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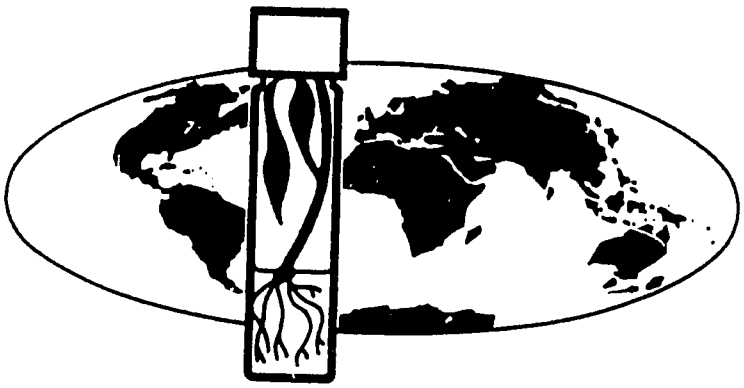
ANNUAL REPORT OF

AGRICULTURAL BIOTECHNOLOGY/PLANT TISSUE CULTURE

OCTOBER 1987 TO OCTOBER 1988

GRANT YEAR FOUR



Tissue Culture for Crops Project

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RESEARCH HIGHLIGHTS

Advancements in tissue culture techniques are the foundation of the research at the TCCP. Advancements may take the form of developing plant regeneration techniques for recalcitrant crops, developing plant regeneration techniques for new explant sources, or improving existing embryogenic callus techniques. Plant regeneration techniques for many cereals were pioneered at the TCCP in the early to mid 1980's, and have recently been developed for legumes. Expansion of culture techniques in rice, from embryogenic callus cultures derived from seed to suspension cultures and anther, have been demonstrated. Improvements in embryogenic callus cultures have been accomplished by the addition of 1% sorbitol to the media. The idea for adding sorbitol was derived from experiments in the legumes at the TCCP and is now being tested in spring wheat and sorghum.

A new idea for improving embryogenic capacity has been tested this past year. Tissue culture traits, such as the ability to form embryogenic callus, may be selected for by passing through the tissue culture process. Seeds from tissue culture derived plants were placed back into cultures. R_1 seed of rice showed a marked improvement in embryogenic callus growth. R_3 seed collected from a sorghum plant grown on acid soils showed the ability to adapt to $AlCl_3$ *in vitro* where the non-regenerate check could not. These experiments indicate that improvements in embryogenic callus growth can be made in two areas: media and genetic selection.

In vitro selection is the second corner stone of the Project. This hypothesis, on which the Project was developed, states that selection of cells on salt, aluminum or PEG would increase tolerance to saline soils, acid soils with high exchangeable aluminum, or drought conditions.

Selection for cells in vitro was addressed in early documents which noted that stress levels which would reduce callus growth by 80% to 95% of the control would be necessary for selection. In the past several years, it has been found that stress levels of greater than 70% generally lead to the death of the culture. In addition, a considerable amount of evidence indicates that callus must be grown a minimum of three passages before the effect of the stress level can be assessed.

Another important goal for the Project is to verify that tissue culture and in vitro selection has a positive effect on improving stress tolerance in the field. This area of research has increased in the past two years with the availability of tissue culture-derived germplasm. This is the third year that sorghum has been planted on an acid soil site in Georgia. This year, two lines of Hegari and one line of RTx-430 sorghum were confirmed as having an increased tolerance to acid soils by surviving in two years of the study. These lines are from either non-selected cultures or cultures selected on NaCl. In an evaluation of R_1 sorghum lines, 5% of lines derived from non-selected cultures exhibited tolerance to acid soils; between 18% and 29% of lines from aluminum-, potassium chlorate-, or hydroxyproline-selected cultures demonstrated tolerance. Potassium chlorate and hydroxyproline are agents which would select for other biochemical factors involved in stress tolerance rather than strictly the ability to tolerate aluminum. As in most environmental stresses, it is a combination of factors that must be tolerated.

A line of sorghum RTx-430 has been identified which is more tolerant than its non-regenerated parent to a combination of drought, heat, and salt present in a field test at Yuma, AZ. Additionally, a line of sorghum which is providing a great deal of excitement is the line of

Hegari which is showing tolerance to the fall armyworm. This line has been confirmed in two seasons as having increased tolerance as compared to its non-regenerated check.

Three lines which have increased drought tolerance (2075), acid soil tolerance (2021) and fall armyworm tolerance (2442) also have tolerance to a second stress. Other R_3 lines of 2075 do well on acid soils and other R_3 lines of 2021 and 2442 are among the top lines on drought. This multiple stress tolerance may have a common origin such as vigor in acid and drought situations, but may also result from very different mechanisms.

Improved tolerance in regenerated plants is not solely restricted to sorghum. Other potentials, although they are in less advanced generations than sorghum, are the rice cultivars 'Calrose' where one regenerated line survived acid soils and blast disease while the non-regenerant parent perished, and IR-36 which showed improved salt tolerance in Pakistan.

The question of the effect of <u>in vitro</u> selection can be answered in part with this year's data from sorghum. Tissue culture-derived material is superior to its non-regenerated checks for acid soil and drought tolerance. Also, a higher percentage of those lines selected on aluminum-containing media survived acid soil conditions than those from non-stressed media. More complete information is expected next year when much of the germplasm developed from <u>in vitro</u> experiments set-up expressly for field testing will be evaluated.

A great deal of effort has been put into the molecular biology project to isolate genes involved in salt tolerance. Rapid progress was made in the last six months prior to the termination of the project. Several proteins have been identified which are produced when the cells

are put under a salt pressure. Isolating the messages for these proteins would be the next step. Those messages could be developed not only to transform plants but also as a diagnostic tool to identify salt-tolerant cells or plants.

The tissue culture, molecular biology, and field research being conducted through the TCCP is exciting and rapidly moving. Partial answers to the central question of the effect of in vitro selection have been obtained and more complete data is expected in the fifth year of the Project.

RESEARCH AND DEVELOPMENT

There are three primary objectives to the research at TCCP. First is to develop plant regeneration techniques for cereals and legumes. Second is to develop cell cultures tolerant to NaCl, AlCl, and PEG and regenerate plants. Third is to verify that tissue culture and *in vitro* selection are useful tools for plant breeders worldwide concerned with saline soils, acid soils, or drought conditions.

TECHNOLOGY DEVELOPMENT FOR TISSUE CULTURE, SELECTION AND PLANT REGENERATION.

A major thrust in the early years of this Project was to develop regeneration techniques for the cereals and legumes. This was accomplished by 1987 in rice, wheat, sorghum, millet, tepary bean, pigeonpea and mothbean. New techniques, such as suspension culture of rice, were developed in 1988.

In vitro stress selection also began early in the Project with the goal of achieving plant regeneration from the highest level of stress, selected for the longest time possible. Since 1986, plants have been regenerated from a systematic set of stress levels and passage times directed toward developing germplasm for field evaluations.

This section contains reports by the staff and graduate students striving to improve plant regeneration techniques and increase the understanding of *in vitro* stress selection.

Tissue Culture of Spring Wheat

Abstract. Two types of experiments were conducted in spring wheat. These experiments are steps in the *in vitro* germplasm development process. First is determination of the selection levels appropriate for a given stress agent and cultivar. This was the goal for the aluminum and PEG research. The next step is germplasm development from selected stress levels and passage times. Since selection levels were known for NaCl selection the object was to produce plants which could provide information on the effectiveness of the *in vitro* selection.

Description of Experiments.

Aluminum. The focus of this study was to determine a critical level of $AlCl_3$ for E calli growth and plant regeneration from these cultivars. A total of 1000 mature embryos of both cultivars were initiated. The E calli produced after third passage were moved to medium containing 0, 100, 200, 300, and 400 ppm $AlCl_3$. Calli weights were recorded after every passage on stress. At the end of the sixth passage the selected E calli were transferred to regeneration media.

Experiments are currently in progress on $AlCl_3$ medium for development of germplasm from different levels of aluminum. These cultures are being maintained on their respective Al levels for nine passages, and plant regeneration will be conducted after every three passages on stress.

PEG. For this study 500 mature embryos of Pavon and Glennson were initiated on non-stressed medium and were maintained for three passages. The E calli from these cultivars were moved to media containing 0, 9, 12, 15, and 18% PEG (8000 MW) for three passages. After a total of six passages in culture the calli were transferred to regeneration medium to test for their ability to produce whole plants.

Salt. A germplasm development experiment was conducted for long-term selection on salt-containing media. Mature embryos of Pavon, Glennson, and Kharchia-65 were used. Kharchia-65 is a salt-tolerant line from India and Pavon and Glennson are CIMMYT releases that are salt-sensitive. In this experiment the E calli produced from non-stressed tissue cultures were maintained for a total of 12 passages, nine of which were on stressed medium containing 0, 4, and 8 g/l NaCl.

Results and Discussion.

Aluminum. The aluminum and PEG experiments are to determine selection levels. Based on the number of plants regenerated as percent of control (Figure 1) Glennson is less sensitive to the addition of $AlCl_3$ to the media than Pavon. Reduction of plant regeneration by 50% occurred at 100 ppm $AlCl_3$. The 200 ppm $AlCl_3$ level has been chosen as the highest selection level for further experiments.

PEG. Pavon is more tolerant to PEG than Glennson. In fact the lowest level used in this experiment, 9%, enhanced regeneration slightly for Pavon (Figure 2). Reduction of plant regeneration by 30-50% occurred at 12% PEG although callus growth was not substantially reduced. This demonstrates that callus growth and plant regeneration are both important criteria for choosing a selection level.

Salt. Critical levels of salt had been determined previously. In fact many lines had previously been regenerated from salt-selected cultures. The purpose of this experiment was to develop germplasm selected from specific stress levels and passage times. The germplasm then could be evaluated for salt tolerance and that tolerance related back to the *in vitro* selection.

Stress levels were selected for this germplasm development experiment so that at the highest level of NaCl plants could be regenerated after 12 total passages and 9 passages on NaCl.

Pavon and Kharchia are more sensitive to in vitro salt than Glennson (Figure 3). By the ninth passage on stress the high level was inhibiting plant regeneration by more than 60% of the control while on 4 g/l plant regeneration was slightly higher than the control.

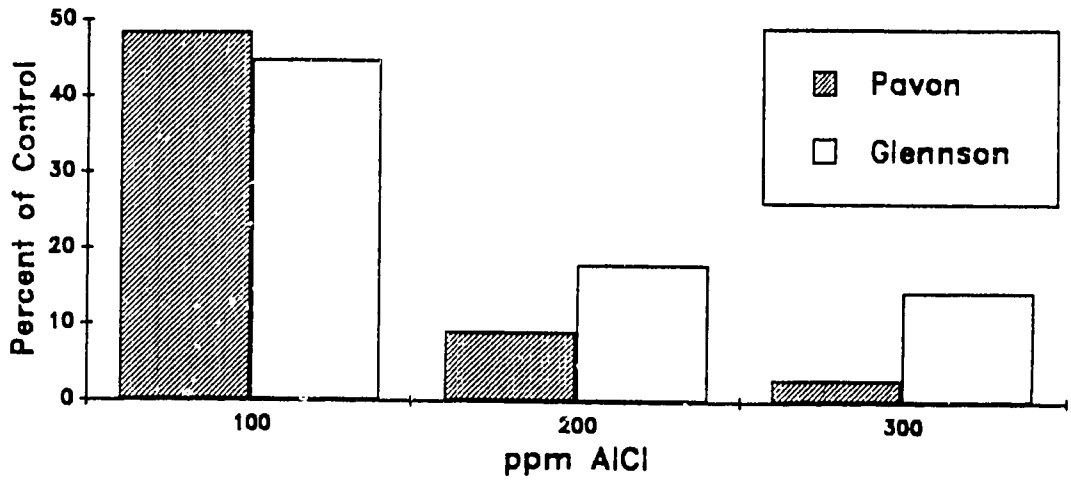


Figure 1. Number of plants regenerated as a percent of control (0 ppm AlCl) of spring wheat cultivars Pavon and Glennson.

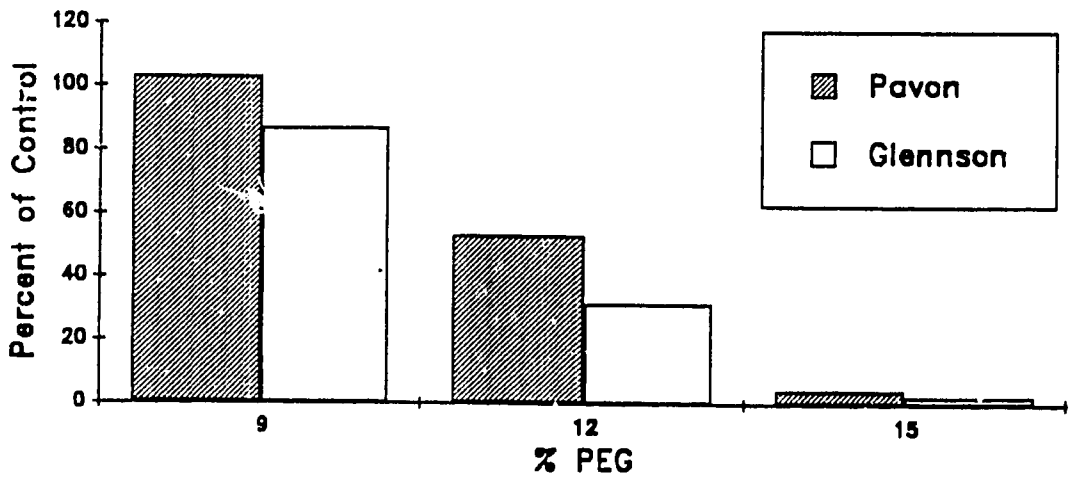


Figure 2. Number of plants regenerated as a percent of control (0% PEG) of spring wheat cultivars Pavon and Glennson.

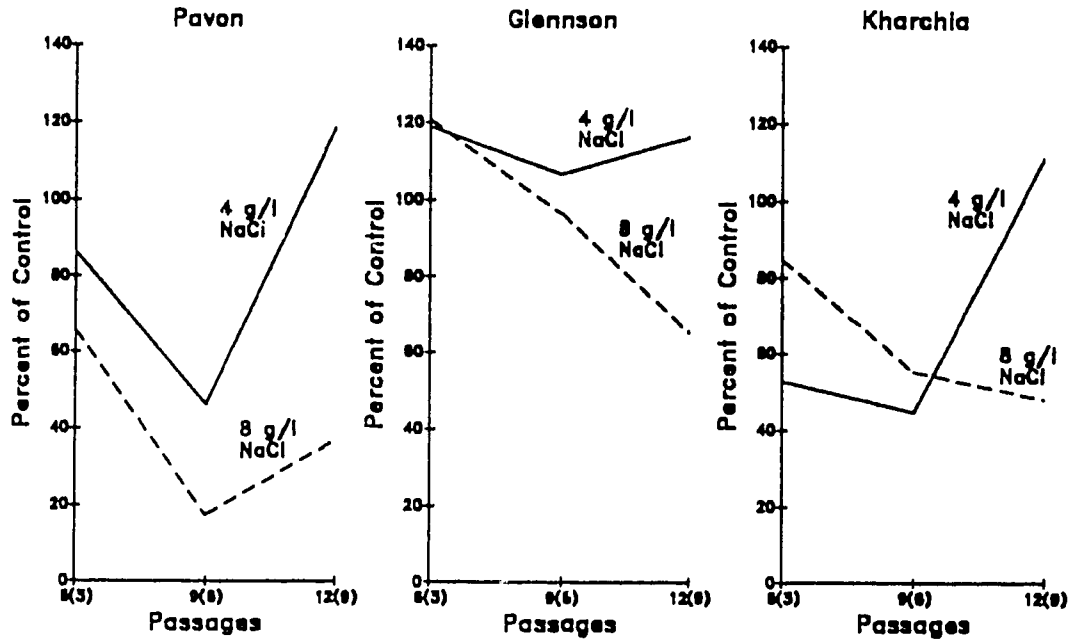


Figure 3. Number of plants regenerated as a percent of control (0% PEG) of spring wheat cultivars Pavon and Glennson.

Tissue Culture of Rice - In vitro Selection

Abstract. Rice tissue culture has been focused on two areas of research. Improving E callus initiation and growth and determining selection levels of aluminum. Improved E callus growth can be accomplished by plating R_1 seed or by the addition of 1% sorbitol to the media. Selection level experiments of AlCl are being repeated with the media containing sorbitol. In experiments without sorbitol, 60 ppm AlCl is very high for IR-36 while 225 ppm is a high level for Giza-159.

Description of Experiments. To test if R_1 seed had a greater ability to produce E callus, R_1 seeds from two plants were put back into tissue culture. The regenerated lines were from previous salt selection experiments. One line, R1A, came from a non-stressed culture, and another line, R1J, came from a culture selected on 9 g/l NaCl.

In a second experiment, 1 or 2% sorbitol was added to the media using established E callus of Giza-159 at tenth passage. Cultures were moved to regeneration medium following the third passage of sorbitol.

Critical level experiments were conducted for both IR-36 and Giza-159 for aluminum tolerance. Embryogenic callus of IR-36 was transferred to media containing 0, 60, 80, 100, and 120 ppm AlCl. Embryogenic callus of Giza-159 was moved to media with 0, 75, 125, 175, 225 and 275 ppm AlCl. After three or four passages, E callus was transferred to regeneration media.

Results and Discussion. By careful selection of E callus, a cell line that can be continued for extremely long periods is occasionally isolated. In February 1982, leaf tissue of Mahsuri was initiated. Those cultures are still producing E callus and occasionally a regenerated plant after 72 passages in culture. A cell line of IR-36 initiated in June 1984 from mature seed has been producing an average of 20 plants per gram of E callus over the past 12 passages. This cell line has been maintained for 47 passages.

Genotypic differences for the ability to form E callus have been reported in several plant species at the TCCP and has been observed in rice. By the fourth passage, out of 100 seeds initiated from IR-36 or Giza-159, 64 or 35 cultures, respectively, will typically be identified which contain E callus. The number of cultures with E callus continue to moderate so that by seventh passage there will be 20 cultures of IR-36 left and the number of Giza will have remained relatively constant. This is a selection process which identifies embryogenic cell lines with the ability to perform in tissue culture. Therefore, in Giza, these cell lines call be identified by fourth passage whereas in IR-36 it would take three more passages.

One possible way to increase the number of cell lines as well as the E callus weight may be to culture seed from regenerated plants. The percent of seed forming E callus did not increase although the mean E callus weight at the end of the third passage increased from 0.37 g/cell line for the non-regenerated parent to 0.49 g/cell line for both regenerated lines combined. The E callus weight distribution (Figure 4) indicates that, from the regenerated lines, there are cell lines which will produce between 1.0 and 1.4 g of E callus per passage as compared to less than 1.0 g for the parent. Plant regeneration was 6 times higher in the regenerated lines as compared to the non-regenerated control.

Another factor being investigated to increase E callus growth is the addition of sorbitol to the medium. Embryogenic callus weights are higher from the sorbitol treatment over the three passages of the study (Figure 5). The E callus from 2%, although significantly more than 0 or 1% sorbitol, was of poor quality and did not regenerate while the 1% sorbitol regenerated more plants than the control. After finding that sorbitol was a significant factor for E callus maintenance, sorbitol was tested for E callus formation. Embryogenic callus growth was significantly improved in Giza-159. At the end of third passage, there was an average of 0.44 g/culture for 1% sorbitol and 0.18 g/culture without sorbitol. In IR-36, E callus weight of the control was 0.06 g/culture and 0.19 g/culture on 1% sorbitol after the second passage. Cultures of IR-36 require less sucrose than Giza-159 but respond equally well to 1% sorbitol indicating that the sorbitol and sucrose have different functions. The sorbitol effect has been reported by Dr. Nguyen Thanh-Tuyen at the Visayas State College of Agriculture (ViSCA), Philippines (personal communication, 1988) where plant regeneration was enhanced with 3% sorbitol.

Critical levels for AlCl. The rice cultivar IR-36 is much more sensitive to AlCl than is Giza-159. At 60 ppm AlCl, IR-36 callus declines dramatically over three passages on stress (Figure 6) while by third passage on 275 ppm Al, the Giza-159 E callus grows as well as the control. Critical level experiments are being repeated with the new-found knowledge of the effect of sorbitol on callus growth.

