wheat lines and of Al+++-, drought tolerent wheat lines.

(d) Initial short visits between International Crop Breeding Centers' personnel and tissue culture labs. Information exchange initiated. Student and personnel training initiated.

(e) National and international tissue culture and plant breeding meetings attended.

(f) Progress report submitted to A.I.D.

c. Third year of contract

(1) Work to meet research objectives.

(a) Begin plant regeneration from Na+-, and Al+++-, tolerant cell lines of rice and Na+ tolerant cell lines of millet.

(b) Conclude greenhouse testing of one Na+-tolerant wheat variety.

(c) Begin greenhouse testing of Al+++-, drought tolerant wheat plants.

(d) Continue information and personnel exchanges with International Breeding Centers.

(e) Attend national and international plant breeding and tissue culture meeting to discuss program and data and present papers.

(2) Expected product.

(a) Some regenerated plants from Na^{+}, Al^{+++} tolerant cell lines of rice, and Na^{+} tolerant cell lines of millet.

(b) A research publication detailing greenhouse testing of Na^{+}-tolerant wheat plants of one variety and the use of tissue culture to select these plants.

(c) Preliminary results from greenhouse testing of Al^{+++}, Na^{+}, drought tolerant wheat.

d. Fourth year of contract

(1) Work to meet research objectives.

(a) Complete plant regeneration from Na^{+}, and Al^{+++}, tolerant cell lines of rice and Na^{+} tolerant cultures of millet. Begin greenhouse and testing.

(b) Begin field testing of one Na^{+}-tolerant line of wheat.

(c) Complete greenhouse testing of Al^{+++}, drought tolerant lines of wheat.

(d) Continue personnel exchanges between International Crop Breeding Centers and tissue culture labs.

(e) Attend national and international plant breedings and tissue culture meetings and present findings.

(2) Expected product.

(a) Na^{+}, and Al^{+++}, tolerant rice and Na^{+} tolerant millet plants obtained from cell cultures.

(b) Preliminary results on field testing of one Na^{+} tolerant wheat variety.

(c) Final results on greenhouse testing of Al^{+++}, Na^{+}, drought tolerant lines of wheat.

(d) A research publication on greenhouse testing results using Al^{+++}, Na^{+}, drought tolerant lines of wheat.

(e) Attended national and international plant breeding and tissue culture meetings and presented findings.

e. Fifth year of contract

(1) Work to meet research objectives.

(a) Complete greenhouse testing of Na^{+}, and Al^{+++}, tolerant lines of rice and Na^{+} . tolerant lines of millet.

(b) Continue field testing of one Na^{+}-tolerant lines of wheat. Release varieties to ICARs and National Crop Centers.

(c) Begin field testing of Al^{+++}, drought tolerant lines of wheat.

(d) Continue personnel exchanges with International Breeding Centers.

(e) Attend national and international plant breeding and tissue culture meetings and present findings.

(2) Expected product.

(a) Na^{+}, and Al^{+++}, tolerant lines of rice and Na^{+} tolerant lines millet, ready for field testing.

(b) Research publication detailing results of greenhouse testing of Na^{+}, Al^{+++}, and drought-tolerant rice and millet

(c) One Na^{+}-tolerant line of wheat released to IARCs and NARCs.

(d) Al^{+++}, Na^{+}, drought tolerant lines of wheat partially field tested.

(e) Research publication on production of field-tested, Na^{+}-tolerant wheat derived from tissue culture.

(f) A review publication covering progress during the five-year contract period.

(g) Attended national and international plant breeding and tissue culture meetings and presented papers.

(i) Submit a final report to A.I.D.

(h) A second Technical Bulletin detailing methodological progress during the five years since publication of the first bulletin.

IV. Project Analysis

A. Technical, Economical and Social Soundness

a. Tissue culture breeding has successfully produced improved varieties.

Several workers (including three investigators in this proposal) have already demonstrated the usefulness of tissue culture for selecting mutant plants: Carlson selected tobacco cell lines resistant to methionine-sulfoximine (a compound with effects similar to the toxin from the xcausal agent of tobacco wildfire disease). Plants regenerated from these cell lines also showed resistance which was inherited into the F2 generation.5 Similarly, Chaleff's lab has obtained picloram-resistant plants (picloram is an herbicide).6 Nabors" lab has obtained NaCl-resistant plants.7 Other workers have produced corn plants resistant to Helminthosporium toxin.8 In all these cases the resistances selected in cell culture have persisted in regenerated plants and have been shown to be inheritable.

b. Tissue culture breeding confers specicity to the mutant selection process.

Tissue culture enables us to specify that a certain type of desired mutant plant will be selected in a desired location (our laboratory flask), at a specific time (when we need it), and in a desired plant variety. This type of selection specificity is almost never available to a plant breeder using whole plants. Breeders frequently screen available varieties in hopes of finding a desired trait. Often the needed characteristic is found in an otherwise undesirable variety necessitating expensive and time-consuming cross-breeding. Many times the desired trait cannot be located in domesticated cultivars, and out-crossing to wild stock must be considered. Too frequently the needed characteristic cannot be located in any available germ plasm lines.
For example, mutations for salt tolerance arise in nature. Perhaps one in ten million plants will show a genetically-based increased level of salt tolerance. An investigator has no way of knowing the location of a

5 P.S. Carlson. Methionine-sulfoximine-resistant mutants of tobacco. Science 180: 1366-1368 (1973).

6 R.S. Chaleff and M.F. Parsons. Direct selection in vitro for herbicide-resistant mutants of Nicotiana tabacum. In Press in Proc. Natl. Acad. Sci. U.S.A.

7 M. Nabors, S. Gibbs, C. Bernstein, and M. Meis. NaCl-tolerant tobacco plants from cultured cells. Submitted for publication. Manuscript attached as Appendix to this proposal.

8 B.G. Gengenbach, C.E. Green, and C.M. Donovan. Inheritance of selected pathotoxin resistance in maize plants regenerated from cell cultures. Proc. Nat. Acad. Sci. U.S.A. 74: 5113-5117 (1977).

desired mutant plant, and time of its production or whether it is found in the variety he desires to improve. Imposing field selection (watering with salt water) to discover the plant can be time consuming and expensive. Sometimes selection using seeds or seedlings instead of adult plants can be used. Tissue culture may enable the investigator to specifically, rapidly, and economically obtain the plant he desires and then to return to the field for whole plant studies.

Also, tissue culture methods enable an investigator to produce a large number of a desired stress-resistant plant whereas field selection might produce one such plant. For example we have imposed NaCl stress on populations of cultured cells. Most cells are killed. However, the few salt-tolerant mutant cells continue to grow and divide until they make up the entire culture. Thus we can produce dozens, hundreds, or thousands of identical, salt-tolerant plants (a clone) by plant regeneration from cell cultures whereas field selection in the same period of time might produce one or a few individual plants.

c. Tissue culture can be used to rapidly clone mutant plants located by conventional means.

Rare, stress-resistant plants encountered in the field can be rapidly reproduced by the tissue culture process. For example, a single drought-resistant wheat plant might be discovered by a breeder in Mexico. By the normal process of collecting seeds and replanting to increase seed stock, several years would be required to obtain sufficient seed for varietal testing and introcution. Tissue culture could shorten this process considerabldy by producing thousands of identical plants within a year.

d. A combination of tissue culture and traditional breeding methods would considerably reduce the cost of producing new varieties.