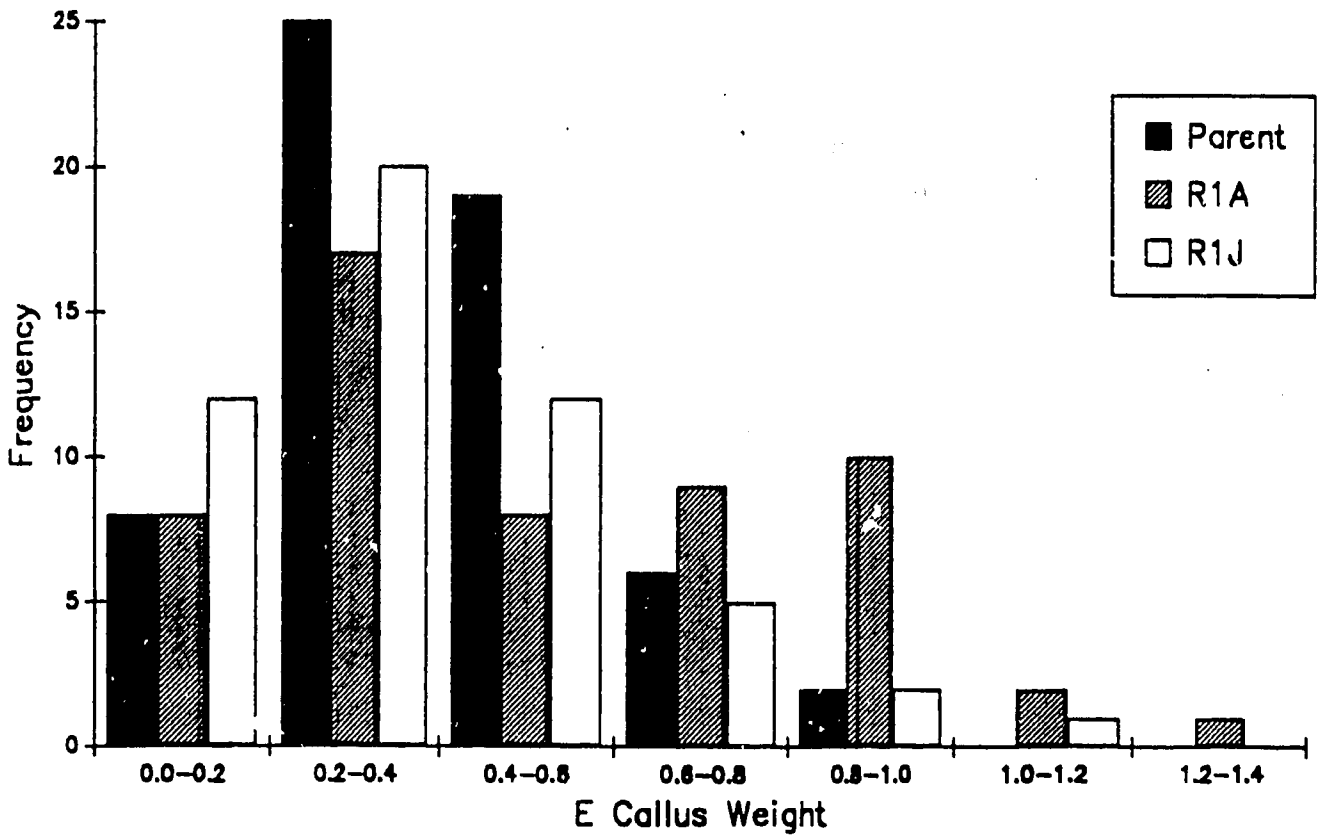


Figure 4. Frequency distribution of embryogenic callus weights. Distribution of E callus weights showing a near-normal curve for the non-regenerated seed (parent) and skewed curves for the two regenerated lines.

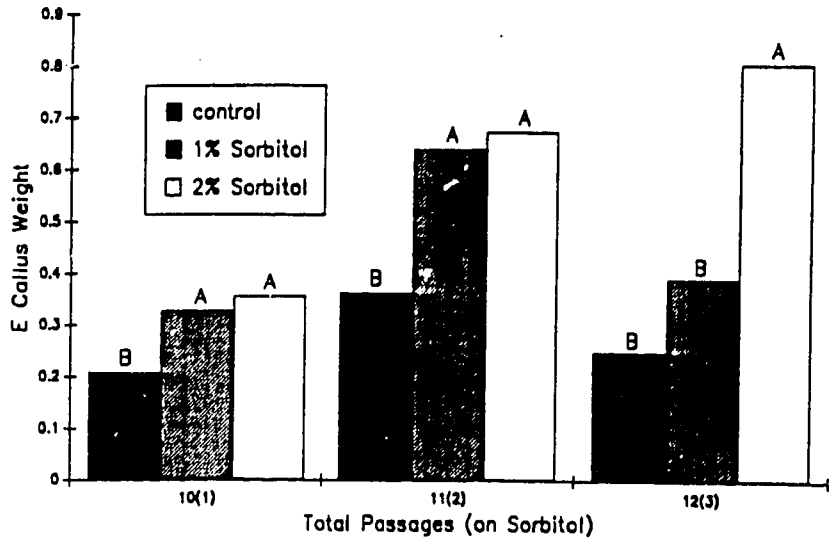


Figure 5. Effect of sorbitol on maintaining E callus of Giza-159. Calli from ninth passage were moved to media containing either 0, 1 or 2% sorbitol.

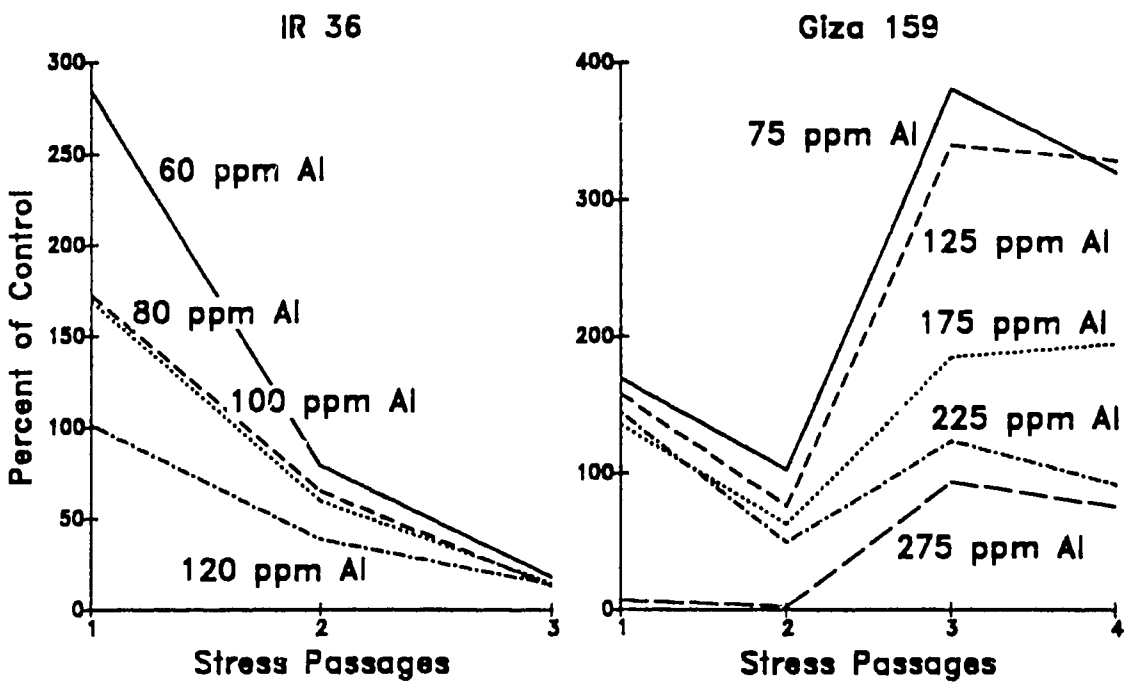


Figure 6. Effect of AlCl on E callus as a percent of control for two rice cultivars.

Tissue Culture of Rice - Suspension Cultures

Abstract. Mature embryos of *Oryza sativa* L. cv Taipei 309 cultured in LS medium supplemented with 2.5 ppm 2,4-D produced pale-yellow, compact, nodular E calli. These calli were subcultured into liquid AA medium supplemented with 2.5 ppm 2,4-D to establish suspension cultures. The cultures were composed of large, elongated and highly-vacuolated cells, and a population of highly-condensed cytoplasmic cells. The highly-condensed cytoplasmic cells appeared in aggregates and single cells while the elongated cells were found as single cells only. Structures resembling embryogenic development were found with highly-condensed cytoplasmic cells. When plated on solid LS medium with or without hormones, these embryoid-like structures differentiated into plantlets. One line has maintained the ability to regenerate after 16 months in suspension.

Materials and Methods. Dehusked rice seeds were surface sterilized in a 20% Clorox solution for 30 minutes and rinsed thoroughly with sterile deionized water. They were then cultured in LS medium supplemented with 2.5 ppm 2,4-D, 4% sucrose, and 8% agar (pH=5.5). Cultures were maintained in the dark at 25±2° C.

Four-week-old calli were used for initiating cell suspension cultures. Calli were initially grown in 10 ml of liquid medium using the same medium used for callus initiation in 50 ml Erlenmeyer flasks. Cultures were agitated at 120 rpm on a gyratory shaker in the dark at 25±2° C. In the early stages of culture establishment, the medium was replaced at weekly intervals. Once cells started dividing, cultures were transferred to 125 ml flasks. Subcultures were made every two weeks by replacing one half of the original medium with AA medium supplemented with 2.5 ppm 2,4-D and 4% sucrose (pH=5.5).

After 3.5, 4.5, 5, 6, 6.5, 10, 10.5, 13.5, and 16 months in suspension cultures, clumps bigger than 500 um were cultured in petri dishes containing solid LS medium without or with 0.5 ppm BA and 0.05 ppm NAA. Cultures were incubated in continuous light at 25±2° C. Regenerated plants were transferred to jars using the same medium from which they originated. They were grown to maturity in the greenhouse.

Results and Discussion. After inoculating calli in liquid medium for four weeks, the cultures contained a mixture of callus clumps, cell clusters, and single cells. The cells formed were either round or elongated. Round cells were rich in cytoplasm. These cells divided and formed small aggregates. They also showed the competence for embryogenesis. Elongated cells appeared vacuolated and large in size and were less competent for embryogenesis. When the medium was replaced with AA medium, round cells divided and resulted in various stages of embryoid formation, such as globular and heart stages (Figure 7A).

Cell clumps bigger than 500 um (Figure 7B) were transferred to solid medium and either turned brown or divided and formed yellowish E calli which gave rise to plants. Some of the yellowish calli formed roots and turned brown, but in some cultures green spots occurred after 2-3 weeks (Figure 7C). These green spots remained green or differentiated into roots or plantlets (Figure 7D).

Three hundred and fifty plants were regenerated from suspension cultures. The number of regenerants decreased after 10 months in suspension. Albino plants were also observed. Regenerants are being grown to maturity in the greenhouse. Sixty-five R_1 somaclones which had matured and been harvested were sent to LSU's Rice Research Station for seed increase and to observe for somaclonal variation. The rest of the somaclones will be increased and observed in the next growing season.

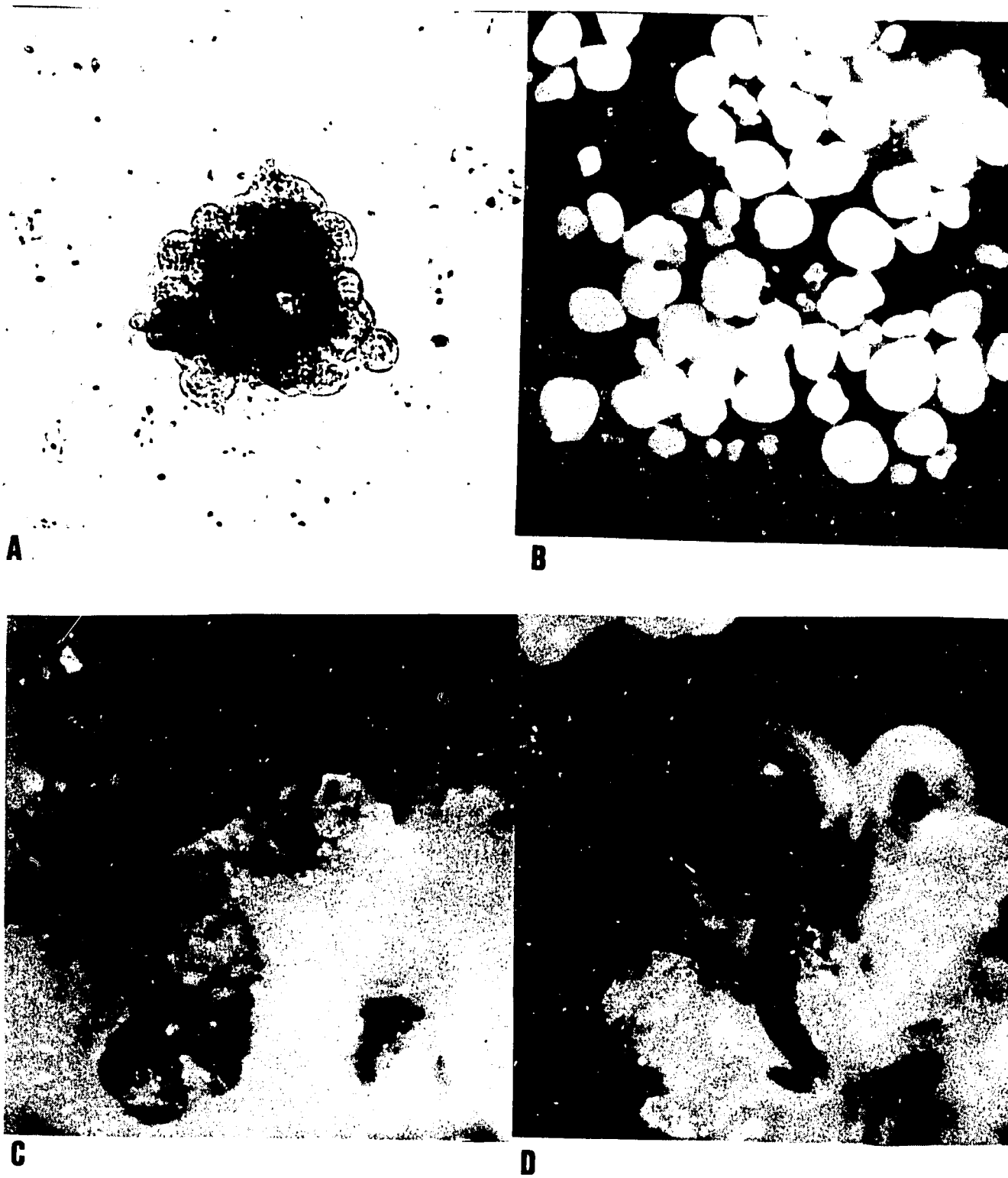


Figure 7. Stages in plant regeneration from suspension cultures of rice. A) Early cell division forming small embryogenic mass. B) Cell clumps larger than 500 um transferred to solid medium. C) Green spots developing on embryogenic callus. D) Shoot formation.

Tissue Culture of Rice - Media Factors

Abstract. Various factors influence the formation and growth of embryogenic callus. The use of a buffer, 5 mM sodium citrate and 5 mM citrate acid or 10 mM sodium citrate enhances E callus growth. Small inocular rates reduce the formation of NE callus. Addition of iron or organic compounds reduces E callus while additional amounts of the trace elements enhances E callus production.

Materials and Methods.

Media Buffers. Mature seeds of IR-36 were dehusked, surface sterilized with 70% commercial Clorox for 30 minutes, rinsed three times with sterile deionized water and plated singly on 10 ml of solidified LS medium. The medium was modified by varying the pH from 5.5 (control) to 4.9 with or without the addition of 10 mM MES, 10 mM sodium citrate, or a combination of 5 mM sodium citrate and 5 mM citrate acid. Embryogenic callus was weighed after 28 days. Calli from the control, 10 mM MES, and 10 mM sodium citrate were maintained on their respective maintenance media for an additional 28 days.

Inoculum Size. Embryogenic callus was inoculated onto LS medium at the rates of 5.5, 6.5 or 7.5 mg/l media. Embryogenic and non-embryogenic calli were weighed and the pH determined after 28 days.

Medium Composition. Major constituents of the medium were modified in these experiments to enhance the effect on E callus formation. In the first experiment the iron, organics (inositol and vitamins) or the trace elements were added at 2 or 3 times the normal concentration. In the second experiment, various types of nitrogen were tested. The basic LS medium is approximately two thirds nitrate, one third ammonium based on the molarity. In this experiment the ratio was reversed, equalled, or equal to the amount of nitrogen was supplied by ammonia acids .

Results and Discussion.

Media Buffers. In the first experiment, 10 mM MES or sodium citrate was added to the basic LS medium. Neither addition enhanced E callus growth during the first passage (Figure 8A). When the callus was maintained for an additional passage, the sodium citrate did enhance the growth of E callus (Figure 8B). The ratio of NE to E for sodium citrate remained equal to the control (Figure 8C).

In a second experiment, lowering the pH in the initiation media or addition of a combination of 5 mM sodium citrate and 5 mM citrate acid enhanced E callus production.

Inoculum Size. Varying the inoculum size between 5.5 and 7.5 did not effect the amount of E callus (Figure 9). It did cause a difference in the amount of NE callus and therefore the ratio between NE and E callus. Less inoculum had less NE callus produced after 28 days. Also the 5.5 mg/ml inoculum had a pH lower than when larger inoculum were used. There are other factors involved besides pH since the pH when 7.5 mg/ml inoculum is used leads to an abundance of NE while the same pH is observed when 10 mM sodium citrate is used which gives very little NE callus. One possible explanation may be that the loss of ions used for callus growth creates the change in pH.

Medium Composition. Additions of iron and the organic reduced E callus production (Figure 10A). The trace elements improved E callus production two and three times. Nitrogen in the form of ammonium or amino acids (Organic N) is not good at inducing E callus.

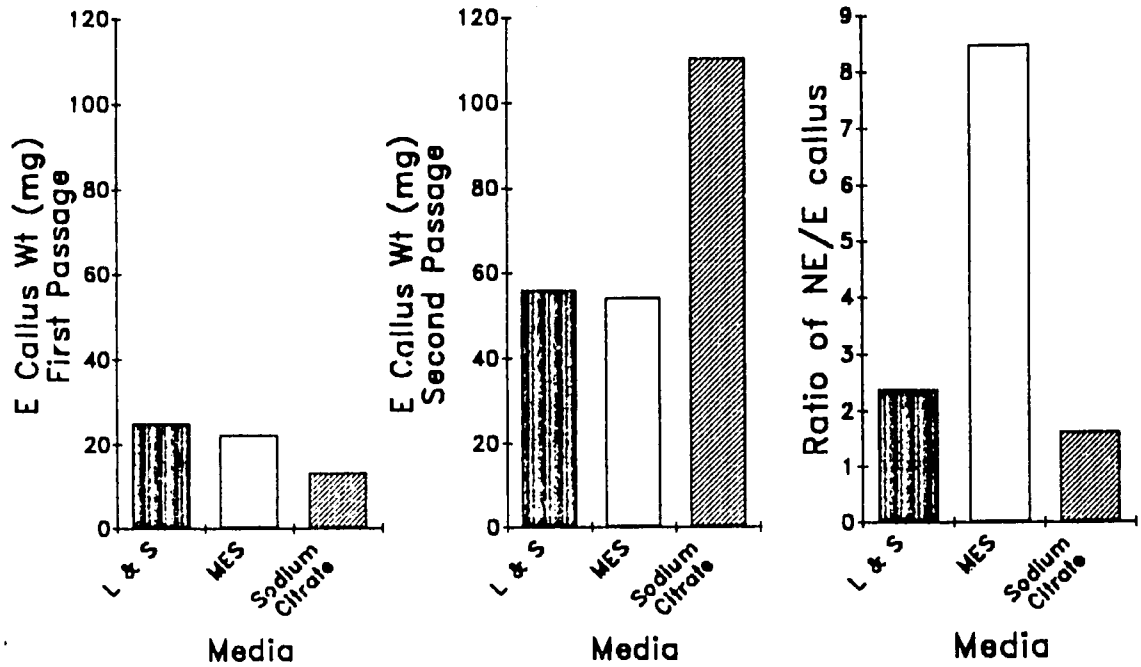


Figure 8. Effect of buffer is the media on embryogenic callus weight and the ratio of NE to E callus.

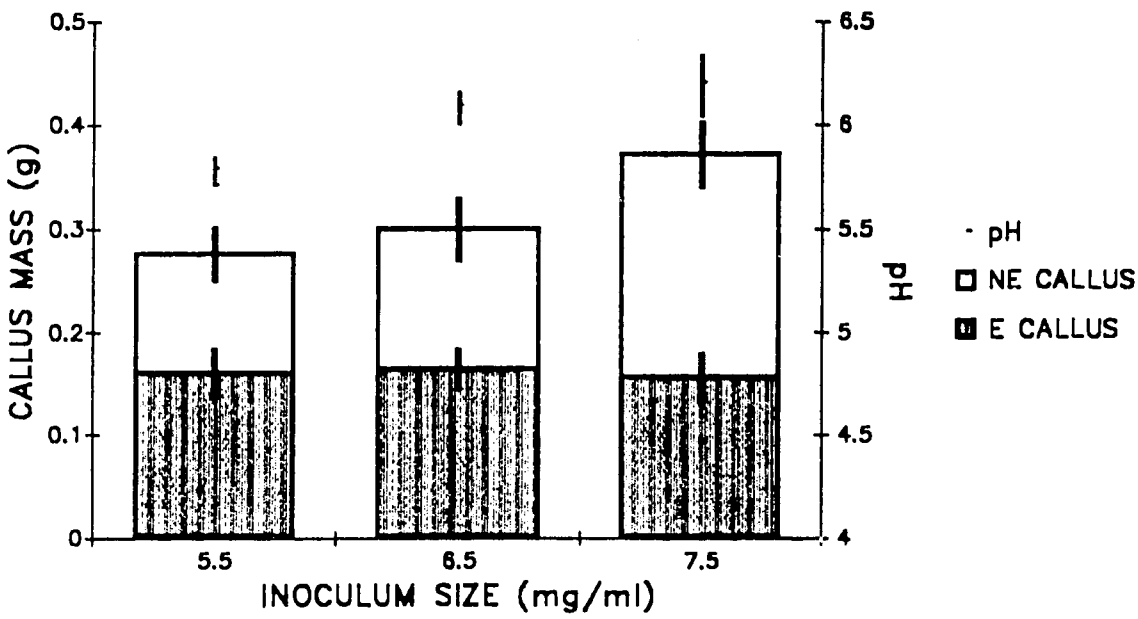


Figure 9. Callus growth and pH at different inoculum rates.

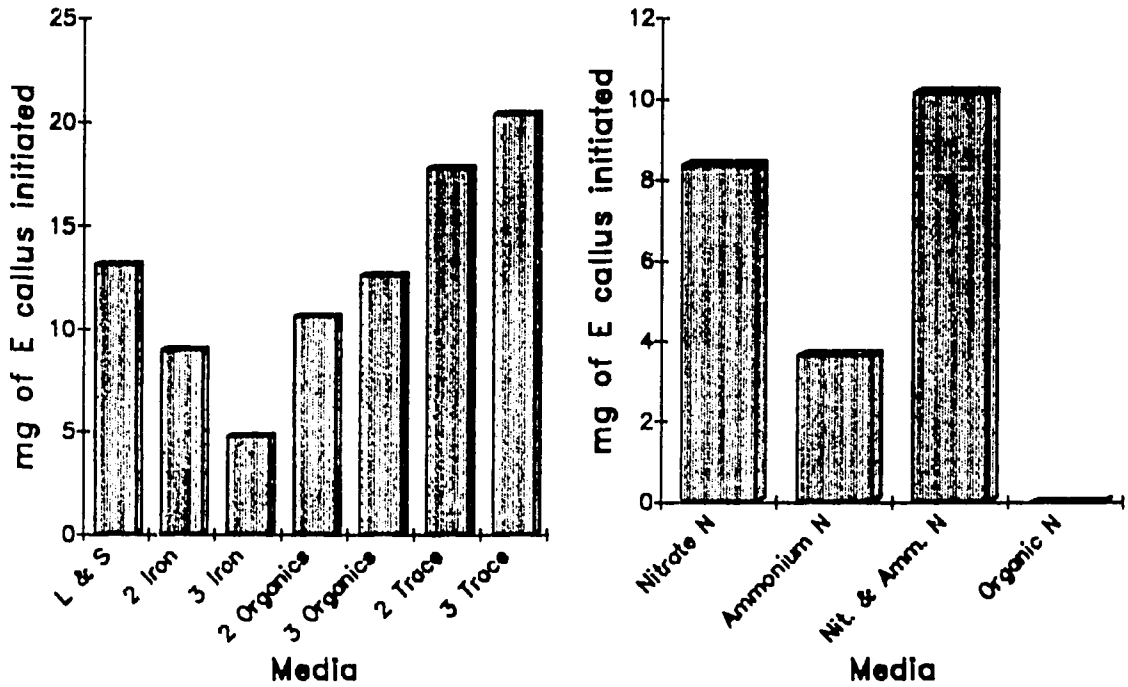


Figure 10. Effect of different nutrient levels and nitrogen source.

Tissue Culture of Rice - Anther Culture

Abstract. Earlier observations that japonica types of rice were more amenable for anther culture than indica types were confirmed in experiments conducted this year. Media study showed that B5 or AA media works equally well for callus initiation. It was also possible to induce callus initiation from anthers of cultivars Yamahoushi and Calrose plated on media containing 0.5% and 0.75% NaCl.

Materials and Methods. Five rice varieties—IR-36, IR-60 (indica types), Mahsuri (indica x japonica hybrid), Calrose and Yamahoushi (japonica types)—were grown under greenhouse conditions from February to August 1988. The temperature was 24±2° C and the relative humidity was 65±5%. Yamahoushi and Calrose produced an inflorescence by 90 days, IR-36 and IR-60 by 110 days, and Mahsuri by 130 days.

Anthers at the uninucleate stage were identified using an Olympus BH-T microscope with a Nomarski optics. Florets bearing anthers at uninucleate stage were then surface sterilized for five minutes using 100% commercial Clorox and rinsed three times in sterile deionized water. Thirty-six anthers were plated on 10 ml of solid callus induction medium contained in a vial, and were incubated at 23±2° C in the dark. Callus induction medium, unless otherwise stated, consisted of 2 ppm NAA, 0.5 ppm 2,4-D and 1 ppm KIN.

Five media, N6, 2AA, B5, AA and LS, were tested for the ability to induce callus formation. Incorporated in this experiment was a test of light plating procedures. Anthers were plated after 0, 1, 3, 4, and 6 days of panicle storage in water at room temperature, or plated immediately after a heat treatment for 2.5 or 5 hours, or plated the next day after a 5-hour heat treatment.

Results. Calli were observed on 10.72% of the Yamahoushi anthers plated, a higher percentage than Calrose, Mahsuri, IR-60 and IR-36 (Figure 11). These results reaffirm that japonica cultivars produce anthers that are more amenable to tissue culture techniques than indica cultivars.

In general, the B5 or AA media induced the greatest percentage of anthers to produce callus (Table 1). An enhanced level of callus initiation was observed when the anthers were plated on the next day, and it was possible to elicit a similar result by subjecting the anthers to head shock for 2.5 hours (Table 1).

The results of the experiment in which anthers were plated on media containing different levels of NaCl is depicted in Figure 12. Anthers that were plated on media containing NaCl at levels of 1.0% and above

failed to initiate callus. It is hypothesized that microspores that contained the gene(s) to resist NaCl had given rise to these calli and the others that did not possess the gene(s) were selected against.

About 60 plants were regenerated from anther-derived callus belonging to the cultivar Yamahoushi and grown in the greenhouse. These plants were all haploid and no albinos were observed. This has not been the case in most of the anther culture experiments performed by others as a considerable percentage of albinos and plants with different ploidy levels are usually produced.

Table 1. Effect of media and plating technique on callusing response of rice anther for Yamahoushi and Calrose.

Treatment*	Cutting	N6	2AA	B5	AA	LS
a	Yamahoushi	11.94	5.67	11.00	11.78	10.00
	Calrose	4.86	3.70	18.52	8.33	5.56
b	Yamahoushi	18.75	7.41	20.37	23.15	6.48
	Calrose	6.94	8.33	5.56	8.36	5.56
c	Yamahoushi	-	-	-	-	-
	Calrose	9.72	2.78	8.33	0.0	4.17
d	Yamahoushi	-	-	-	-	-
	Calrose	8.33	0.0	8.33	4.18	1.39
e	Yamahoushi	-	-	-	-	-
	Calrose	0.0	-	-	-	0.0
f	Yamahoushi	16.67	9.72	20.83	23.61	4.17
	Calrose	-	-	-	-	-
g	Yamahoushi	0.0	1.39	1.39	4.17	1.39
	Calrose	-	-	-	-	-
h	Yamahoushi	-	-	-	-	-
	Calrose	-	16.67	11.11	2.78	-

* Treatments
a. plated same day
b. plated next day, panicles stored in water at room temperature
c. plated the third day, panicles stored in water at room temperature
d. plated the fourth day, panicles stored in water at room temperature
e. plated the sixth day, panicles stored in water at room temperature
f. 32° C for 2.5 hours, plated same day
g. 32° C for 5.0 hours, plated same day
h. 32° C for 5.0 hours, plated next day, panicles stored in water at room temperature

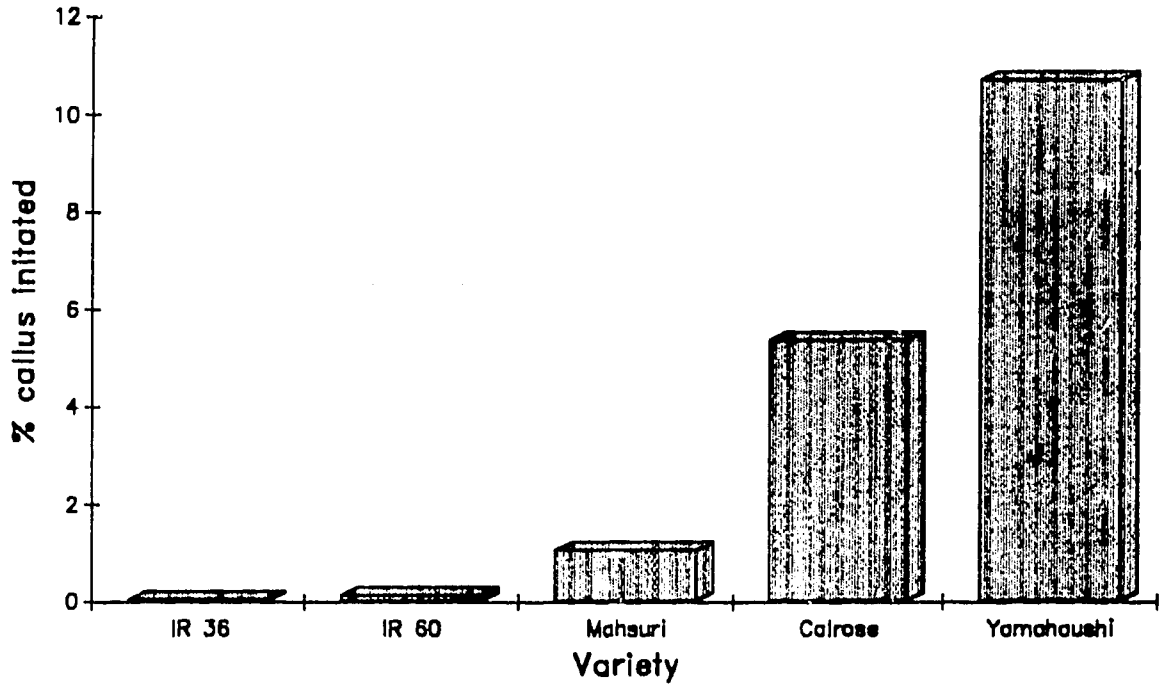


Figure 11. Callus induction response from anthers of five cultivar of rice.

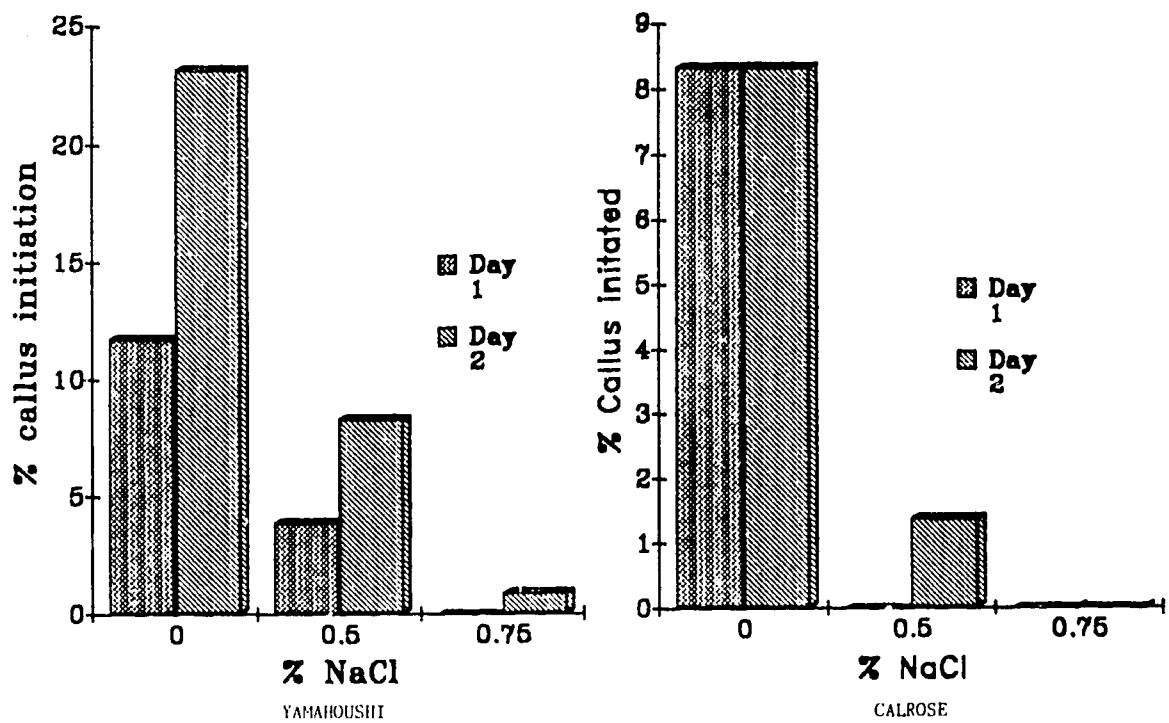


Figure 12. Effect of NaCl on callus initiation of rice anthers.

Tissue Culture of Rice - Selection for Increased Nitrate Reductase Activity

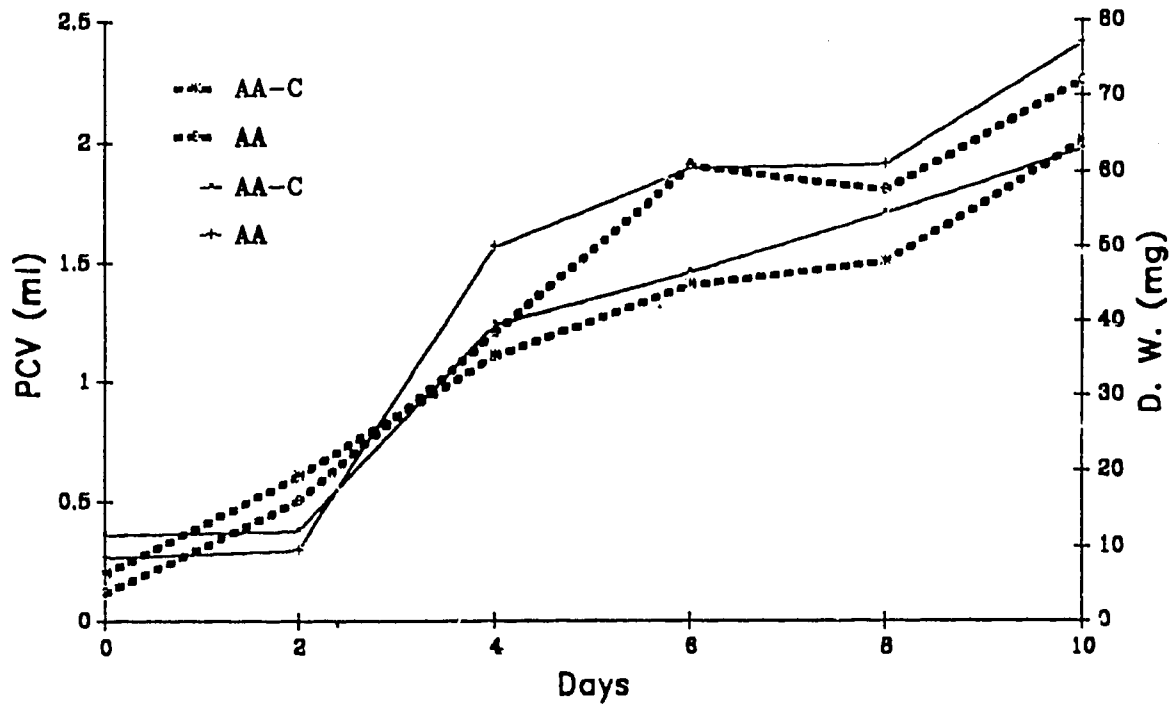
Abstract. Rice variants (cv IR-36) with increased nitrate reductase activity and efficient substrate utilization are being investigated using tissue culture techniques. One approach is through the use of chlorate which is a specific inhibitor of nitrate reductase. In callus culture, IR-36 cells survived only up to 50 ppm chlorate whereas in cell suspension they tolerate much greater levels. Nitrate reductase activity proves to be greater in the presence of chlorate which indicates an altered substrate binding activity.

Description of Experiments. Cell selection techniques for obtaining rice variants with increased nitrate reductase activity and more efficient substrate utilization are being developed. The problem is being approached in two ways. First, cells which continuously grow in abnormally low nitrate levels might have nitrate reductase with increased activity. Results from the first year of research showed that IR-36 callus cultures can grow in nitrate levels that support 15% of the normal growth. Plants were regenerated from callus grown at this low nitrate level and are intended for whole plant assay. Secondly, cells which grow in the presence of a specific inhibitor like chlorate might have nitrate reductase with altered substrate binding activity. Cells which are able to reduce chlorate produce chlorite toxic to cells, inducing mutants without nitrate reductase. The few cells that survive selection are then subjected to the lowest nitrate level they can tolerate to check for increased nitrate reductase activity.

Results and Discussion. In callus culture, IR-36 cells have survived chlorate levels of up to 50 ppm for five passages. Plants were regenerated at this level. Cell suspension cultures survived up to 100

ppm chlorate but no plants were regenerated. They are presently being subjected to increasing chlorate levels to check changes in NR activity.

Figure 13 shows the growth pattern of an IR-36 cell suspension culture in relation to its nitrate reductase activity. The cell suspension was initiated and maintained in amino acid medium. Cells in the presence of chlorate (AA-c) have shown lesser increases in dry weight (DW) and packed cell volume (PCV) but greater increases in the amount of nitrite formed over the control (AA). When the cells were transferred to LS medium, the growth patterns of the treated (LS-C) remained superior to the control (LS) (Figure 14). There was a high initial increase in NR activity of the control which dropped on the eight day. In the presence of chlorate, NR activity had periodic increases until the tenth day. Visual observations also revealed browning cell clumps in the control media. Cell suspension cultures are now growing in 200 ppm chlorate.



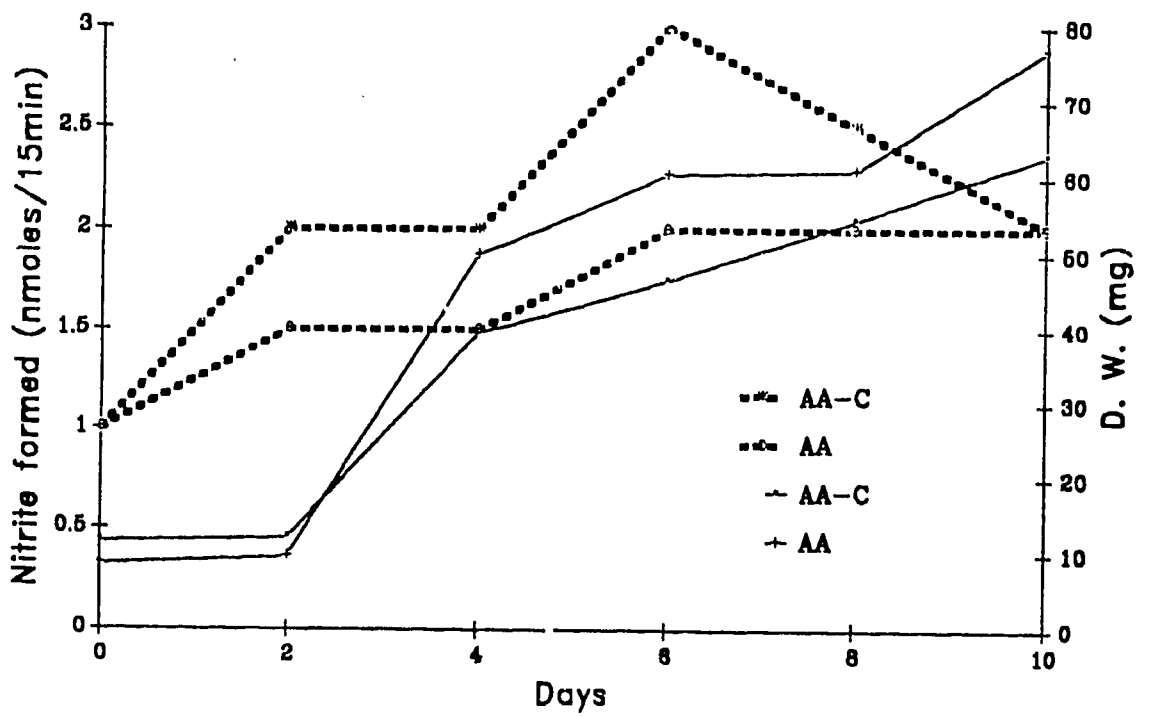


Figure 13. Growth pattern and change in nitrate reductase activity of rice IR-36 cultured in amino acid medium with (AA-C) or without (AA) chlorate at 100 ppm.