Tissue culture compresses space and time requirements for selecting stress-resistant plants. In addition, it adds a desired specificity to the selection process. For these reasons the incorporation of tissue culture techniques could considerably reduce the time and money required for introcution of new plant varieties. A new variety presently requires around $400,000 and 8-10 years for introduction to growers. We estimate that utilizing tissue culture techniques could easily save 2-3 years and $150,000 in many such programs. If tissue culture were utilized in all breeding programs at a considervative savings of $50,000 per variety the yearly return realized would be (480 varieties x $50,000 =) $24 million in the breeding programs alone. This dollar figure is miniscule compared to that realized by the introduction of stress-resistant varieties could be used to further agricultural production or other projects requiring input of capital or labor.

e. Potential for effective utilization of research results in developing countries worldwide and suggested extension strategies.

Developing nations can all effectively utilize energy-efficient plant varieties designed for specific environmental conditions. Such plants would lower fuel and other energy costs of food production and would enable individual farmers to successfully and economically utilize existing soil and water conditions.

Worldwide A.I.D. personnel and scientists and growers in CGIAR Breeding Centers would participate in identifying the most critical stress conditions and levels inhibiting food production for particular crops in their region. They would also select varieties which have agronomically desirable traits, but which lack a desired level of stress resistance. For the tissue culture selection and greenhouse testing phases of the research, students and scientific personnel from developing nations and from CGIAR Centers would be funded for short visits and for extended training periods to our three tissue culture laboratories during the five year project duration. Following the addition or augmentation of specific stress resistances in varieties by tissue culture breeding and greenhouse testing of the resulting plants, the new cultivars would be field tested in the particular region for which they were developed by CGIAR personnel.

f. Possible effects of proposed work on other specific concerns of A.I.D.

In general the proposed work should be a step toward the economic independence of developing nations and of the individual farmers within these nations. Crops which require less than normal enviornmental modification are more economically and easily grown. Yields on currently arable land should increase; land formerly non-arable can be brought into cultivation; years with marginal environmental factors should be less damaging; and the willingness of individual farmers to plant should increase. The net result of energy-efficient crop plants which increase food production will be to release A.I.D. and other funds currently encumbered in both plant breeding and in environmental modification in energy-intensive agriculture for other desirable projects.

For example, considerable effort and resources are now expended by A.I.D. and other agencies worldwide to understand and control soil salinity, particularly in irrigated regions.[9,10] Some of this work is managed by Colorado State University. Production of NaCl-tolerant varieties is mentioned by many workers as one primary method for increasing the efficiency of irrigation and salinity management efforts. Thus use of tissue culture to rapidly incorporate salt tolerance into useful varieties will interface directly with A.I.D. concerns in water quality management.

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9 For example see FAO Soils Bulletin #31. Prognosis of Salinity and Alkalinity. Food and Agricultural Organization of the United Nations, Rome, 1976.

10 Also see published papers from an A.I.D.-sponsored symposium, Plant Adaptation to Mineral Stress in Problem Soils. Ed. M.J. Wright. Cornell U. Exp. Sta., 1977.

There are approximately one billion hectares or about one third of all potentially arable land on the earth's surface which could profit agriculturally from drought-and salt-tolerant plant varieties.

For these and other reasons the role of the plant breeder becomes a crucial factor in increasing agricultural production. Since tissue culture has now the proven capacity to rapidly produce plants with salt tolerance and efficient water utilization. Its incorporation into plant breeding contributes directly to all A.I.D. concerns relating to food production.

B. Review by International Centers

The proposal was reviewed and endorsed by all four international agricultural research centers to which it was sent. The excerpts of the comments from these centers is given here under:

IITA (Nigeria). "IITA finds the proposal technically very sound and logical . . . IILTA interested in this work as relates to maize, rice, cowpeas and soybeans with references to sodium and aluminum, toxicity and drought stress. Similar work crops with limited genetic diversity such as yam and cocoyams will be of interest . . . IITA very interested prospects screening for virus resistance at cellular stage and in rapid propagation techniques tuber crops, yam in particular."