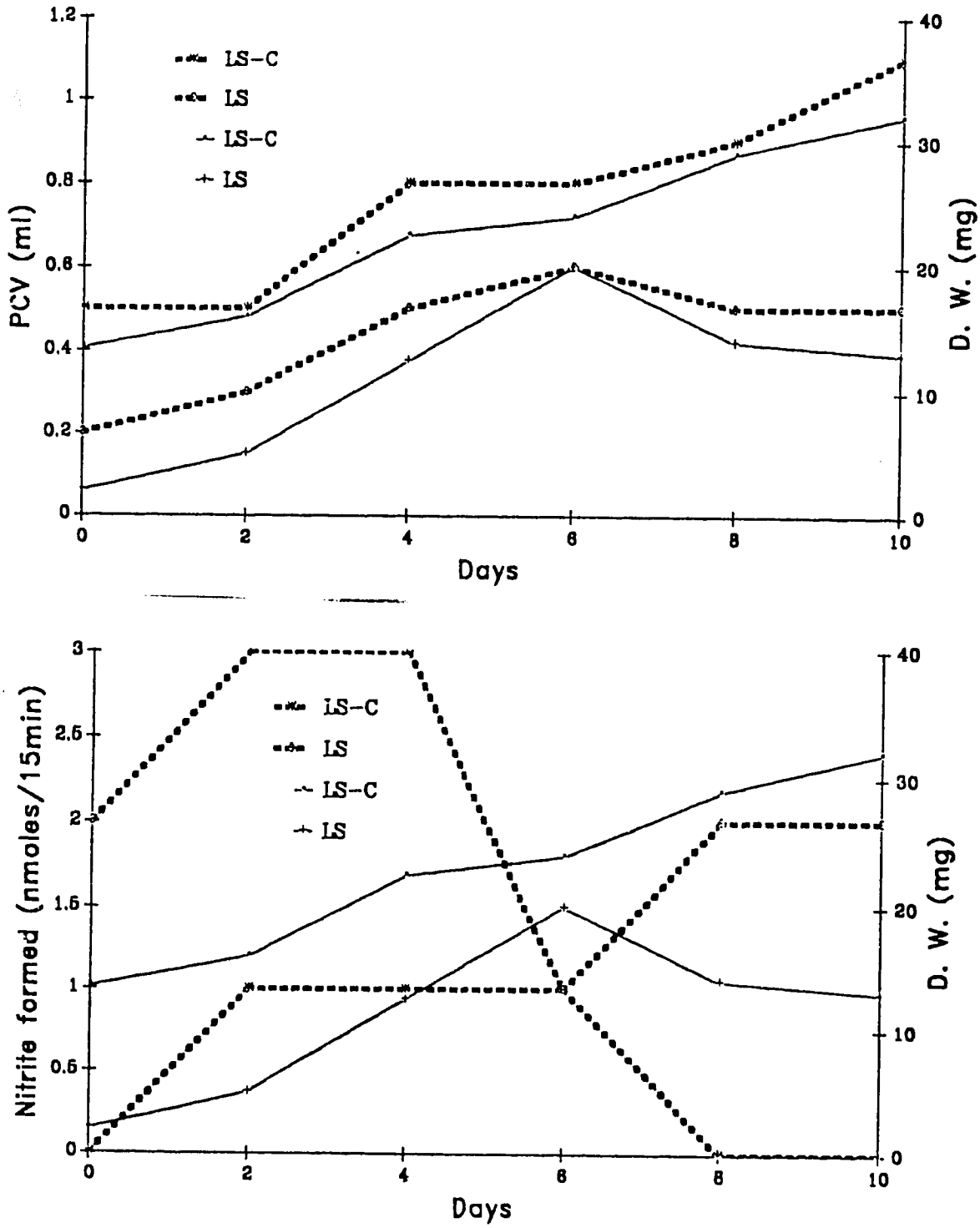


Figure 14. Growth pattern and change in nitrate reductase activity of rice cells (cv. IR 36) cultured in LS medium with (LS-C) or without (LS) chlorate at 100 ppm.

Tissue Culture of Millet

Abstract. Millet tissue culture has proceeded in two areas in the past year: 1) *in vitro* selection for salt and PEG for the open pollinating cultivars of HMP-559 and Senegal Bulk, and 2) development of a regeneration system for an inbred line, Tift-23 DBE, used in more breeding programs than the open-pollinating types.

Description of Experiments.

Tift media study. Callus cultures were initiated from mature caryopses on LS medium supplemented with 1.1% agar and 4% sucrose and were tested on nine media combinations utilizing 3, 5 and 7 mg/l 2,4-D and 1, 2, and 3 mg/l IAA. All media contained .3 mg/L KIN for callus induction and maintenance. In addition, IAA and BAP combinations were studied as media components for plant regeneration.

Mature seeds were initiated and maintained on the nine media for two passages. Following second and subsequent passages, NE callus was dissected away from various other callus types and discarded. Embryogenic (E) callus fresh weights from each culture vessel were determined by placing the E callus on a petri plate and weighing with a Mettler analytical balance.

Salt. Callus cultures were initiated and maintained for seven passages before sufficient E callus was obtained to begin the stress selection. Salt levels of 0, 6, and 8 g/l NaCl were the treatments for both HMP-559 and Sengal Bulk. Embryogenic callus was moved to regeneration media every third passage.

PEG. Mature embryos were initiated and callus maintained for four passages. After the fourth passage, embryogenic callus was moved to media containing 0, 5, 8, 11, 13, 17 or 20% PEG with .45% Gel-Gro® in place of the 1% agar. Media with PEG above 10% will not solidify with

agar but will with Gel-Gro®. All cultures were moved to regeneration media after 5 passages on stress.

Results and Discussion.

Tift Media Study. At the end of the first subculture, embryoids were virtually non-existent, making it difficult to isolate and weigh the callus pieces. Therefore, further growth of callus formation during first passage was allowed and the entire mass of callus, minus shoots and caryopses, were transferred onto fresh media. Non-embryogenic callus growth was rapid during the second subculture and was dissected away and discarded for the third transfer. Embryoids and various other callus types were obvious following the third transfer. Yellow, hard, nodular, compact callus was often the most prevalent callus type formed followed by the production of a green, hard, compact, nodular, type. In most cases the "true" embryogenic, creamy, white, compact, callus was the least amount formed. All three callus types were weighed by cell line. Although E callus growth fluctuated over time, it did proliferate and was maintained on several media combinations for five or more passages (Figure 15).

For the production of whole plants, E callus with surrounding NE callus completely removed was subcultured on regeneration media. An average amount of 0.1 gm of callus was placed on 20 mls on LS media plus combinations of IAA and BA or BA alone to allow formation of shoots. When shoots reached a height of 1 1/2 to 2 inches, they were placed on media containing 3 mg/L IBA, allowing formation of a complete plantlet. Regenerates with an established root system were then transplanted to a soil-less mix and placed in the greenhouse for seed increase.

Salt. This experiment was set-up to develop germplasm from specific *in vitro* treatments. The number of cultures and E callus weights were observed (Figure 16). The number of cultures declined without stress being imposed although the decline from 6 and 8 g/l NaCl was greater. Germplasm development experiments started with an unequal number of cultures to handle this rapid decline and that the callus under stress will regenerate loss. By observing the embryogenic callus weights, HMP-559 is less sensitive to NaCl *in vitro* than Sengal Bulk. The HMP callus on 6 g/l NaCl recovered and grew better than the control for the 7th and 8th passages.

PEG. The first step for *in vitro* selection is to determine the selection levels. Adequate stress must be applied without causing the death of the callus. Levels up to 13% PEG for HMP 559 would be useful selection levels (Figure 17), but higher levels would not allow for long-term culture.

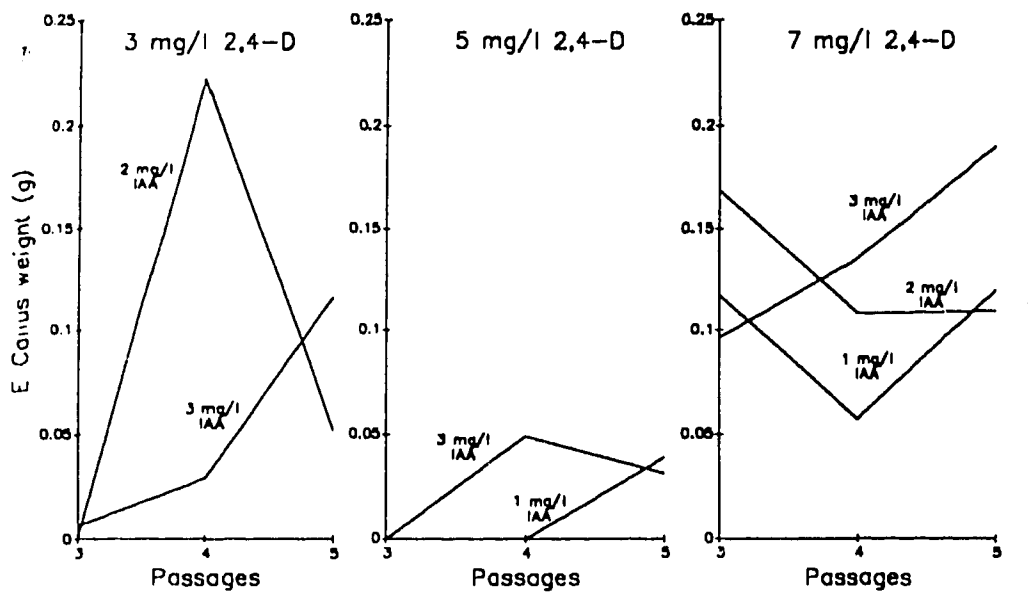


Figure 15. Effect of 2,4-D and IAA on E callus growth of millet cultivar Tift 23 DBE.

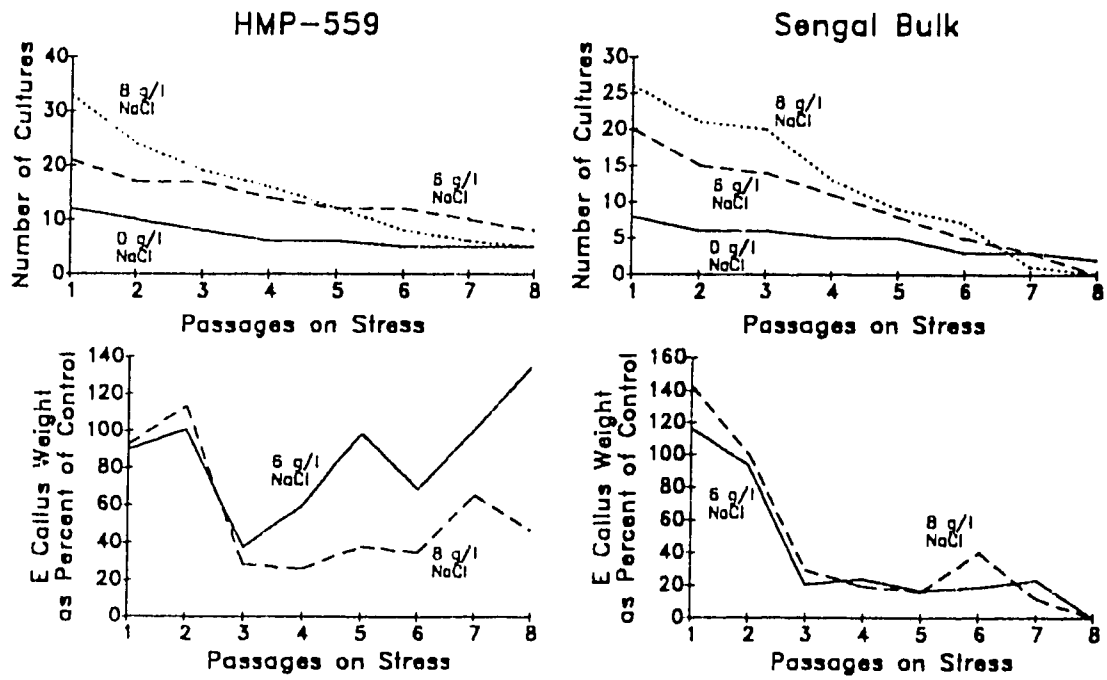


Figure 16. Effect of NaCl on number of cultures and E callus fresh weight as percent of control for the millet cultivars HMP 559 and Sengal Bulk. Embryogenic callus from seventh passage was used to initiate the salt evaluation.

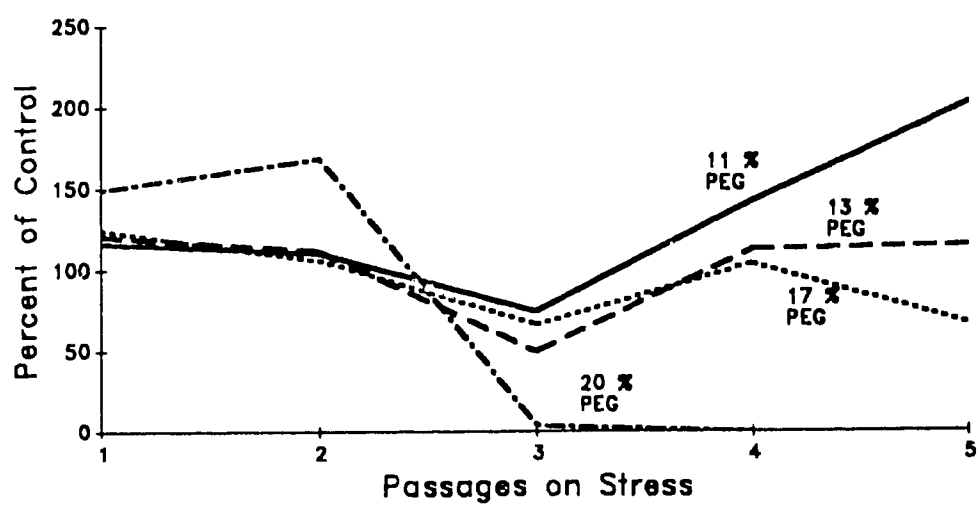


Figure 17. Embryogenic callus fresh weight on PEG containing media as percent of control of millet cultivar HMP-559.

Tissue Culture of Sorghum

Abstract. In 1987, two new lines were brought into the program. One line, Sc 283, is highly tolerant to acid soil, and the other, M-35, is drought-tolerant. In 1988, Sc-283 was tested *in vitro* on AlCl and M-35 on PEG. It appears that 75 ppm AlCl is a good selection level for Sc-283; higher levels do not allow for adaptation and produce death of the callus. For M-35, none of the levels tested allow for recovery of callus growth. In a 1987 field trial, one plant grew remarkably well on acid soil. Seed from this plant were tested as to its ability in tissue culture and its *in vitro* tolerance to AlCl. Seed from the R_2 plant growing on acid soil showed a better ability to adapt to *in vitro* AlCl stress than did non-regenerated seed.

Description of Experiments.

Aluminum Selection Levels. Embryogenic callus of Sc-283 was moved to media containing 0, 75, 150, 225, and 300 ppm AlCl. Embryogenic callus weights were taken for three passages on stress. Callus was transferred to regeneration media after the third passage.

PEG Selection Levels. Embryogenic callus of M-35 was moved to 0, 9, 12 and 15% PEG and weighed after each passage. Callus was moved to regeneration media after the third and sixth passages.

Testing Regenerated Seed. Seed from a plant (2024-7-19) derived from tissue culture was grown on the acid soil at Griffin, Georgia. This seed, as well as non-regenerate seed, was placed into tissue culture. Embryogenic callus of both lines was placed on 0 and 200 ppm AlCl. Embryogenic callus weights were taken for three passages on stress then moved to regeneration media.

Results and Discussion.

Aluminum Selection Levels. The genotype Sc-283 exhibits acid soil tolerance in the field. Our goal was to evaluate its tolerance to AlCl *in vitro* and to regenerate plants with varying morphology. Stress levels of 150 to 300 ppm limits callus growth. Only the 75 ppm AlCl level has a reduction in E callus weight and then an increase demonstrating adaptation to the stress (Figure 18). Plants have been recovered from all treatments except the 300 ppm level.

PEG Selection Levels. The line M-35 is a genotype developed by Dr. Charles Sullivan at Nebraska for drought tolerance. We have obtained the line to evaluate its *in vitro* tolerance to PEG and to see if the regenerated plants have an increased tolerance to drought or new mechanisms for drought tolerance.

The levels of 12 and 15% PEG reduced callus growth from the initial passage on stress (Figure 19). The 9% PEG treatment enhances callus growth for three passages before restricting callus growth. By the sixth passage callus growth on all levels was less than 20% of the control. Plants have been obtained from the third passage on stress from all treatments. Callus has again been placed onto regeneration media after the sixth passage but there has not been sufficient time for shoots to appear.

Testing Regenerated Seed. The E callus weight of the regenerated line (denoted as 2024) was similar to the Hegari. The regenerated seed was able to adapt to the stress while the Hegari could not (Figure 20). Shoots have been obtained from 2024 placed on 200 ppm Al but not from Hegari on 200 ppm Al.

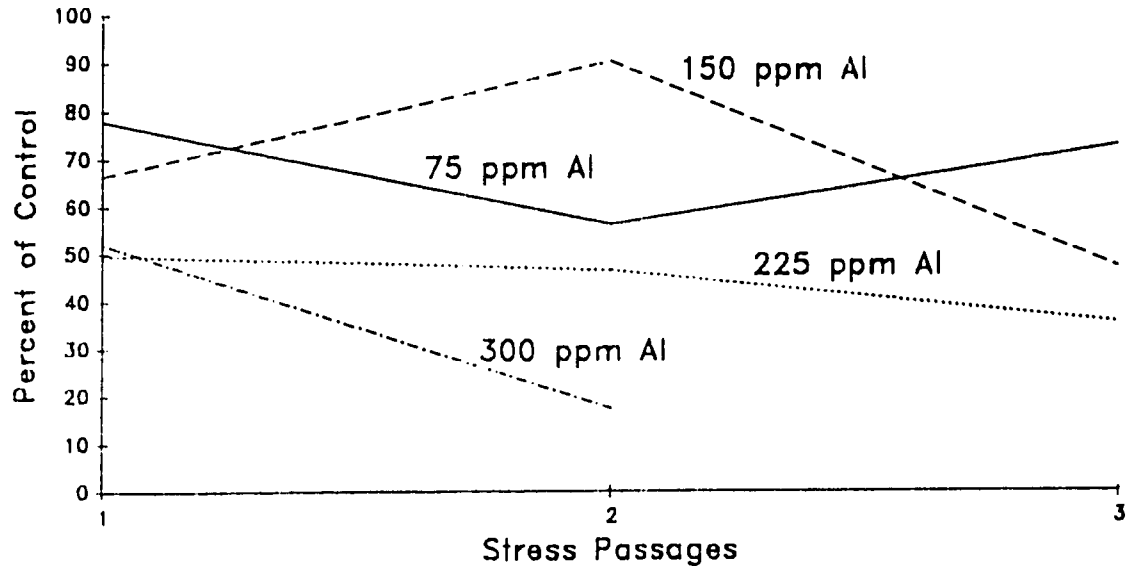


Figure 18. Effect of AlCl on E callus growth as percent of control for the sorghum cultivar Sc-283.

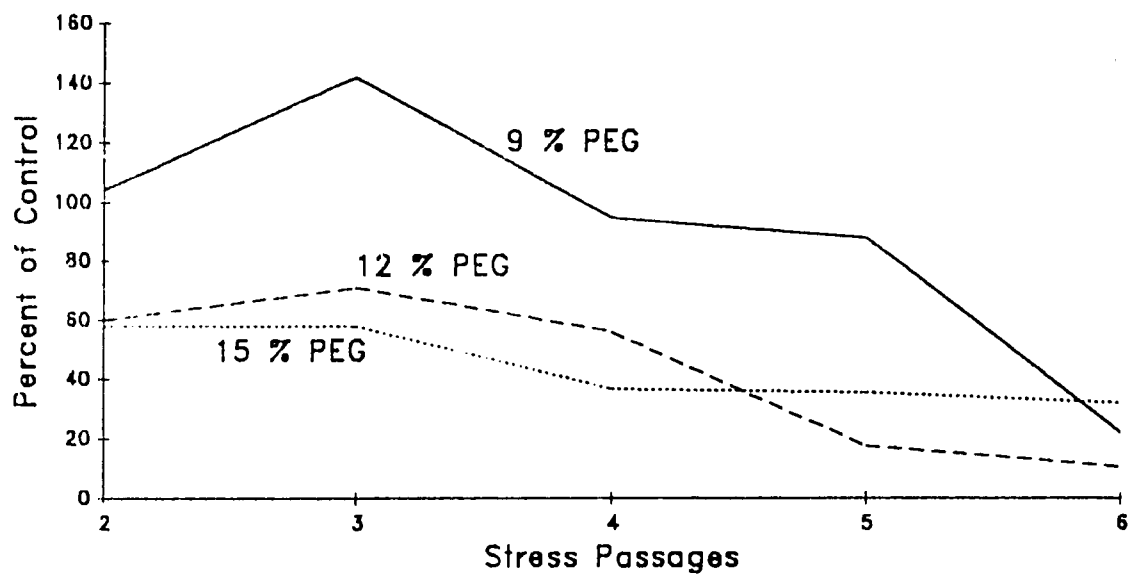


Figure 19. Effect of PEG on E callus growth of the sorghum variety M-35.

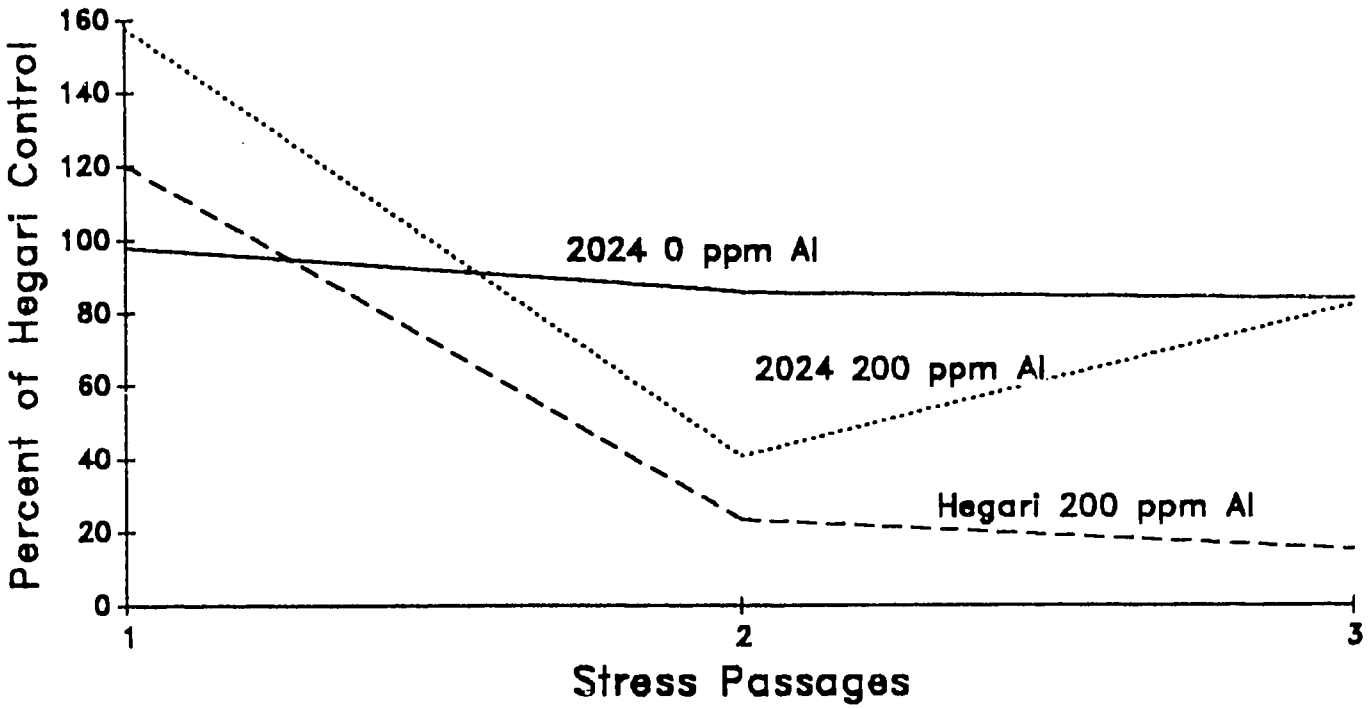


Figure 20. Effect of AlCl on Hegari and an R_3 Hegari-derived line harvested from acid soil.

Tissue Culture of Legumes

Abstract. Increasing salinity of soil and water threatens agriculture in arid and semi-arid regions of the world (Epstein *et al*). Recent advances in genetics and biotechnology offers the possibility of developing salt tolerant crops, which, in conjunction with environmental manipulation, could improve agriculture production in saline regions of the world. After establishing plant regenerating cell cultures from three grain legumes, experiments were continued to select for salt-tolerant cell lines.

Cell lines of *Vigna* and tepary beans were obtained which have been adapted to grow in the L-6 media with 1.0 and 2.0% NaCl. Salt-tolerant cell lines exhibited enhanced growth expressed as fresh and dry weight

increases in saline medium compared to non-selected cell lines. Tolerant cell lines were regenerated into plants on agar media with 20 gm/l NaCl, while none were produced from non-selected cells plated on L-6 agar medium with similar concentrations of salt. Toxic effects of salinity on cell growth and differentiation of Vigna aconitifolia were more pronounced in the presence of $NaSO_4$ and mixed salts compared to similar concentrations of NaCl. A total of 250 plants of Vigna and 90 plants of tepary bean were transferred to the greenhouse.

Efforts were made to isolate $AlCl_3$ adopted cell lines of tepary beans. The toxicity of Al was evaluated under different pH regimes. Packed cell volume and dry weight reduction was maximum at pH 4.5 with 100 mg/l $AlCl_3$ when compared to controls. The decrease in growth rate was more pronounced when the medium was buffered with MES at pH 4.5. After growing in 100 mg/l of $AlCl_2$ for seven passages with approximately 50 cell generations, a green plant regenerable cell line was recovered.

In pigeonpea, a plant-regenerating cell suspension was developed by initiating the suspension in modified L-6 medium with different concentrations of PEG. The plant-regenerating ability of the PP-987 cell line increased significantly when the cells were grown in 10% PEG in L-6 medium. The cell line retained its regenerating ability for 9 passages when maintained in modified L-6 medium with 50 gm/l of PEG. Several regenerated plants of pigeonpea were grown to maturity in the greenhouse.

Experimental Methodology. In last year's annual report we reported the procedures for the development and maintenance of cell suspension cultures capable of plant regeneration in three different grain legumes including Vigna, tepary bean and pigeonpea. To develop a salt-tolerant cell line of Vigna, the VA-986 cell line was cultured in L-6 medium with 20 gm/l of NaCl for two weeks. Green, surviving cells were recovered by

growing the brown, stressed cells in salt-free medium for two weeks. The recovered cells were then transferred to L-6 medium containing 20 g/l NaCl. A salt-tolerant cell line (VA 986ST) was isolated by repeating the above procedure four times. The resulting tolerant cell line was maintained in L-6 medium with 10 and 20 gm/l of NaCl, respectively. Similar procedures were used to develop tolerant cell lines in tepary bean; however, the stability of the salt-tolerant trait was lost repeatedly in the TB-986 cell line. Measurements of PCV, dry weight and regenerability of the adopted and non-adopted cell lines were made by transferring exponentially growing cells of VA-986C and VA-986ST cell lines in L-6 medium with 0, 10 and 20 gm/l of NaCl, respectively. Tolerance was evaluated by determining fresh and dry weight of cells in media with different concentrations of salt and by plating the cells at the end of the growth cycle on agar regeneration medium with and without salt. The tolerance of VA-986ST cell line to equal concentrations of NaCl, $NaSO_4$ and mixed salts (containing 1M NaCl, 0.3M $CaCl_2$ and 0.2M $MgCl_2$) were determined by growing the cells in 0, 10 and 20 gm/l of different salts in L-6 maintenance medium. The long-term regenerability of the control and tolerant cell lines were monitored by plating cell lines on regeneration medium once in every three weeks. The regenerated plantlets were transferred to pots containing vermiculite and soil mixtures and grown to maturity in the greenhouse.

To isolate Al-tolerant cell lines of tepary bean, the TB-986 and TB-1087 cell lines were cultured in L-6 maintenance medium with 0, 50, 100 and 200 mg/l of $AlCl_2$ with four pH regimes (4.5, 5.0, 5.5 and 6.0) with and without buffering. Ten mM MES was used for buffering. At the

end of the three weeks the cells were subcultured and data collected on PCV and dry weights.

In pigeonpea, cell suspension cultures were initiated by inoculating the seven-day old leaf explants in modified L-6 medium with 10 mg/l 2,4-D. The suspensions were then filtered through 500 um sieve and cells collected were transferred to L-6 medium with 50, 100 and 200 gm/l of PEG. After three subcultures, the cells were plated on solid L-6 medium with 2.25 mg/l BA, 0.5 mg/l NAA and 0.25 mg/l GA_3. The plants regenerated after four to six weeks were established in the greenhouse.

Results and Discussion. A salt-tolerant cell line was obtained in Vigna in 18 - 20 weeks, after that the cell line was maintained in saline L-6 medium with 10 and 20 gm/l of NaCl. Although no significant change was observed in the lag phase between the tolerant and the original cell lines, significant differences were noticed between the cell lines in their rate of growth. The cell doubling time was approximately two days for both the cell lines in control medium, while it was approximately six and two days for control and tolerant cell lines, respectively, in L-6 medium with 10 gm/l of NaCl. Growth of the non-selected cell line was almost negligible for twelve days in 2% NaCl medium, while the tolerant cell line grew with a four-day cell doubling time in L-6 medium containing 20 gm/l of NaCl. A similar pattern was observed for packed cell volume and dry weight.

The tolerant cell line was able to regenerate plants even on regeneration medium with 20 gm/l NaCl while the non-selected cell line did not produce any plants. An average of 11.7 and 3.5 plants per gram were regenerated from the tolerant cell line on 10 and 20 gm/l NaCl-containing L-6 medium, respectively. While only 2.6 and 0 plants were produced from control cell line.

Plant regeneration ability of both the control and tolerant cell lines regenerated on normal media remained relatively constant for 30 passages (about 30 months); however, the regeneration ability of the tolerant cell line decreased slightly during that period.

In addition to NaCl, the saline soils in different parts of the world contain either $NaSO_4$ or combinations of several salts at toxic concentrations. An experiment was conducted to compare the growth of the NaCl-tolerant cell line to different kinds of salts. The toxic effects on cell growth and plant regeneration were found to be higher for $NaSO_4$ followed by mixed salts. The reduction in dry weight increase was 52% for $NaSO_4$ and 48% for mixed salts compared to control, whereas reduction in cell dry weight gain was only 25% for NaCl. Similar observations were also made for plant-regenerating ability of the tolerant cell line. This type of response might be due to the fact that the cell line under investigation was selected for NaCl tolerance. Plants regenerated from different treatments were grown to maturity in the greenhouse. The R_1 seeds were multiplied at Fort Collins, Department of Agronomy Farm, Colorado State University. The R_2 will be screened in salt stress effected soils.

In tepary bean, the adopted cell line grew faster in saline L-6 maintenance medium compared to the non-selected control. The tolerance of the selected cell line, however, was lost repeatedly after eight to nine subcultures. After maintaining the cell line for eight to nine subcultures, or approximately 60 to 70 cell generations, salt inhibited growth and regenerability of the cell line. Several dozen plants were regenerated from the cells grown in saline medium for 50-60 cell generations and were grown to maturity in the greenhouse. The R_2 plants will be screened on the stressed soils of CIAT, Cali, Colombia.

To select for an Al-tolerant cell line, the TB-986 and TB-1087 cell lines were cultured in the L-6 maintenance medium with different concentrations of $AlCl_3$. A linear decrease in dry weight of TB-986 and TB-1087 was observed with increasing concentrations of Al. Growth of these two cell lines was completely inhibited at 400 mg/l of $AlCl_3$ in the maintenance medium. The proportion of densely-cytoplasmic and isodiametric cells decreased with increasing concentrations of Al. The proportion of highly-vacuolated and elongated cells was highest at 200 mg/l of $AlCl_3$.

An experiment was conducted to determine the effect of pH of the medium on Al toxicity of the tepary bean cell cultures. The toxicity was maximum when the pH of the medium was buffered at 4.5 with MES compared to the cells grown at the same Al concentration in unbuffered media. The MES was not toxic at pH 4.5. The inhibition was 58% at 100 mg/l of $AlCl_3$ when the growth medium was buffered at 4.5. In the unbuffered medium with the same initial pH and Al concentration, the dry weight reduction was only 32%. Highly significant differences were observed among Al-containing maintenance media with pH 4.5, 5.0, 5.5 and 6.0 buffered with MES, while, no such differences were observed among the unbuffered media with initial pH of 4.5, 5.0, 5.5 and 6.0. A green, $AlCl_3$-tolerant cell line of TB-1087 was isolated and is being cultured for regeneration.

In pigeonpea, a plant-regenerating cell suspension line was developed using PEG. Initial efforts to develop such a system were not successful. The cell lines lost plant-regenerating capability within two to three subcultures. The addition of PEG in the initiation and maintenance medium not only enhanced regeneration by 27.5%, but also retained the regenerability for 9-10 passages. Of the different

concentrations of PEG tested, 50 gm/l was optimal. The plant-regenerating cell suspensions are being used to develop a salt-tolerant cell line. The regenerated plants were grown to maturity in the greenhouse. The R_2 plants regenerated from the callus grown on salt and salt-free media are being tested at ICRISAT both in field trials and in greenhouse hydroponic screening.

COLLABORATIVE RESEARCH

A strong component of the TCCP is the training of graduate students and visiting scientists as well as providing small grants to other labs to strengthen their capabilities in tissue culture. This section contains reports from this broad area.

Salt-Tolerant Rice Through Protoplast Fusion

Abstract. The research is a collaborative program with NIAB in Pakistan and is now limited to activities at NIAB. Dr. Yusuf Zafar, supported by USAID/Pakistan, returned in December 1987 to NIAB after one year at TCCP. The suspension cultures which he established are being maintained at TCCP. Samples of both rice and kallar grass cultures have been sent to NIAB. Dr. Zafar is concentrating on developing the necessary protoplast technology for rice and kallar grass.

Results and Discussion.

Tissue Culture. Experiments were carried-out to confirm the earlier results of regeneration of basmati rice (B-370) in N_6-medium. The LS medium with 1.0 mg/L 2,4-D and 0.25 mg/L KIN most commonly used at TCCP for different cultivars of rice was included for comparison. Eighty-five to ninety percent callusing was obtained from the cut end of excised embryos of rice in N_6 and LS media. Regeneration experiments were carried out on eight- to ten-week-old callus. Embryogenic callus was placed on the earlier-described N_6 and LS media with 0.5 mg/L IAA and 0.25 mg/L BA. A higher rate of regeneration was obtained in LS than in N_6 medium. The callus initiation and growth was poor in kallar grass. In order to study the reasons for the low response of callusing, experiments were initiated by using different sources of seeds collected

from three locations at different seasons. The results showed that seeds gradually lose the potential for callus formation during storage.

Cell Suspensions. Experiments were continued to confirm the plant regeneration from the newly-developed cell suspension of rice (RB-187). A multistep procedure of transferring calli originating from cell suspensions to different media was adopted. Plant regeneration was observed in some plates. This technique, however, needs to be further improved.

Attempts to get regeneration from the kallar grass cell suspension (KG-187) were unsuccessful. Only roots were developed in some plates. A cell line grown on 2% NaCl (KG 587-S) was established.

Protoplast Work. Techniques were established earlier for the isolation of protoplasts from the cell line of RB-187. In spite of using various methods of culturing, cell division in isolated protoplasts was not achieved. Attempts were made to culture the protoplasts in 25 ml and 50 ml flasks containing protoplast culture medium. After two days, the flasks were placed on a shaker at a slow rate of 20 rpm. Frequent observations of protoplasts were carried out under microscopes. This technique was not successful.

Tissue Culture Technology for Chickpea Breeding

The chickpea tissue culture program is a collaborative project between ICRISAT and TCCP. Ms. Sheila Vijayakumar from ICRISAT was at TCCP from April 1987 to March 1988 and was supported by ICRISAT. She is a member of the chickpea breeding program of Dr. H. A. van Rheenen at ICRISAT. The objectives of the program were:

1. To develop a reliable procedure for rapid clonal propagation of elite lines of chickpea.
2. To develop embryo culture methods for interspecific crosses.
3. To develop a plant regeneration procedure for chickpea.

The research performed was compiled in a report entitled, "Chickpea Tissue Culture Program" by V. K. Sheila, O. L. Gamborg, H. A. van Rheenen, and Y. L. Nene. The following is a summary of the report:

1. Research in tissue culture was conducted at TCCP with ten genotypes of chickpea and four accessions of wild Cicer species.
2. Several techniques were adopted and attempts were made to define the conditions for methods that have application in chickpea breeding.
3. Shoot cuttings of chickpea genotypes and wild Cicer species produced roots efficiently in rooting media containing NAA and IBA, respectively.
4. Clonal propagation from shoot tip explants was efficient in L-6 medium containing 2.0 mg/l BA and 0.5 mg/l IAA.
5. Attempts to cross wild Cicer species with chickpea genotypes were unsuccessful; however, a medium was developed to culture immature embryos of chickpea.
6. Embryo-like structures were obtained from leaflet and cotyledon callus initiated on MS and L-6 media containing 2,4-D.
7. Plantlets of K-850 and C. judaicum (No. 185) were produced from immature cotyledon callus.
8. Cell suspensions were initiated from leaf callus in MS medium containing 2,4-D and cell aggregates were produced.

The research is continuing at ICRISAT where Ms. Sheila Vijayakumar is now a member of the Biotechnology Group at ICRISAT.

Tissue culture for genetic improvement of bean (Phaseolus spp)

Abstract. The research is part of a collaboration between CIAT and TCCP and has been performed by Mr. Paul Chavarriaga, a research scientist from CIAT. The program comprises the transfer of the plant regeneration technology for grain legumes developed at TCCP. Mr. Chavarriaga was at TCCP from April 4 to September 30, 1988. The objective of the research was to apply the methods of plant regeneration of tepary bean (*Phaseolus acutifolius*), developed at TCCP and define the conditions which are necessary for achieving plant regeneration in common bean (*P. vulgaris*) cultivars.

Research in bean tissue culture was conducted at TCCP with three genotypes of *P. vulgaris* and one of *P. acutifolius*. The seeds were germinated on L-6 basal medium. Callus was initiated on L-6 basal medium with hormone composition varied according to the purpose of the experiment. Different explants from seedlings were used to produce callus. Forty different cell suspension cultures of both species were originally initiated from callus or directly from explants. This number was reduced to four *P. vulgaris* cell lines and one of *P. acutifolius*. The process of filtration was carried out using 500 um and 250 um filters. Cells above both sizes were cultured separately in the same initiation medium. The cultures were transferred to maintenance medium after a minimum of three filtrations. Cell suspensions were cultured in PEG to enhance plant regeneration using three different cell lines of *P. vulgaris*. Cultures of both species were transferred to embryo induction medium after six to seven filtrations. Plating was carried out after eight to nine filtrations. Fourteen regeneration media were tested for *P. vulgaris* cell lines and three for *P. acutifolius*. Plants were regenerated from *P. acutifolius* cultures.

Materials and Methods.

Plant Sources. Four genotypes of *P. vulgaris* and one of *P. acutifolius* representing a wide genetic and geographic diversity were selected for this study (Table 2).

Table 2: Selected genotypes used in the research.

Species	Cultivar	Country	Seed source
P. acutifolius	Sonora 56	Mexico	CSU
P. vulgaris	Iniap Bayito	Ecuador	CIAT
P. vulgaris	Ex-Rico 23	Colombia	CIAT
P. vulgaris	Tamazulapa	Guatemala	CIAT

Callus Production. Explants were obtained from fifteen-day-old *P. vulgaris* and seven-day-old *P. acutifolius* seedlings. Callus formation was induced on L-6 agar medium plus 2mg/l 2,4-D and 0,5mg/l KIN. The cultures were placed under continuous cool, white fluorescent light (2200 lux) at 22 $\pm$ 2° C. The kinds of explants used are listed in Table 3.

Table 3: Explants used as callus sources.

Variety	Explant tissue
Sonora	Leaves and epicotyls
Iniap Bayito	Leaves and epicotyls
Ex-Rico 23	Whole embryos
Tamazulapa	Hypocotyls

Suspension Cultures. The suspension cultures were initiated either from 20-day-old callus or directly from seedling explants. A total of five cell suspension lines were chosen from up to 40 lines originally established. L-6 basal medium with two different hormone combinations was used to initiate the suspensions (Table 4).

Table 4: Hormones and kind of explants used to initiate the suspensions.

Variety	Suspension initiated from	L-6 medium hormone composition (mg/l)	cell lines
Sonora	Epicotyl callus	10 NAA/ 2 K	PA-009-E
Iniap Bayito	Leaf Callus	10 2,4-D	PV-014-L
Iniap Bayito	Epicotyl callus	10 NAA/ 2 K	PV-015-E
Ex-Rico 23	Whole embryo callus	10 2,4-D	PV-017-WE
Tamazulapa	Hypocotyl callus	10 2,4-D	PV-020-H

Filtration Procedure. Cell suspension cultures were subjected to filtration using 500 um and 250 um filters. Cells which did not filter were subcultured separately in initiation media. This procedure was repeated three or four times at 10- to 15-day intervals. After the fourth filtration the cells were transferred to L-6 medium plus 1 mg/l 2,4-D and 0.5 mg/l Z (maintenance medium). They were cultured in this medium for the next three or four filtrations. At this time many embryo-like structures were observed in all the cultures. Further experiments and filtrations were conducted with the main objective of selecting an embryogenic cell line with several embryo-like structures. Selection of such cell clumps had resulted in embryogenesis in P. acutifolius.

Embryo Induction. After the seventh or eighth filtration cells larger than 500 um were inoculated into L-6 liquid medium plus 2.25 mg/l

BA, or 1 mg/l Zeatin. Cells were maintained in the media for two to three weeks and then plated on agar regeneration media.

Suspensions with PEG. The cells collected on a 500 um filter were also inoculated into the embryo induction media with 2.25 mg/l BA. Initially, the PEG concentration in the medium was 10%; after seven days the concentration was reduced to 2.5%, replacing the old medium by decanting.

Plating on Regeneration Media. The cell suspension cultures were filtered through 500 um filters. The cells larger than 500 um were plated on different regeneration media. The cell material which passed through 500 um size filters but collected on the 250 um filter, was then transferred to maintenance media and/or embryo induction media. Screw-cap jars containing 20 ml of agar regeneration media were used to plate all the cell materials including the suspensions with PEG. All the plated cultures were kept under continuous light (3500-5500 lux) at 22 $\pm$ 1° C.

Results and Discussion. A total of 53 callus cultures were produced on L-6 agar medium. Most of the suspension cultures were initiated from three-week-old callus. Several cultures were also initiated directly from seedling tissues. All of the cultures were initiated in L-6 medium with 10 mg/l 2,4-D alone or 10 mg/l NAA in combination with 0.5 mg/l KIN. The 2,4-D medium was the more effective in stimulating cell division and the production of cell suspension cultures; however, good results were also obtained with NAA and KIN, especially with the *P. acutifolius* cell lines. A total of 22 cultures were initiated. The cultures established directly from explants had considerably more debris than those from callus.

Initially, the cultures consisted of three major cell types. They were 1) single isodiametric cells, 2) elongated cells, and 3) clusters of rounded cells. The latter category is made up of relatively small cells with a dense cytoplasm and small nuclei. The clusters varied in number of cells and size. Rounded cells were predominant in cultures from leaf and epicotyl callus and became the most rapidly growing.

In the procedure for regeneration of the P. acutifolius, the cell type which usually became embryogenic consisted of clusters of small cells with dense cytoplasm. Upon plating on a regeneration medium, the clusters would produce E callus and subsequently plantlets.