CIAT (Colombia). "I am pleased to forward, with my endorsement these comments: . . . see a direct application of this type of research to CIAT's Beef Program. Selection for resistance to aluminum toxicity in forage legumes, both among species and among varieties of the given species, would be of great value in developing suitable pastures in the Llanos. . . . the general idea is attractive since the tissue culture system would have the advantage over convential selection/breeding methods in its case of handling large populations of individual cells instead of large numbers of intact plants."

IRRI (Philippines). " . . . may I say that this proposal is of great interest to us at IRRI. As a matter of fact for a period of about two years, we have had discussions with the scientists concerned in the development of this project and with administrators from their universities. We strongly support this research effort and would do what we could to cooperate with the scientists in supplying appropriate genetic materials and in reproducing materials which come from their research efforts."

CIP (Peru). "We feel an attempt to use tissue culture techniques as an additional tool for breeding is highly welcomed and should be supported. . . . We all want to sue this develop on a solid basis without the overplay that was done with irradiation concerning the potentials . . . work should be conducted in association with good breeders who are using conventional methods and working on the same crop plants."

C. Administrative Responsibility

This project will be administered by Colorado State University (CSU). Dr. Murray W. Nabors, Associate Professor of Botany and Plant Pathology will be the principal investigator.

CSU will develop close working relationships with sutiable international and national crop research centers to obtain germ plasm and to disseminate outputs of this project.

A.I.D. technical office (ST/AGR) will monitor the progress of the project and will interact on a continuing basis with the contractor. It will schedule on-site evaluation as deemed necessary.

D. Environmental Impact

The activities of this project fall into the area described in environmental procedure regulations paragraph 216.2(c) "Analysis, studies, academic or investigative research, workshops and miidings." These classes of activities will not normally require the filing of an Environmental Impact Statement or the preparation of an Environmental Assessment. It is possible that an output of this project will be a set of procedures, guidelines or research results which when used would require such as assessment. However, the project itself only proposes research and directly supportive activities. Under these guidelines, this activity clearly qualified for a Negative Determination at the time when a threshold decision is determined.

E. Women in Development

It is the goal of this project to encourage the widest possible participation of women workers. The past small research contract AID/ta-C-1223 on tissue culture employed women professionals in the Department of Botany and Plant Pathology to support the research work. A continuation of these strategies under this proposed research project is assumed. Colorado State University has an aggressive affirmative action program to assure opportunities of employment for qualified personnel, including minorities and females, in compliance with Title IX. Colorado State University has an officer responsible for program implementation.

Overall thrust of the program to advance knowledge in crop production particularly on marginal lands holds great promise for increased involvement of poor farmer, especially women, who have traditionally played a major role in such areas.

V. Evaluation Plan

The project will be reviewed by ST/AGR, the A.I.D. Regional Bureaus, A.I.D. Missions, LDC countries and the international crop research centers. Highly qualified outside consultants may also be included in the review process, if A.I.D. consisered such assistance desirable.

Summary

We have obtained NaCl-tolerant cell lines by exposing tobacco cell suspensions to increasing levels of NaCl. Tolerance to 8.8 g/l NaCl is the maximum obtainable in cell suspensions. Normal lines are tolerant to about 1.6 g/l NaCl. Plants regenerated from resistant cell lines transmit tolerance to two subsequent generations. The level of NaCl-resistance in regenerated plants is higher than that of cell cultures. Few nontolerant F2 plants survive watering with solutions containing more than 15.4 g/l NaCl whereas most tolerant plants survive 33.4 g/l NaCl.

Introduction

The prospects of utilizing tissue culture to increase food production are bright, but depend on demonstrations that agriculturally desirable traits, selected in cell cultures, persist in regenerated plants and are inheritable. Carlson (1973) has shown persistence and inheritability for methionine-sulfoximine (MSO) resistance in tobacco. MSO causes effects much like those of the toxin causing tobacco wildfire disease. Chaleff and Parsons (1978) have provided a similar demonstration for herbicide (picloram) resistance in tobacco. Gengenbach et al. (1977) have shown resistance to Helminthosporium toxin in corn.