Cell filtration. The procedures of filtration previously developed for P. acutifolius were applied. The cell suspensions were filtered on a 500 um filter and the filtrate was put through a 250 um filter. The cell fraction collected on the 500 um and on the 250 um, respectively, were cultured on a maintenance medium of L-6 with 1 mg/l 2,4-D and 0.5 mg/l zeatin. After three or four filtrations, depending on the line, the cultures contained embryo-like structures. The embryo-like structures were observed in the Tamazulapa and in the Iniap cultures (P. vulgaris). Some of the clusters produced structures which appeared to be roots (see Research Report III). The cell clusters and aggregates of some cultures of P. vulgaris had a tendency to turn brownish, which was likely due to phenolics and phenolase activity. The problem was overcome in part by increasing the frequency of subculturing to seven to ten days.

Because PEG had been shown to enhance plant regeneration in cell cultures of pigeonpea, some of the P. vulgaris lines were subcultured into media with PEG. A concentration of 2.5% PEG did not affect the growth rate and the cultures had less tendency for browning. In

pigeonpea, a concentration as high as 10% PEG enhanced plant regeneration efficiency by 77.5% (unpublished).

Induction of embryogenesis and plating. After the third, fourth, and subsequent filtrations, the cells collected on the 500 um filter were transferred to L-6 media with 2.25 mg/l BA. After two to three weeks the cell materials/clusters were plated on L-6 agar media. Several hormone combinations were tested using NAA or 2,4-D with either zeatin or BA and GA_3. After two to three weeks on these media, some of the callus grew into structures which were globular and greenish.

Some of the cells from the L-6 (BA) liquid media were also plated on 0.4% Gel-Gro® with 2.5% PEG as well as the three hormones. At the conclusion of the research period (September 30), the plated cultures had not produced shoots in any of the media compositions.

On the other hand, cells from the TCCP cell line TB-787 plated on L-6 agar medium with 0.5 mg/l of BA had produced shoots. The TB-787 suspension culture was established July 1987 and has been cultured on L-6 with 2 mg/l 2,4-D and 0.5 mg/l zeatin. The cell line was subjected to filtration every second month. The cells were filtered on a 500 um screen followed by a 250 um screen. The cell clusters collected on the 250 um screen were subcultured. Prior to plating, the cell materials from a 250 um filtration were cultured on L-6 with 2.25 mg/l BA.

Stress Tolerance Selection in Tissue Culture of Spring Wheat.

This project was undertaken by Dr. Ahmed Medat Al Naggar. Dr. Naggar is from the Faculty of Agriculture, Cairo University, Egypt. He was at TCCP as a Senior Fulbright Scholar from October 1987 to April 1988.

The objective of Dr. Naggar's project was to determine the levels of PEG for in vitro selection for drought tolerance in wheat. The research involved a stepwise selection with increasing percentages of PEG. In the second approach, the callus was initiated on media with 0.25% PEG. A smaller project was also performed on salt stress selection.

The drought tolerance study was made on spring wheat cultivars Pavon, Shorawaki, and PK-16171 at critical levels of 0, 10, 15, 20 and 25% PEG. Approximately 200 mature embryos of each cultivar were initiated on solidified PEG medium using Gel-Gro® as a substitute for agar. Two drought selection methods, viz. step-wise and long-term, were used and continued for five passages. Total and E calli were weighed before transferring to fresh medium at the end of each passage.

Generally, the higher the PEG concentration the slower the growth of the E callus; however, the growth of E callus at 10% PEG was about equal to that of the control in PK-16171. The critical PEG concentration for selection of tolerant calli appeared to be 15% for Pavon and PK-16171 and 20% for Shorawaki. The higher concentrations of PEG reduced plant regeneration from E callus. A portion of calli from each stress level, starting from 15% PEG, was used for plant regeneration and the remaining calli were moved to fresh medium. The regenerated plants from the step-wise experiment were transplanted into soil in the greenhouse and were grown to maturity. The same was done for those plants regenerated from the long-term experiment. The seeds will be sent to Dr. Naggar, who will perform the screening of the R_1 plants.

NaCl Selection. Mature embryos from five cultivars of spring wheat (Pavon, Glennson, Shorawaki, PK-16171 and Kharchia) were initiated on LS medium supplemented with 2 mg/L 2,4-D with and without salt. Different salt selection methods were used with 0, 3, 6, or 9 g/L^{-1} NaCl. Calli

were weighed and E calli moved to fresh medium. After the fifteenth passage, the E calli were transferred to regeneration medium. Plants were then transferred to the greenhouse and grown to maturity.

Tissue Culture of Winter Wheat

Abstract. This tissue culture project is supported by the Colorado State Experiment Station. The objective is to determine the tissue culture techniques for winter wheat, determine PEG selection levels, and then regenerate plants from PEG-tolerant callus. These regenerated lines will be evaluated for drought tolerance.

Experiments in the past year have demonstrated that plant regeneration is most efficient at 1 mg/l 2,4-D in the maintenance media and that KIN is not beneficial for callus growth or plant regeneration. For selection on PEG, 12% (MW=8000) was the level chosen.

Material and Methods. The investigation on *in vitro* selection for increased drought tolerance of Colorado winter wheat cultivars continued this year. After initially screening for tissue culturability of five cultivars of Colorado winter wheat, two, Hawk and Bennett, were selected for further investigation.

Subculture techniques. Results from previous experiments seemed to indicate that higher levels of 2,4-D in the initiation and maintenance media needed to be lowered sufficiently for efficient regeneration to take place. An experiment was designed to study methods of affecting dilution of 2,4-D in the E callus by using several subculture methods prior to plating on regeneration medium. Callus was initiated on 1,2 and 5 mg/l 2,4-D and maintained for four four-week passages on initial levels of auxin before subjecting to different subculture methods.

Kinetin. In another experiment, the presence of KIN in the initiation and maintenance media was investigated. Beneficial effects of KIN in the initiation medium was indicated for some cultivars in earlier experiments.

PEG Selection. Embryogenic callus was moved to media containing 0, 3, 6, 9, 12 and 18% PEG (MW=8000). Embryogenic callus weights were taken after each passage and plants regenerated after a total of three passages on stress.

Results and Discussions.

Subculture techniques. Based on the number of regenerated plantlets after each treatment, it is apparent that the subculture methods have not improved the regeneration rates of E callus originating from 2 and 5 mg/l 2,4-D. In the 1 mg/l 2,4-D, however, it seems more beneficial to maintain the E callus on either the initial or half the initial levels of 2,4-D rather than dilute out the auxin (Table 5). It also seems that there is a critical level of 2,4-D necessary to initiate as well as maintain E callus, and for the cultivars tested this seems to be closer to 1 mg/l 2,4-D in comparison to 2 mg/l for the spring wheat cultivars tested at TCCP.

Kinetin. For the cultivar Bennett, the number of plantlets regenerated from callus initiated with 0.5 mg/l KIN and 2,4-D is more in number than in the absence of KIN (Table 6). In Hawk, however, the presence of KIN seems to be deleterious. Initiating and maintaining callus on 1 mg/l 2,4-D is optimal.

PEG Selection. After obtaining reasonable information on the initiation and maintenance of E callus of Hawk and Bennett as well as some regeneration procedures, we proceeded to make an examination of the critical levels of PEG (MW=8000) that could be applied for an *in vitro* selection scheme. The concentrations tested were 0, 3, 6, 9, 12 and 18%

PEG (8000). From these initial experiments, 12% PEG was chosen as a suitable level to carry out *in vitro* selection for drought tolerance.

Table 5. Effect of subculture techniques on plant regeneration of Hawk.

Media of Passages			Plants Regenerated
1-4	5	6	
1 mg/l 2,4-D	0 mg/l 2,4-D	.1 mg/l IAA .5 mg/l BA	2
2 mg/l 2,4-D	0 mg/l 2,4-D	.1 mg/l IAA .5 mg/l BA	1
5 mg/l 2,4-D	0 mg/l 2,4-D	.1 mg/l IAA .5 mg/l BA	0
1 mg/l 2,4-D	.1 mg/l IAA .5 mg/l BA		7
2 mg/l 2,4-D	.1 mg/l IAA .5 mg/l BA		0
5 mg/l 2,4-D	.1 mg/l IAA .5 mg/l BA		0
1 mg/l 2,4-D	0.5 mg/l 2,4-D	.1 mg/l IAA .5 mg/l BA	31
2 mg/l 2,4-D	1.0 mg/l 2,4-D	.1 mg/l IAA .5 mg/l BA	2
5 mg/l 2,4-D	2.5 mg/l 2,4-D	.1 mg/l IAA .5 mg/l BA	1
1 mg/l 2,4-D	1 mg/l 2,4-D	.1 mg/l IAA .5 mg/l BA	34
2 mg/l 2,4-D	2 mg/l 2,4-D	.1 mg/l IAA .5 mg/l BA	0
5 mg/l 2,4-D	5 mg/l 2,4-D	.1 mg/l IAA .5 mg/l BA	0
1 mg/l 2,4-D	0.5 mg/l TIBA	.1 mg/l IAA .5 mg/l BA	3
2 mg/l 2,4-D	0.5 mg/l TIBA	.1 mg/l IAA .5 mg/l BA	0
5 mg/l 2,4-D	0.5 mg/l TIBA	.1 mg/l IAA .5 mg/l BA	0

Table 6. Effect of KIN on plant regeneration of two cultivars of winter wheat.

Media for Passages		Plants Regenerated	
1	2-3	Bennett	Hawk
1 mg/l 2,4-D	1 mg/l 2,4-D	2	24
1 mg/l 2,4-D	1 mg/l 2,4-D .5 mg/l Kin	2	6
2 mg/l 2,4-D	2 mg/l 2,4-D	1	3
2 mg/l 2,4-D	2 mg/l 2,4-D .5 mg/l Kin	1	13
5 mg/l 2,4-D	5 mg/l 2,4-D	1	0
5 mg/l 2,4-D	5 mg/l 2,4-D .5 mg/l Kin	0	0
1 mg/l 2,4-D .5 mg/l Kin	1 mg/l 2,4-D	6	4
1 mg/l 2,4-D .5 mg/l Kin	1 mg/l 2,4-D .5 mg/l Kin	9	11
2 mg/l 2,4-D .5 mg/l Kin	2 mg/l 2,4-D	0	10
2 mg/l 2,4-D .5 mg/l Kin	2 mg/l 2,4-D .5 mg/l Kin	5	5
5 mg/l 2,4-D .5 mg/l Kin	5 mg/l 2,4-D	4	0
5 mg/l 2,4-D .5 mg/l Kin	5 mg/l 2,4-D .5 mg/l Kin	0	0

Somaclonal variation and In vitro selection to elevated temperature in tropical wheat - Dr. A. Zamora, Institute of Plant Breeding, Philippines.

Abstract. Cultures of Trigo 3, a wheat cultivar developed for the Philippines, were tested for tolerance to elevated temperatures. The optimal temperature for E callus growth is 25° C and temperatures of 37 to 40° C are suitable for selection. Regenerated plants and their R_1 progeny were evaluated in the field. Morphological variations were noted in R_1 lines.

Materials and Methods. Embryogenic calli of Trigo 3 were selected from callus stock which have been established from immature embryos. The maintenance medium was MS supplemented with 2 mg/l 2,4-D, 30% sucrose, 25 mg/l arginine and 15% coconut water.

After subculture onto fresh medium, the E calli were maintained under an eight hour photoperiod at 20, 20-25, 25, 25-35, 35-40, and 40° C. For temperature treatments of 20 and 40° C, photothermal cabinets were utilized. These cabinets were placed at varying distances from an air conditioner to vary temperature. The cabinets for 25-35° C and 37-40° C were enclosed with styrofoam and black plastic with a side of the cabinet covered with a black plastic bag to allow air circulation and minimize overheating. Temperature increase was achieved by adding an incandescent bulb to the fluorescent bulb inside the cabinets.

After four weeks, the calli were observed and subcultured onto fresh medium. Subculture of E callus was such that half of what was recovered after callus sorting was inoculated onto fresh maintenance medium and the other half onto the regeneration medium.

Results and Discussion. After four weeks at 20° C, 20% of the inoculated E callus cultures exhibited E callus type of growth. The percentage of cultures with E callus was higher at 20-25 and 25° C (30.8

and 28.9%, respectively) than in other treatments. The percentage of cultures with E callus was lowest at 40° C incubation (9.7%).

The percentage of cultures with predominantly E callus was lower than cultures with predominant NE callus in all treatments except at 25-35° C. The percentage of cultures with predominant browning was high in the experiment (50 to 74.2%). Browning of callus which eventually lead to callus death increased with increasing temperature.

At the end of the second passage of cultures incubated at different temperatures, the percentage of cultures with E callus was highest (51.5% at 20° C and declined with increasing incubation temperature (31.3-36.8% at 25-35 at 37-40° C, respectively. Browning increased with increases in incubation temperature. All cultures incubated at 40° C turned brown. Embryogenic calli in the 37-40° C temperature regime were associated with the brown calli, the E callus appeared as "toppings" on the brown callus. It was more typical in the other calli that browning appeared as patches on the whole callus or the whole calli was brown; however, it was only in this treatment (37-40° C) that E callus occurred on top of brown callus.

Field Evaluation of Regenerated Plants and Their Progeny. Generally, R_1 plants performed better than their parent plants (R_2) in terms of measurable characters. This could be attributed to the difference in planting material evaluated. R_1 plants were germinated from seed while the R_0 plants were subjected to more stresses when they were transferred from culture vessels to soil. Some physiological and biochemical pathways could have been affected by the drastic change in condition resulting in inferior agronomic performance of the R_0 plants.

The R_0 progenies performed better than their parents, based on mean plant height, spike length, number of florets per spikelet, number of

spikelet per spike, number of tillers, and number of seeds harvested. R_0 line number 11 was the shortest (28 cm); however, its progenies did not deviate much from the average plant height, although it was still smaller than seed-derived plants. This was also obvious for line 22. Plant height for the R_1 was generally shorter than the -derived but taller than the R_0 parent. Some R_1 lines were as tall as seed-derived plants.

The number of spikelets per spike increased by three or four times in the R_1 than what was observed for their parents. Line 6 (R_0) gave the lowest number of spikelets; however, the number of spikelets per spike increased for its R_1 by almost three times. The same trend in increase was also noted for spike length. Except for line 11, all the R_0's were longer than the seed-derived plants.

Tiller number generally increased except for R_1 lines 3, 23 and 25. For these lines, there was a decrease in tiller number. Lines 11, 22 and 26 had fewer tillers than seed-derived plants. Of all the R_1 lines, it was observed that line 25 did not vary much in performance from its parent.

The ability of these R_1 lines to withstand elevated temperature conditions will be further assessed. The harvested seeds will be apportioned such that each line is further evaluated in two environments. Performance of R_1 lines will be evaluated in two locations: at high elevation (La Trinidad, Benguet, 1372 m asl) and at low elevation (UPLB, Los Baños, 21 m asl). Morphological and physiological characteristics will be noted and this will be correlated with tolerance to elevated temperatures.

Corn Tissue Culture - Dr. Thanh-Tuyen; Visayas State College of Agriculture, Philippines

The interaction effects of genotype and culture medium on somatic embryogenesis of corn were investigated with two commercial varieties and four promising lines. Among the six culture media adopted from Fahey et al. (1986), none was the best for all tested genotypes. Nevertheless, in terms of percent embryogenic response and regenerative ability of the cultured embryos, MS Pro and PHL Pro were found suitable for more than one genotype. The two medium formulations were therefore used in subsequent experiments related to optimization of culture medium for high-frequency and long-term plant regeneration from callus cultures of corn.

For the six genotypes which were evaluated for their culturability, IPB Var 2 was most promising followed by V8426 and Improved Tiniguib. IPB Var 2 and Improved Tiniguib are popular white corn varieties in the region while V8426 is still a recommended line awaiting approval of the Philippine Seed Board.

Refinement of the culture procedures for high-frequency, long-term plant regeneration from corn callus has been initiated this year. In particular, various experiments have been set-up to investigate the effects of osmoticum (e.g. mannitol, sorbitol) and abscisic acid (ABA) on callus initiation, maintenance and plant regeneration from immature embryos of corn. Addition of abscisic acid at various concentrations before regeneration medium did not show positive effects on retention of E callus initiated from embryos of IPB Var 2 and V8341. Mannitol, and mannitol plus sorbitol added to the standard MS Pro medium, improved the frequency of plant regeneration and retained the regenerative ability of the callus cultures of V8341 to the fifth and fourth passage, respectively.

A broad spectrum experiment was set-up with the combination of sucrose (3, 6, 9 and 12%), mannitol (0, 1.5, 3.0 and 5%) and ABA (0 and 0.5 MM) for immature embryos of Improved Tiniguib and IPB Var 2. Partial results showed that in terms of percent embryogenic response, an increase in sucrose concentration beyond 3% was not beneficial to the culture even in the presence of mannitol and ABA; however, at the 3% sucrose level, an increasing mannitol concentration resulted in a higher percent embryogenic response. Likewise, the E callus was also maintained longer on medium containing 3% sucrose and mannitol. The same trend was noted for the regenerative ability of the cultures. In addition, plant regeneration has been retained to the sixth passage.

Regenerated plants derived from immature embryo cultures of IPB Var 2, V8341 and Improved Tiniguib from various experiments have been raised to maturity in greenhouse. The two common abnormalities which have been observed among R_o plants are 1) the emergence of an unshelled ear together with the tassel at the top of the shoot, and 2) very short stature plants.

Self-fertilized progeny (R_1, R_2, R_3 and R_4) of an IPB Var 2 R_o plant obtained in one experiment in 1986 were evaluated under greenhouse (R_1) and field conditions (R_2, R_3 and R_4). No abnormality of the reproductive structures were observed in the progeny; however, wide variation in number and weight of kernel per ear was noted.

Heat Screening and Plant Regeneration from Leaf Tissue of Diploid Solanum Species

Abstract. Two lines of *Solanum chacoense*, one line each of *S. microdontum* and *S. bulbocastanum*, were selected for heat screening based on *in vitro* screening and electrolyte conductivity measurement. A better

protoplast yield was obtained from leaf tissues of shoot cultures of these lines which were grown in MS medium with no sucrose, and incubated in isolation medium with 0.125% cellulase, 0.05% hemicellulase and 0.0125% pectolyase with 0.6M glucose, for 48 hours.

Plant regeneration was achieved from suspension cultures of diploid lines Gp. Tuberosum x Gp. Phureja (US-W-9310-3) and Gp. Phureja/Stenotonum (77-19) by a multi-step regeneration procedure. The regeneration medium was MS with 0.1 mg/l IAA, 1 mg/l zeatin, and 0.1 mg/l GA_3. Protoplasts of four suspension cultures formed cell walls, but only line P135287-26.1 underwent division and formed a microcolony.

Materials and Methods.

Heat screening for diploid Solanum species. Shoot cultures were obtained from shoots excised from seedlings of diploid Solanum species and meristem cultures of tetraploid cultivars. One-week-old shoot cultures of each diploid Solanum species and the tetraploid cultivars 'DTO-33' and 'Red Pontiac' were treated at 35°/22° C, with a 12-hour photoperiod for three weeks. This experiment was repeated three times. In further experiments, the electrolyte conductivity measurements were applied to leaf disc samples from greenhouse-grown plants of selected lines. Lines which were selected based on electrolyte conductivity tests were re-evaluated with additional treatments at 35°/22° C for 24 and 48 hours.

Protoplast isolation of diploid Solanum species. The protoplast isolation procedures, including growing conditions for shoot cultures, enzyme combinations and concentrations, osmolarity, and incubation period of four selected lines from the heat screening, S. chacoense 2, S. chacoense 8, S. microdontum 5, and S. bulbocastanum 187.21, were

determined. The aim of these experiments was to optimize protoplast yield in quality and quantity.

Plant regeneration from leaf tissues of diploid Gp. Tuberosum x Gp. Phureja and Gp. Phureja/Stenotonum. One gram of expanded leaves from one-month-old shoot cultures were exc'sed and chopped, and incubated in 5 ml isolation medium containing 1.25% cellulase, 0.5% hemicellulase and 0.125% pectolyase, with 0.5M glucose in H-KM medium, at 27° C in the dark. After two hours, the mixture was filtered through 50 and 30 um screens, and washed with 5 ml washing medium followed by centrifugation. The protoplasts were purified by adding 5 ml of 20% Percoll to the washing medium, and centrifuged. The band was pipetted and washed with 5 ml washing medium as previously described. One ml of protoplast culture medium (PCM) was added to the pellet. Protoplasts were counted in a hemocytometer, the final concentration adjusted to 10^6 with the addition of PCM, and plated 300 ul per plating medium. Microcolonies were transferred onto fresh plating medium, and later calli were transferred to regeneration medium (MS medium supplemented with NN vitamins, 0.1 mg/l IAA, 1 mg/l zeatin and 0.1 mg/l GA_3). Regenerated shoots were excised and rooted in MS medium with no hormones, and the remaining calli were moved to regeneration medium.

Results.

Heat screening for diploid Solanum species. There were 25, 40, 11 and 5 shoot cultures of S. bulbocastanum PI 275196, S. bulbocastanum PI 275187, S. chacoense and S. microdontum, respectively, screened in vitro. After three screenings, 14 lines were subjected to electrolyte conductivity measurements on leaf disc samples which were treated at 50°C for 30 and 60 minutes (Table 7). Based on the percentage of injury after heat treatment, four lines of diploid Solanum were evaluated on heat

treatment responses. S. chacoense 2 and Red Pontiac showed similar responses (a decreased percentage of injury with heat induced treatment). The percentage injury of S. chacoense 8 and S. microdontum 5 slightly increased after a 24-hour treatment and then slightly decreased after a 48-hour treatment. S. bulbocastanum 187.21 showed lower percentage of injury before heat treatment which was greatly increased after heat treatment. Tetraploid DTO-33 demonstrated the lowest percentage of injury (Table 8).