Specific ion toxicity in soil and water is probably the largest single environmental factor restricting agricultural production. In particular, Na+ toxicity currently affects 50% of the irrigated land in the western United States and restricts crop production on 25% of this land (Wadleigh, 1968). Casey (1972) cites estimates that 33% of irrigated land worldwide is salt-affected. He questions the usefulness of irrigation schemes in general and suggests that breeding for salt tolerance is highly desirable. We believe this to be the first report that NaCl resistance contained in cultured cells is retained and inherited by subsequent generations of regenerated plants.

Materials and Methods

Cultured cell lines of Nicotiana tabacum var. Samson resistant to various levels of NaCl were obtained as previously reported (Nabors et al., 1975). Both spontaneous and induced (using ethyl methane sulphonate) lines have been obtained. Populations of 10 million cells contain salt-tolerant cells in about 90% of cases tested. Thus the spontaneous mutation rate would be not less than 1×10^{-7} per six months (the time required to obtain a cell suspension before selection begins). We have now produced lines resistant to as much as 8.8 g/l NaCl. Resistance to higher levels is not obtainable in our system. Shoots were regenerated from both NaCl-tolerant and NaCl-sensitive cultures by placing cell suspension aliquots on solid regeneration medium. Our regeneration medium consists of Linsmaier and Skoog's (1965) basic medium supplemented with 0.5 mg/l kinetin. Shoot regeneration is noticeably restricted on medium containing NaCl. Shoots were

rooted by transfer to an identical medium that contained in addition either 5 or 10 mg/l indoleacetic acid (IAA). Reducing the level of major salts to half or tenth normal does not promote rooting in our system. Again, rooting is noticeably less vigorous in NaCl-containing medium.

Regenerated plants were removed from culture vials, potted in soil, and hardened in the lab for several weeks before transfer to the greenhouse. Three groups of plants were used: (1) Plants regenerated from NaCl-sensitive cultures and watered with solutions containing no salt; (2) NaCl tolerant plants selected from cultures tolerant to 6.4 g/l NaCl, regenerated in the presence of NaCl, and watered with solutions containing the same level of salt; (3) Plants like those in group 2 but regenerated and rooted in medium containing no salt. Seeds from each group were collected and planted to obtain the F1 generation. F1 plants were potted in soil in 4 inch pots and groups were watered with various concentrations of salt water containing from 0.0 to 19.0 g/l NaCl. When it became apparent that the tolerance of whole plants was considerably higher than the tolerance of cultured cells, the concentrations of the watering solutions were adjusted upwards to contain from 0.0 to 32.8 g/l NaCl.

Results and Discussion

Plant survival rates at 13 weeks at two levels of NaCl are shown in Table 1. Seeds from the three groups of F1 plants were collected whenever possible. Flowering and seed set are considerably more sensitive to NaCl than plant survival, so seeds were not obtained from all plants. Seeds from F1 plants of the three groups watered with a salt-free solution were planted to obtain the F2. Groups of F2 plants were salt-stressed at various levels as for the F1. Data at two levels of NaCl are shown in Table 1.

Our results demonstrate persistence of tissue-culture-selected NaCl resistance in plants one and two generations beyond regeneration. Since we have been able to repeatedly select spontaneous NaCl-tolerant cell lines from cultures of 10^7 diploid cells we believe the resistance results from a dominant or co-dominant allele. Given typical eukaryotic mutation rates of 1×10^{-5} (for specific phenotypic changes [Strickberger, 1968]) to 1×10^{-9} (for changes in specific DNA bases [Vogel, 1970]), the probability of obtaining two or possibly four (since tobacco is an allotetraploid [Smith, 1968]) recessive alleles in the same cell is vanishingly small.