Table 7. Percentage of injury of selected Solanum lines, DTO-33 and 'Red Pontiac'.

Lines	50° C, 30 minutes	50° C, 60 minutes
'Red Pontiac'	19.0 ± 1.31	44.1 ± 13.80
DTO-33	22.1 ± 2.36	24.3 ± 3.56
S. chacoense 1	30.0 ± 3.11	62.0 ± 3.35
S. chacoense 2	16.2 ± 1.90	41.0 ± 6.72
S. chacoense 8	15.0 ± 0.60	51.4 ± 2.74
S. microdontum 1	56.1 ± 4.94	67.2 ± 1.91
S. microdontum 5	22.6 ± 1.88	32.2 ± 1.29
S. bulbocastanum 187.1	39.7 ± 0.87	62.6 ± 17.34
S. bulbocastanum 187.2	68.3 ± 1.50	78.3 ± 2.43
S. bulbocastanum 187.5	55.5 ± 3.24	75.9 ± 5.03
S. bulbocastanum 187.16	66.5 ± 2.10	82.8 ± 1.10
S. bulbocastanum 187.21	19.7 ± 0.03	44.2 ± 0.75
S. bulbocastanum 196.5	30.7 ± 2.57	64.3 ± 2.76
S. bulbocastanum 196.11	22.0 ± 2.08	52.5 ± 2.31
S. bulbocastanum 196.16	25.4 ± 1.25	68.3 ± 0.67
S. bulbocastanum 196.17	20.9 ± 1.91	55.8 ± 3.01

Table 8. Percentage of injury of selected Solanum lines, DTO-33 and 'Red Pontiac' grown in the greenhouse and treated at 35° for 24 and 48 hours.

Lines	Greenhouse	35° C, 24 hrs.	35° C, 48 hrs.
'Red Pontiac'	50.9 ± 1.86	43.0 ± 3.05	13.5 ± 1.97
DTO-33	25.2 ± 5.40	31.4 ± 7.40	35.9 ± 4.68
S. chacoense 2	80.0 ± 5.41	46.1 ± 15.33	24.6 ± 2.58
S. chacoense 8	65.7 ± 7.02	78.1 ± 2.19	69.3 ± 10.92
S. microdontum 5	70.7 ± 9.84	78.1 ± 3.34	58.2 ± 2.36
S. bulbocastanum 187.21	35.6 ± 6.63	85.6 ± 0.49	68.8 ± 6.51

Plant regeneration from leaf tissues of diploid Gp. Tuberosum x Gp. Phureja and Gp. Phureja/Stenotonum. Protoplasts formed cell walls one day after plating, and began to divide two days after plating. Fifty-three days after plating, microcolonies which had formed were transferred to callus induction media. Masses of calli in MS medium supplemented with 0.1 mg/l IAA and 1 mg/l zeatin gave the best results, producing green, compact callus with many nodule-like structures. After calli were transferred to regeneration media, primordia were developed from nodule-like structures and in a later stage grew to form shoots with or without roots. All of the remaining calli were placed on MS medium with 0.1 mg/l IAA, 1.0 mg/l zeatin and 0.1 mg/l GA_3 and were regenerated into shoots with roots.

MOLECULAR BIOLOGY

The goal of the molecular biology program at TCCP and its collaborating institutions is to obtain the genes which can be used to confer salt tolerance to crop plants. The rationale is to investigate species which tolerate high levels of salt and attempt to isolate the genes responsible for the salt tolerance trait. Since one of the principal objectives is to transfer salt tolerance genes to cereals such as rice and wheat, the salt-tolerant species being used is kallar grass, which is highly salt-tolerant (halophytic) (Leptochloa fusca). The cell materials being used are suspension cultures. Cells grown in media with 20 g/l NaCl show a 50% reduction in growth. Such cells are considered stressed. One of the assumptions being used is that genes involved with salt stress tolerance are activated under salt stress. Subtraction (cascade) hybridization has been used to obtain an enrichment of mRNAs from salt-stressed cells of kallar grass for messages which are salt stress induced.

Experimentation and Results.

Growth rates of kallar grass cells under salt stress. Experiments were set-up to determine which salt stress is reasonable for making a cDNA library from L. fusca cells. Cell suspension cultures of equal packed cell volume were transferred to media containing 0, 20, and 30 g/l NaCl. The cells were harvested after 3, 6 and 9 days. In each case, change in dry weight (mg/ml) was plotted against time (days). The results showed that 20 g/l NaCl caused about 50% reduction in growth rate. Thus 20 g/l salt stress has been used as a standard for salt-stressed cells.

cDNA synthesis and cloning. Before making cDNA from salt-stressed cells of L. fusca and cloning into lambda vector, test experiments were

performed. cDNA was synthesized from poly(A^+) RNA (B globin gene) to check the efficiency of the synthesis system. A test insert pRheo was cloned into lambda ZAP vector to make sure that ECORI digested lambda ZAP arms and in vitro packaging systems yielded the expected results. The packaging system and lambda ZAP gave the expected results. A cDNA library has now been prepared using the poly (A^+) RNA from cells that have been salt stressed.

Model Experiment for making probe to screen cDNA library. Subtraction hybridization is being used for making the probe. This technique involves hybridization of poly(A^+) RNA from cells without salt stress and single stranded (ss) cDNA from salt-stressed cells and then fractionation on a hydroxylapatite column. A model experiment using ss cDNA and double stranded (ds) cDNA was set-up. The hydroxylapatite column was packed and ss DNA and ds DNA were separated.

Enrichment of salt-stress induced cDNA species from suspension cultured cells of kallar grass. Single-stranded cDNA (650 ng) produced from poly (A) RNA isolated from salt-stressed (20 g/l, 24 hours) kallar grass cells was hybridized for 20 hours with a 10-fold excess (6500 ng) of poly A RNA isolated from control cells. The hybridization mixture was diluted and applied to a hydroxylapatite (HA) column in 0.12 M phosphate buffer. cDNA species corresponding to mRNA species present in both salt-stressed and control cells formed RNA-DNA duplexes with control cell poly(A) RNA. The resulting double stranded molecules bound to the HA column under the conditions used. cDNA species corresponding to mRNA species that were present at elevated levels in the poly(A) RNA isolated from salt-stressed cells remained single stranded and did not bind to the HA under the conditions used.

This experiment indicates that 1) approximately 17% of the mRNA population in salt-stressed cells is increased in abundance relative to the mRNA population in control cells. This observation provides support for the assumption that salt-stress induces the expression of specific genes, and 2) HA fractionation will be useful in preparing salt-stress enriched probes for use in screening a cDNA library produced from poly(A) RNA isolated from salt-stressed cells.

Preparation of cDNA Library. Amersham cDNA synthesis system was used to synthesize cDNA from 2.0 ug of poly (A^+) RNA isolated from salt-stressed cells of kallar grass. The cells had been exposed to 20 g/liter NaCl for 24 hours. Mass and size distribution analyses showed that the amount of cDNA obtained was 685 ug, and the average size of cDNA was in the range of about 1 KB. To clone this cDNA into EcoRl sites of lambda ZAP-II, the internal EcoRl sites of cDNA were methylated with EcoRl methylase. The EcoRl tailings were then ligated followed by EcoRl digestion to generate cohesive termini. Excess linkers were separated and cDNA was ligated to lambda ZAP-II EcoRl digested arms. Gigapack plus and gold were used for *in vitro* packaging. Titration results indicated that the library consisted of about 0.5 million recombinants.

Identification of genes by isolating salt stress-induced proteins. An alternate method for isolating salt stress-induced genes consist of identifying proteins which are produced during exposure to salt. The plant material used for this purpose was a cell suspension culture of rice (*Oryza sativa*, Basmati B-370). The cells were cultured in 0, 1.0, 2.0, 3.0% NaCl, and harvested after culture periods of 6, 12, 24 and 48 hours, respectively. The total RNA and poly(A^+) RNA were isolated from the different treatments and controls. The poly(A^+) RNA's were then used for *in vitro* translation to produce polypeptides, which were then

analyzed by SDS polyacrylamide gel electrophoresis. The profile in rice indicates the presence of several polypeptides induced by salt stress.

Salt Stress Regulated mRNA Induction In Rice

Abstract. Gene expression in response to salt stress at the m-RNA level was investigated in seedling roots, shoots and a cell line of Basmati Rice. Cells and seedlings in the hydroponics were exposed to different concentrations of salt for specific time periods and translatable poly (A^+) RNA was isolated. A reticulocyte lysate cell-free system was programmed with rice poly (A^+) RNA and the *in vitro* translated products were resolved on 10% polyacrylamide gel. Differential transcription of m-RNA was observed in different tissues. In rice shoots, 5 different mRNA were induced that encoded proteins of 17 to 51 kDa within 12 hours of salt stress. Three proteins of 53, 51, and 31 kDa, respectively, were over produced in seedling roots in response to salt stress. The m-RNA which encoded 51 kDa protein was induced both in shoots and roots exposed to salt stress for 12 hours or more. In cells exposed to salt, the proteins of low molecular weight ranging from 16 to 24 kDa were over-produced. Most of the m-RNA's coding for this proteins were induced within 12 hours in the cells exposed to $\geq$ 0.75% NaCl. These proteins also accumulated in response to $CaCl_2$, $NaSO_4$ and KCl, but not with $AlCl_3$, $MgCl_2$ and heat shock; however, two other proteins of 39 and 26 kDa were accumulated in the cells exposed to $AlCl_3$ and $MgCl_2$. The cells exposed to ABA for 24 hours triggered the transcription of mRNA's found in both salinity and non-osmotic, metallic, stress-exposed cells. The findings suggest that tissue specific transcriptional mechanisms regulate gene expression in rice when exposed to salinity stress.

Description of Experiments. The cell line RB-387 was maintained in modified AA medium. Exponentially growing (six days after subculture) cells were exposed to various concentrations of salts (0, 5.0, 7.5, 10, 20 and 30 gm/l NaCl) for various time periods (0, 1.5, 3.0, 6.0, 12.0, 24.0, and 48.0 hours). After treatment, the cells were harvested, immediately frozen in liquid nitrogen, and stored at -80° C until RNA isolation. Cells from the same stage of growth were exposed to different salts including $NaSO_4$, KCl, and $CaCl_2$ (30.0 gm each), PEG (300 gm/l), $AlCl_3$, $MgCl_2$ (400 PPM) and temperature (65° C for 6 hours). The cells were harvested after 24 hours, except those which were exposed to high temperature, and the samples were stored at -80° C.

Eight-day-old seedlings of Basmati rice were established in hydroponics and grown in the greenhouse. After five days, the nutrient solution was replaced with 0, 10, 20 and 30 gm/l NaCl-containing nutrient media. Shoots and roots were harvested separately and frozen immediately in liquid nitrogen.

RNA from different tissue were isolated using phenol extraction procedure (Ramagopal, 1984) and purified several times with alcohol and NaOAc or LiOAc precipitations. The purified RNA was resuspended in column binding buffer. Poly (A^+) RNA was selected by affinity chromatography on a poly U Sephadex column. The mRNA was purified with ethanol and NaOAc/KOAc precipitations and re-dissolved in RNA grade water.

Poly (A^+) RNA was translated in a nuclease treated, rabbit reticulocyte lysate from Promega Biotec. A 25 ul of reticulocyte lysate mixture containing 1.0 ug of poly (A^+) RNA, 17.5 ul of Lysate, 40 uCi of ^{35}S (Amersham Corporation, 1.0 uCi = 37 MBq.) was incubated at 30° C for one hour. Incorporation into TCA-insoluble precipitates was determined in 1.5 ul aliquots. Sample buffer was added to the remaining translation

mixture to make a final volume of 40 ul and boiled for 3 minutes, cooled immediately on ice, and stored at -20° C.

Proteins synthesized in vitro were resolved on 10% polyacrylamide gel along with molecular weight standards. An equal amount of radioactivity based on trichloroacetic acid precepitable CPM of protein sample was applied to each gel. The gels were stained with Coomassie blue, dried and exposed to Kodak X-ARS film.

Results and Discussion. Cells of RB-387 yielded more total and Poly (A^+) RNA compared to shoots and roots; however, no set pattern was observed in RNA levels in response to different types of stress. The translational activities of poly (A^+) RNA from different treatments were compared in reticulocyte cell-free system. The translational efficiency of poly (A^+) RNA from the cells exposed to NaCl increased up to 7.5 gm/l, followed by a linear decrease up to 30 gm/l of NaCl. Translational efficiency of the mRNA isolated from the cells exposed to stress for 6 and 12 hours was less than their respective controls; however, for 24 and 48 hours, mRNA isolated from stressed cells translated better. The mRNA from roots stressed for 12 and 24 hours translated 50% more efficiently than their respective controls. After 48 hours, however, the mRNA from the controlled cells incorporated more label than the stressed roots. Poly (A^+) RNA from the non-stressed shoots translated better than the stressed shoots. Translational efficiency of the mRNA isolated from the cells exposed to ABA, KCl, Na_2SO_4, $MgCl_2$ and PEG was better than the control mRNA.

The in vitro translational products encoded by the mRNA's of cell, root, and shoot tissues were resolved on 10% poly acrylamide gel. Salinity stress induced four mRNA's, which in turn encoded proteins of

24, 21, 19 and 16 kDa. All of these mRNA's encoding for the four proteins were induced within 6 hours of salt stress of 0.75% or above. Similar sets of mRNA' were also induced in response to other salts including $CaCl_2$ and Na_2SO_4 but not with other osmotic stress like PEG, and non-osmotic metallic ions like $AlCl_3$, and $MgCl_2$. Accumulation of two other proteins were observed in the translational products of mRNA isolated from the cells stressed with $AlCl_3$ and $MgCl_2$. The Poly $(A)^+$ RNA's transcribed in response to metal ion stress encoded 39 and 25 kDa proteins. The cells exposed to ABA (10 mg/l) triggered the transcription of six different mRNA, four of which were common to salt stress and two unique to metallic ions such as $AlCl_3$ and $MgCl_2$. This results indicate that salinity stress and metallic stress triggers the expression of different genes, while ABA alone induces both sets of genes.

Salinity also induced five shoot mRNA's and three root mRNA's. Shoot poly (A^+) RNA encoded proteins of 17 to 51 kDA proteins while root mRNA's produced three proteins of 53, 51, and 31 kDa proteins. Although both roots and shoots harbor the unique sites for activation and expression of several genes induced by salt, both tissues induced a poly (A^+) RNA which had translated into 51 kDa protein.

Salts and other factors induced the expression of selected mRNA's in cells, shoots, and roots of rice. The identification of gene products altered by salinity offers the potential to understand gene expression related to salinity by their isolation and characterization.

EVALUATION OF REGENERATED PLANTS

Field evaluation of regenerated material is the final aspect of *in vitro* selection. Based on the field evaluation, determination of the efficiency of the *in vitro* selection can be made. There are three levels to the question of the effect of tissue culture and *in vitro* selection on increased stress tolerance: 1) identification of individual plants which are outstanding for field tolerance which may be useful in breeding programs; 2) comparison of plants derived from *in vitro* selection and plants derived from non-stressed tissue culture to non-regenerated checks; 3) identification of a specific stress level or passage time which would lead to a larger percentage of the regenerated lines being tolerant in the field.

Evaluation of Regenerated Sorghum Lines for Tolerance to Al-Toxic Soils

Abstract. The main objective of this study was to evaluate regenerated sorghum lines for tolerance to aluminum toxic soils. A total of 372 regenerated lines ranging from the R_1 to the R_4 generation were tested in Griffin, Georgia on acid soil. The soil was an ulitsol (typic rhodudult) at pH levels ranging from 4.2 to 4.6 with a range of 110 to 275 mg/L of exchangeable aluminum. Compounding the problem of aluminum toxicity was severe drought which occurred in the first two months of plant development. A total of 5 populations were tested on acid soils plus one R_1 population which was increased for field evaluation in 1989. Visual stand and vigor ratings were reported at 42 and 109 days after planting. Germplasm with tolerance to aluminum toxic soils was identified from these various populations. A total of 47 lines from the first year screen of R_1 and R_3 populations survived to set seed. From the R_3 confirmation study, line number 862021-4-1 performed consistently

across replicates and within plots. This line also performed well in the 1987 acid soil experiment.

Field Conditions. Field studies of stressed and non-stressed regenerated sorghum lines have been conducted in Georgia since 1986 in collaboration with Dr. Ronny Duncan from the University of Georgia. A total of five regenerated populations were tested on acid soils, and one regenerated population was increased for future testing. The following is a list of these populations:

1) R_1 lines regenerated from Hegari germplasm;
2) R_3 lines regenerated from Hegari but with no previous history of acid soil testing;
3) R_3 lines regenerated from Hegari and selected for tolerance to acid soil conditions in 1987's acid soil test;
4) R_4 lines tested in 1987 for tolerance to acid soil conditions and increased in the greenhouse;
5) R_4 and R_2 lines increased in Puerto Rico;
6) R_1 and R_3 seed increase on normal pH soil.

A general flow diagram is shown in Figure 21 which shows the relationship among generations of sorghum lines tested in 1987 and 1988, and for 1989's trial.

Balanced incomplete block and completely randomized designs were used to test these lines; there were a total of 1170 plots on acid soil to evaluate 372 lines. Statistical analysis was conducted with SAS, (Statistical Analysis System), by using analysis of variance, mean separations, and chi-square. The site was visited twice by TCCP personnel—once at 42 days and again at 109 days after planting—to visually rate for vigor and stand. The combination of drought conditions

during May and June, along with the acidic soils, resulted in high mortality of sorghum lines.

Soil samples were taken at each trip. Samples from the first trip ranged from soil pH of 4.25 to 4.5 and the exchangeable aluminum ranged from 120 to 250. Samples from the second trip ranged from soils pH of 4.3 to 4.45. Exchangeable aluminum was not analyzed for samples at the second trip.

Plots were 3.0 m long with 0.75 m between the rows replicated 3 times unless noted otherwise. Visual ratings were reported for 3 characteristics: 1) stand 42 days after planting, 2) vigor 42 days after planting, and 3) vigor 109 days after planting. For stand, the following rating scale was used: 1=no stand, 2=1%-25%, 3=26%-50%, 4=51%-75%, and 5=76%-100% stand. The vigor ratings were rated as: 1=dead, 2=barely alive with severe nutritional deficiencies, 3=plants stunted with some nutritional deficiencies, 4=slight nutritional deficiencies, and 5=healthy plants. Square root transformations were performed on the data to conform to a normal distribution of error.

Evaluation of R_1 Population on Acid Soils. The objective was to screen 131 R_1 lines and to identify lines and plants which were tolerant to acid field conditions. The R_1 population was derived from three <u>in vitro</u> stressing agents: aluminum chloride (Al), hydroxyproline (Hyp), and potassium chlorate (Cl). Aluminum chloride is a stressing agent which may select for cells tolerant to high aluminum concentrations. Hydroxyproline is an analog of proline which is thought to be involved in several stress tolerance mechanisms. Potassium chlorate is a stressing agent which may select for more efficient nitrate utilization.

Lines differed significantly for visual stand rating ($p < .01$). Lines did not differ significantly for first and second visual vigor

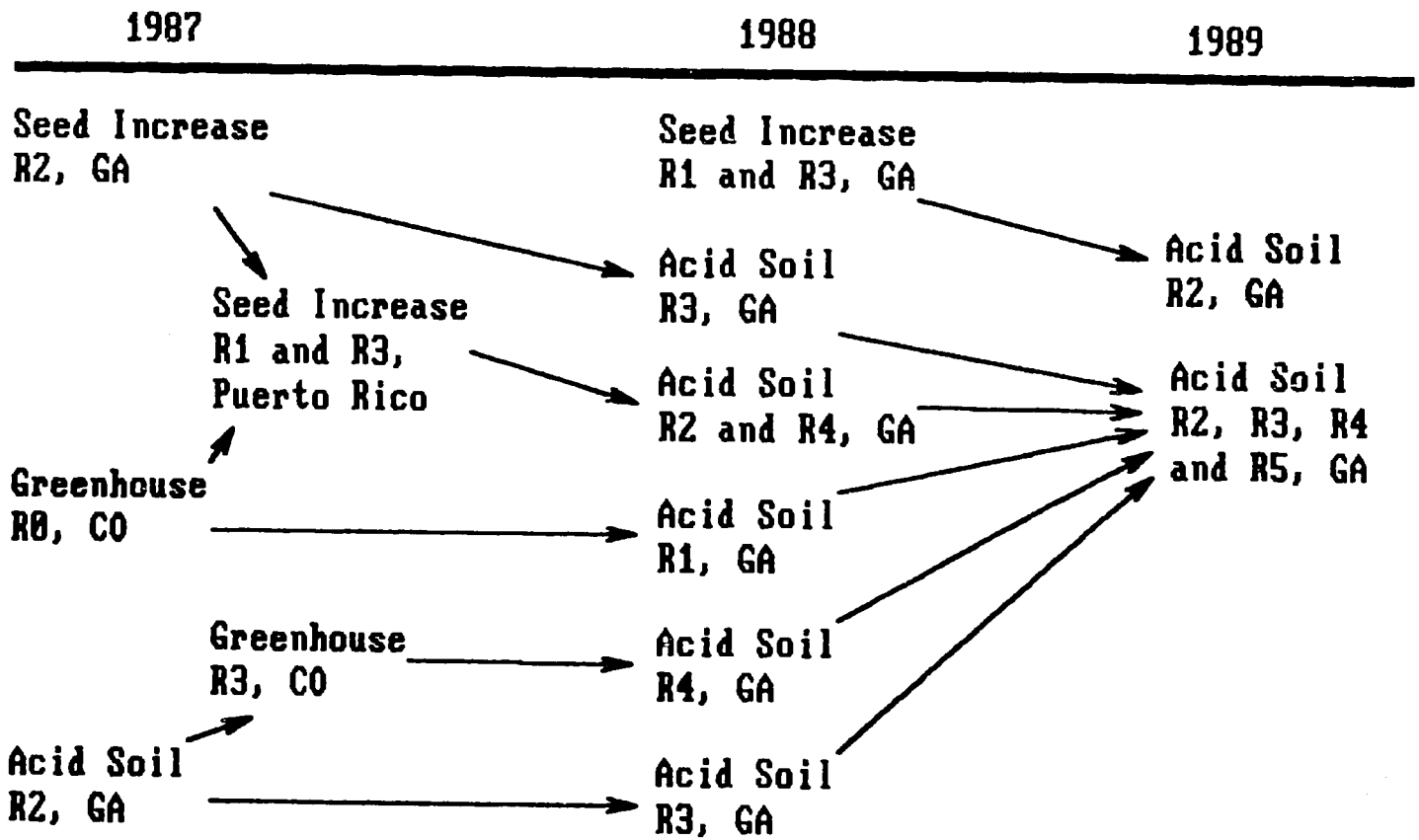


Figure 21. Flow chart of field sites related to the acid soil evaluations in Georgia in 1987 and 1988 and proposed evaluations for 1989.

ratings ($p = .193$ and $p = .477$ respectively). Blocking was effective and significant ($p < .01$). Lines that survived the severe stress conditions (acid and early drought) at Griffin, Georgia, will be tested again on acid soil in 1989. Table 9 shows R_1 sorghum lines ranked by seed weights per plot.