If a single dominant allele is responsible for the observed salt tolerance a 3:1 ratio of tolerant to sensitive plants would be expected in the F1 assuming the parent plant (regenerated from a salt-tolerant cell culture) was diploid and heterozygous. Continued self-fertilization would lead to an increasing proportion of both salt-sensitive and salt-tolerant homozygotes. This assumes that seeds for each succeeding generation were collected from non-stressed plants so that both salt-sensitive and salt-tolerant plants survived. Data of our F1 and F2 generations (Table 1) support neither this hypothesis at the 95% level nor a similar hypothesis based on a tetraploid parent.

Table 1. Survival rate under high or no salt stress of salt-tolerant and non-salt-tolerant tobacco plant lines derived from cultured cells. Plants regenerated from tissue cultures were designated the parental generation (P) and were selfed to obtain the F1 generation. The F1 plants of each group in the 0 g/l NaCl column were selfed to obtain the F2 generation. F1 had 43 plants per group. F2 had 20.

	% alive after 13 weeks			
	F1		F2	
	Watering solution		Watering solution	
	26.2 g/l NaCl	0 g/l NaCl	29.8 g/l NaCl	0 g/l NaCl
Original culture #1 resistant to NaCl with NaCl continu ally present	65	100 (selfed to produce F2	100	100
Original culture #2 resistant to NaCl with NaCl not present during regeneration	35	100 (selfed to produce F2)	90	100
Original culture #3 not resistant to NaCl	20	100 (selfed to produce F2)	15	100

Our findings that NaCl-resistant cell lines give rise to plants that transmit resistance to future generations has many potential practical implications. Incorporation of Na- tolerance is desirable in many cultivars of crop plants, particularly those grown in arid, irrigated regions with salt accumulation problems. Utilizing typical field selection techniques, salt tolerance is time and space consuming as well as expensive to cotain. The time-space compressibility and the selection specificity inherent in tissue culture techniques when used in conjunction with traditional methods should remarkably improve the efficiency of the plant breeding process.

This kind of genetic analysis probably does not apply to salt tolerance in these plants for two reasons. First, the ratio of healthy to non-healthy (or dead to alive) plants for any salt-sensitive or salt-tolerant population is dependent on (1) stress level and (2) duration of stress as well as genetic factors. Second, our method of stress selection involves application of high salt levels to populations of 10 million cells. This leads to a period of no growth followed by slow adaptation of the culture to the stress, presumably (but not necessarily) by growth and division of the few naturally occurring NaCl-resistant cells in the population. Following a resumption of normal or near normal growth rates even higher stress is applied. Thus, a stepwise selection pattern of stress application and accommodation results. Because of this pattern our mutants may well be multiallelic or multigenic and not suitable for a Mendelian-based analysis of inheritance. Backcrosses and other F1 and F2 crosses are being made to provide further data on the inheritance pattern.

Two lines of evidence indicate that phenotype persistence is in some manner dependent on or enhanced by the presence of NaCl. First, Table 1 shows that plant lines derived from NaCl-tolerant cell cultures are noticeably less tolerant if salt was omitted during the original regeneration process. This omission was made as noted earlier to improve regeneration and rooting rates. The observation could be explained if different mutations are involved in each plant line. This interpretation is supported by the fact that under salt stress the two lines have somewhat differing morphologies with one being more compact in growth habit than the other.

Second, F2 plants are noticeably more salt tolerant if seeds are collected from F1 plants that were exposed to salt (data not shown). This observation cannot be explained by different mutations since only one plant line is involved. The results could be explained in one of several ways. (1) Further selection, for pollen with even higher salt tolerance for example, could have occured in F1 plants. (2) A high reverse mutation rate could be promoted by the absence of NaCl. (3) The F1 plants could be chimerical, composed of salt-tolerant and salt-sensitive cells. Thus selection would still occur in F1 plants exposed to salt. This explanation seems unlikely to us since the salt-tolerant cell lines which gave rise to parental plants were exposed to high salt levels for almost a year prior to regeneration. After this period of selection all cells in the culture should have been salt-tolerant. (4) Both observations could be explained if the observed salt tolerance were not genetic but the result of increased levels of a particular enzyme or some other physiological adaptation maintained by the presence of NaCl. Alt et al. (1978) have reported on such a system involving duplication of a particular gene in cultured animal cell lines, although their system does not involve regenerated organisms. We consider the first and last possibilities to be most likely and thus candidates for future research.

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PROJECT DESIGN SUMMARY
LOGICAL FRAMEWORK

Project Title & Number: Utilization of Tissue Culture to Increase Food Production

NARRATIVE SUMMARY	OBJECTIVELY VERIFIABLE INDICATORS	MEANS OF VERIFICATION	IMPORTANT ASSUMPTIONS
Program Sector Goal: The broader objective to which this project contributes: 1. To improve the efficiency of food production with developing nations through increased utilization of land resources, especially by growing crop varieties more suited to the prevailing environments.	Measure of Goal Achievement: 1. Greater use of marginal lands with minimum inputs. 2. Higher crop yields in marginal lands. 3. Increased food production in the developing nations.	1. Government records. 2. Spot checking for use of areas and crop yields.	Assumptions for achieving goal targets: 1. A.I.D. and developing nations view plant breeding for stress resistance as an important alternative to energy-intensive agriculture involving environmental modification.
Project Purpose: 1. To test the use of tissue culture as a technique to add stress tolerance characteristics to wheat, rice and millet.	Conditions that will indicate purpose has been achieved: End of project status: 1. Cell cultures tolerant to the various stresses. 2. Regenerated plants from these cultures. 3. Greenhouse testing of these plants for stress tolerance and inheritability of stress resistance. 4. Field testing of the resistant plants.	1. International centers and national centers start using the tissue culture technique in their breeding programs.	Assumptions for achieving purpose: 1. Tissue culture technology is now sufficiently developed to make a practical contribution to plant breeding.
Outputs: 1. Wheat variety(s) tolerant to salt, aluminum toxicity, drought 2. Rice variety(s) tolerant to salt and aluminum toxicity. 3. Millet variety(s) tolerant to salt 4. Linkages between tissue culture labs and worldwide plant breeding personnel.	Magnitude of Outputs: 1. At least one wheat variety tolerant to salt, aluminum toxicity, drought respectively, released to IARCs and NARCs for testing and use. 2. At least one rice variety tolerant to salt and aluminum toxicity released to IARCs and NARCs. 3. At least one millet variety tolerant to salt released to IARCs and NARCs. *see 4 below.	1. Laboratory, greenhouse, and field-testing progress reports. 2. On site visits. 3. Scientific publications. 4. Verification from IARCs & NARCs.	Assumptions for achieving outputs: 1. Specific outputs and assessments of anticipated technical problems are not unsurmountable. 2. Past progress rates are indicative of future rates.
Inputs: 1. Expertise and facilities in tissue culture breeding at Colorado State University. 2. Expertise in plant breeding at the CSU. 3. Physical plants and administrative staffs at CSU. 4. Financial support from A.I.D.	Implementation Target (Type and Quantity): 1. Project approved by BAC - 1980. 2. Project funded by AID - 1980. 3. Staff and facilities in place - 1980. * 4. Linkages with CIMMYT, IRRI, ICRISAT and at least 3 national agricultural research centers (IARCs)	1. Annual reports. 2. On-site visits. 3. Technical publications. 4. Reports to interested breeders. 5. Cooperation with interested breeders.	Assumptions for providing inputs: 1. Qualified investigators and technical staff continue with project. 2. IARC personnel are interested in tissue culture input. 3. University support is forthcoming. 4. A.I.D. support and monitoring provided.

APPENDIX E

Best Available Document

NaCl-Tolerant Tobacco Plants from Cultured Cells

by

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(submitted for publication)