Table 9. R_1 sorghum lines that survived and set seed under acid soil at Griffin, Georgia 1988.

RANK	LINE	TOTAL PASSAGES	PASSAGES ON STRESS	STRESSING* AGENT	VIGOR2**	SEED WEIGHT (G/PLOT)
1	02410079	13	3	100 CL	4	51.3
2	02301049	12	3	100 AL	2	15.6
3	022866	11	1	3 HYP	4	13.4
4	02410041	10	2	150 CL	2	12.5
5	02420005	12	2	3 HYP	5	11.0
6	2030604	8	2	300 AL	2	10.3
7	2000109	15	6	100 AL	3	9.5
8	2030310	8	1	300 AL	3	8.3
9	02410033	13	3	150 CL	3	7.9
10	02410025	11	3	100 CL	2	7.4
11	2030310	8	1	300 AL	3	6.8
12	02301050	13	4	100 AL	4	6.3
13	2030309	8	1	300 AL	3	5.9
14	02420005	12	2	3 HYP	3	5.0
15	02301038	12	3	100 AL	2	5.0
16	022868	12	2	5 HYP	4	4.7
17	02410048	10	2	150 CL	4	4.4
18	02410028	12	4	100 CL	3	4.4
19	2030302	8	1	300 AL	2	1.8
20	02410005	13	3	100 CL	2	0.8
21	862905	12	2	3 HYP	3	0.6
22	02410005	13	3	100 CL	2	0.3
23	02001011	12	0	0	3	0.2
24	2000110	15	6	100 AL	3	0.2

* CL=potassium chlorate(ppm); AL=aluminum chloride(ppm); HYP=hydroxyproline(mM)

** VIGOR2 109 days after planting 1=dead 5=healthy

Individual lines were identified which were outstanding in the R_1 evaluation. From non-stressed cultures, 6% of the lines sent set seed. But from Al, Cl and Hyp-selected cultures 22, 16 or 31% of the lines evaluated set seed, respectively. This data indicates that identifying lines tolerant to acid soils from non-stress is a rare event; however, from Al, Cl or Hyp-selected cultures, identification is more likely. This also confirms Dr. Duncan's research showing that there are other nutritional factors involved in acid soil tolerance, not strictly Al tolerance.

Evaluation of R_3 Population Not Previously Tested on Acid Soils. A total of 107 R_3 sorghum lines which were not previously tested on acid soils were evaluated in 1988. Most were sib lines of those selected on acid soil in 1987 and were re-evaluated to identify other material which was acid-tolerant. These lines were advanced to the R_3 generation on normal soil conditions in 1987 (see Flow Chart Figure 21). This R_3 population consisted of Hegari lines regenerated from cultures with or without NaCl.

Lines did not differ significantly for stand or for the first and second vigor ratings ($p=.309$, $p=.408$ and $p=.270$ respectively). Blocking was effective ($p<.01$). Lines that survived and set seed will be tested again in 1989 on acid soil. Table 10 shows R_3 lines ranked by seed yield in grams/plot, <u>in vitro</u> history and corresponding 109 day vigor rating. There were ten lines that ranked higher than the Hegari check. The purpose of this experiment was to identify lines which could provide material for breeders with acid soil tolerance. Ten lines produced more seed than the Hegari check and three lines produced four times as much seed.

Table 10. R_3 (not previously stressed on acid) sorghum lines that survived and set seed under acid soil at Griffin, GA, 1988.

LINE #	TOTAL PASSAGES	STRESS PASSAGES	STRESS LEVEL g/l	VIGOR *	SEED WT. g/plot
862125-4-7	6	0	0	4	81.1
862043-1-2	6	0	0	4	37.3
862125-4-6	6	0	0	3	25.1
862131-4-5	6	0	0	4	19.4
862116-3-4	6	0	0	3	18.0
862024-6-7	6	0	0	3	14.6
862045-2-1	6	0	0	3	12.7
862043-5-3	6	0	0	4	10.1
862132-2-3	6	0	0	3	9.5
862516-1-2	13	0	9 NaCl	2	8.1
hegari check	-	-	-	3.3**	6.6
862131-4-11	6	0	0	3	5.6
862515-8-4	13	7	9 NaCl	3	3.7
862220-1-10	12	0	0	3	3.3
862118-3-5	6	0	0	3	2.3
862102-5-3	6	0	0	3	1.7
862131-6-9	6	0	0	3	1.4
862110-6-5	6	0	0	4	1.2
862113-2-1	6	0	0	3	1.0
862223-1-8	12	0	0	3	1.0
862110-2-5	6	0	0	3	0.7
862118-2-1	6	0	0	3	0.6
862131-1-5	6	0	0	3	0.3
862516-1-2	13	7	9 NaCl	3	0.3
862043-1-5	6	0	0	3	0.2

* 109 day vigor rating
** Mean of 3 plots

R_3 Acid Soil Tolerance Confirmation Study, Griffin, Georgia, 1988. The objective of this study was to confirm acid soil tolerance of 38 R_3 lines selected in 1987. These 38 lines were derived from cultures with and without NaCl <u>in vitro</u> selection. Soil samples taken had a mean pH of 4.27 and 181 ppm exchangeable aluminum.

Standard analysis of variance (Anova) procedures could not be conducted on this population due to lack of a normally distributed

sample. Comparisons between the regenerants and the non-regenerant Hegari checks were not possible by Anova because all Hegari checks failed to survive. Therefore a 2 x 2 chi-square analysis was conducted on the following categories: 1) alive versus dead, and 2) regenerants versus Hegari checks. The results are shown in Table 11.

Among the 39 regenerated lines tested, only four lines survived and set seed. These lines are shown in Table 12. Line number 862021-4-1 performed consistently across the three replications. This line ranked third in seed production for the 1987 acid soil study. The results from this year's test confirms that 862021-4-1 has acid soil tolerance. A 2x2 chi-square analysis conducted on 1) alive versus dead and 2) line 862021-4-1 versus Hegari check is shown in Table 13. The chi-square value was multiplied by 260 (number of lines in the original R1 population) according to Bonferoni's procedure. The Bonferoni procedure accounts for the fact that a selected line is being compared against the check. This procedure multiplies the number of lines in the original population by the probability from the chi-square.

Line 2075 is derived from RTx-430 which is very sensitive to acid soils. This line set seed in 1986's acid soil study in the R_1 generation. In 1987, the line did not survive but was located in an area of the field in which the tolerant check, Sc-283, also did not survive.

Table 11. Frequencies of regenerated and non-regenerated (check) Hegari lines by alive and dead.

	ALIVE	DEAD	TOTAL
HEGARI NON-REGEN. CHKS.	0	13	13
ALL REGENERANTS	32	75	107
TOTAL	32	88	120

CHI-SQUARED VALUE P=.021

Table 12. Surviving lines from R_3 sorghum confirmation study.

LINE NUMBER	TOTAL PASSAGE	PASSAGES ON STRESS	STRESS AGENT(g/l)	VIGOR 2*	SEED WT (g/plot)
862021-4-1(REP 1)	6	0	0	4	12.8
862021-4-1(REP 2)	6	0	0	3	9.6
862539-4-15	13	7	9 NaCl	4	6.7
862021-4-1(REP 3)	6	0	0	4	4.7
862075-46-1	3	0	0	4	4.0
862075-44-3	3	0	0	4	2.6

* 109 day vigor rating

Table 13. Frequencies of Line #862001-4-1 and non-regenerated Hegari (check) by alive and dead.

	ALIVE	DEAD	TOTAL
862021-4-1	3	0	3
HEGARI NON-REGEN. CHKS.	0	13	13
TOTAL	13	13	16

CHI-SQUARE VALUE P<.0001 Bonferoni TEST P=.026

Evaluation of R_4 Population on Acid Soils. Six R_4, regenerated sorghum lines were tested for acid soil tolerance. These lines were selected from lines which survived and set seed in the 1987 acid soil test. They were increased to the R_4 generation in the TCCP's greenhouse. The area of the field where the R_4 population was planted had a pH of 4.25 with 250 ppm of aluminum. In this area no plants survived including the Sc-283 check.

Evaluation of Puerto Rico Population on Acid Soils. Twenty-nine R_2 and 24 R_4 regenerated sorghum lines were tested for acid soil tolerance. These lines were increased in a Puerto Rico winter nursery. The R_1 lines were selected from $AlCl_3$ in vitro. The R_4 lines were selected from the 1987 seed increase field at Griffin and then increased to R_4 at the Puerto Rico winter nursery. This plot was put together by Dr. Duncan and was separated from the rest of our material. This area was also low in pH and none of these lines survived to set seed.

R_1 and R_3 Sorghum Seed Increase. Four-hundred and eighty regenerated sorghum lines consisting of both R_1 and R_3 populations were planted in May 1988 on a normal pH soil in Griffin, Georgia to increase the seed for further field testing. The lines were derived from callus tissue which had been exposed to different numbers of passages and levels of stress from the following stressing agents: 1) potassium chlorate, 2) hydroxyproline, 3) sodium chloride, and 4) aluminum chloride. The sorghum lines were planted for seed increase in single row plots 3 m. long and .75 m. between rows. Appropriate weed and insect control was provided. The soil pH of the seed increase was 5.1.

Visual ratings for vigor and observations for variability were made by TCCP personnel on June 28 (42 days after planting). The mean vigor rating for all 480 lines was 2.88. There were four lines which rated 5

for vigor (1=dead 5=high vigor). One line from RTx germplasm (862076-46-1) had exceptionally high vigor compared to its non-regenerated check. This line also had the best vigor ratings in the 1988 Yuma, Arizona drought tolerance study. Variation was also observed for earliness in one regenerated Hegari line (862019). This line was headed at 42 days after planting; whereas normal heading for Hegari occurs 60-70 days after planting.

Seed harvested from the $AlCl_3$ lines will be combined with remnant seed of the R_2 lines from Puerto Rico and field tested in 1989 on acid soils. This experiment will have a substantial number of lines and will be specifically designed to answer the question of what *in vitro* process results in the highest acid soil tolerance.

Millet and Sorghum Drought Studies in Zimbabwe

The seed for these drought experiments was originally sent to Dr. Maruli of the University of Nairobi, Kenya. Due to a problem in obtaining a field permit, Dr. Maruli was unable to plant the experiment. The TCCP contacted Dr. Ian Robertson of the University of Zimbabwe regarding the possibility of conducting this drought study, to which he agreed. Dr. Robertson, in collaboration with ICRISAT, planted this seed in June, 1988 for a seed increase.

The seed for these experiments originally consisted of 20 R_3 regenerated Hegari sorghum lines derived from NaCl *in vitro* selection and Sc-283, Hegari and M35-1 non-regenerated checks. The sorghum and millet checks were sent to determine their day-length sensitivity and survivability.

Sorghum Field Trials in Botswana

Abstract. Ten R_3 Hegari grain sorghum lines and six check lines were evaluated on drought and acid soil stress environments in Botswana during the 1988 season. Regenerated lines were derived from Hegari material selected *in vitro* on NaCl and from non-stressed cultures. Checks used for comparison included three Botswana adopted lines, the Hegari parent line, a drought-tolerant line, and an aluminum-tolerant line. The study was planted in December 1987 at two sites; one field primarily under drought stress, and one field having low soil pH. All lines were evaluated for traits related to stress tolerance including germination, stand, height, maturity, disease, insect infestations, biomass, and grain yield. Hegari had the highest mean grain yields but was statistically no different than R_3 lines 862110-8-6, 862110-2-5, 862024-7-2, 862043-9-8, 862384-6-10, 862518-4-9, and 862131-1-5. Three of the lines originating from non-stressed cultures and moderate passage time out-yielded all lines from long-term (>11) salt-stressed cultures.

Field Conditions. This test was conducted in collaboration with Dollina Malepa of the Botswana Department of Agricultural Research. She evaluated our material in two test sites. The first site was located at Sabele, Botswana near their main research facility in Gaborone. This site has a soil pH of approximately 6.0 and is rainfed. Periodic drought of prolonged duration is the major factor limiting plant growth; however, the 1988 field season was wetter and cooler than average. The plots were planted at Sabele on December 7, 1987. Anthesis occurred around February 16, 1988 and harvest was on May 4, 1988. Sabele is approximately 1000 meters above sea level and is located at latitude 24° 34' south. One of the major objectives of this study was to determine if our material could be successfully tested in southern Africa.

The second test site in Botswana was near the town of Goodhope, approximately 120 km south of Gaborone. This station is located in a more productive agricultural region which generally receives greater rainfall than Sabele. Our plots were planted on a moderately acidic soil (pH 5-6) with aluminum saturation of 30-40 percent. Small areas of much lower pH and high aluminum saturation caused variability within the field, resulting in somewhat erratic stands. Planting occurred at Goodhope on December 15, 1987. Plants began to flower on January 27 and the later lines continued flowering until early March. The Goodhope plots were harvested on May 13, 1988.

Both tests were planted in a randomized complete block design with four blocks. Individual plots were single rows approximately 6 m long. Planting densities in southern Africa are very low by our standards in order to compensate for inadequate moisture. Notes were taken by Ms. Malepa throughout the study for such factors as emergence, stand, disease incidence, insect infestation, height, flowering dates and head number. Dry matter and grain yields were taken at harvest.

The lines chosen for inclusion in this study were R_3 generation Hegari derived from salt-stressed and non-stressed cultures. The regenerated lines had rated among the best for vigor and yield on acid soil stress in Griffin, Georgia during 1987. Only 10 lines were sent for testing because our collaboration in Botswana was new and we were unsure how well Hegari would perform in southern Africa. The check lines were included because they possessed known traits which were used for comparison to the regenerated materials. Line M-35-1 is known to be drought-tolerant, while Sc-283 is known to be acid-soil-tolerant. Hegari was included as the parental check line and three Botswana adapted lines

were included for yield comparison. Table 14 lists the lines used in this study and their tissue culture background. The same lines were evaluated in both Sabele and Goodhope.

Table 14. Sorghum Lines Included in Botswana Study

Line	Background	*in vitro* Stress Level g/l NaCl	Total Passages	Stress Passages
862024-7-2	R_3 Hegari	0	6	0
862043-9-8	R_3 Hegari	0	6	0
862110-2-5	R_3 Hegari	0	6	0
862110-8-6	R_3 Hegari	0	6	0
862128-5-8	R_3 Hegari	0	6	0
862131-1-5	R_3 Hegari	0	6	0
862384-6-10	R_3 Hegari	9	15	4
862471-7-6	R_3 Hegari	9	11	5
862518-4-9	R_3 Hegari	13	9	7
862522-10-4	R_3 Hegari	13	9	7
Hegari	Parental Check			
M-35-1	Drought tol.			
SC283	Al tol.			
Marupantse	Botswana			
Segaolane	Botswana			
65-D	Botswana			

Results. The small field test conducted in Botswana this year was successful for several reasons. We learned more about what is involved in field testing and sorghum research in Africa. We also successfully identified a network of scientists in southern Africa which we can work with to do field testing of sorghum, millet, tepary beans, and moth beans. It was further established that Hegari material performs fairly well in southern Africa. Lines M-35-1 and Sc-283 are not quite as well adapted as Hegari, but will grow and complete their life cycles.

The regenerated material sent for evaluation performed well in general, but was not significantly superior to the parental check. This may be explained in part by the fact that the stress levels in Botswana

this year were not nearly as high as usual. More plentiful rainfall kept drought conditions from becoming extreme and may have masked any significant differences in drought tolerance within the regenerated lines. Table 15 summarizes the grain weight per plot for both locations. Lines are ranked in descending order with Duncan's grouping numbers to the side. Hegari tops the list at both sites; however, it is not significantly different from lines 862110-8-6, 862110-2-5, 862024-7-2, and 862043-9-8 at both locations. R_3 lines 862384-6-10, 862128-5-8, and 862518-4-9 were not significantly less than Hegari for grain yield at Sabele. The four R_3 lines which performed well at both sites are from cultures maintained for six passages with no <u>in vitro</u> stress. Lines 862522-10-4 and 862471-7-6, which performed poorly in both sites, were originally regenerated from salt-stressed cultures maintained for 11 or more passages.

Table 15. Mean* Total Grain Weight per Plot**

Location: Sabele			Location: Good Hope		
Line #	Grain Wt. g	Duncan Grouping	Line #	Grain Wt. g	Duncan Grouping
Hegari	847.5	A	Hegari	475.0	A
862110-8-6	740.0	A	Segaolane	462.5	A
862024-7-2	677.5	AB	862110-2-5	342.5	AB
862110-2-5	660.0	AB	862024-7-2	332.5	AB
Marupantse	657.5	AB	862110-8-6	325.0	AB
862384-6-10	612.5	ABC	65D	277.5	ABC
862128-5-8	595.0	ABC	Marupantse	270.0	ABC
862518-4-9	592.5	ABC	862043-9-8	260.8	ABC
862043-9-8	570.0	ABC	862518-4-9	217.5	BC
862131-1-5	417.5	BCD	862384-6-10	215.0	BC
Segaolane	360.0	CDE	862131-1-5	210.0	BC
65D	352.5	CDE	SC283	196.5	BC
862471-7-6	332.5	CDE	862522-10-4	167.5	BC
862522-10-4	275.0	DEF	862128-5-8	160.0	BC
M35-1	127.5	EF	862471-7-6	145.0	BC
SC283	40.0	F	M35-1	54.0	C

*Means are for 4 blocks in a RCB design.
**ANOVA for grain weight significant at .01 level for both locations.

Data collected on stand, dry matter, yields, head number, and head weight generally followed the trends for grain yield. Head weight was seen to be inversely proportional to stand and probably not a good indication of stress tolerance. Data on height, flowering date, insect and disease resistance was not significant.

Drought Tolerance Screening of Sorghum

Abstract. Two test plots of R_2 and R_3 sorghum lines were evaluated for drought tolerance at the Yuma Valley Experiment Station. Sorghum entries were derived from salt-stressed and non-stressed tissue culture selection. Data were taken and analyzed for stand, vegetative vigor, reproductive vigor, height, and maturity. When completed, data on grain yields and seed weights will also be evaluated. Preliminary results indicate significant progress has been made in stress tolerance through tissue culture on both Hegari and RTx-430. One particular RTx-430 line is extremely promising for its overall tolerance to acid, saline, and drought soils.

Field Conditions. Field testing of TCCP regenerated sorghum lines continued this year in Yuma, Arizona, in collaboration with Dr. Robert Voigt of the University of Arizona. Last year's trial at Yuma proved that completely uniform salt stress was not possible to obtain at this site, so it was decided that the primary factor to be evaluated this year was drought tolerance. Yuma is an ideal location for high temperature drought evaluation since it generally receives less than three inches of precipitation during the summer growing season and temperature commonly exceeds 40° C. Adequate irrigation water allows drought stress to be maintained near desired levels. Salt stress, high temperatures, and insect infestations significantly increase the overall

stress on the regenerated material tested at this site. Salt levels, however, are variable throughout the field and necessitate many replications for adequate evaluation.

The sorghum trials this year were divided into two separate studies. The first, experiment 8802, was a screening of 95 R_2 sorghum lines. The second, experiment 8803, consisted of 96 R_3 sorghum lines. These experiments were planted adjacent to each other in a randomized complete block design on April 27 and 28, 1988. Three complete blocks for each experiment were planted across the furrow irrigation gradient. A total of 760 plots, including checks and borders, were established on single rows three meters long, with one meter centers on a clay loam soil with pH 7.9. Three irrigations on alternate rows were necessary to ensure good stands. Thereafter, irrigation was withheld until plots were under severe drought stress. Three additional irrigations were applied between emergence and soft dough in order to keep the plants alive and maturing. Drought stress was severe enough at this level to delay flowering by approximately 40 to 50 days past the normal flowering date.

Salt stress on these plots was considered to be a secondary stress factor; however, salinity levels built to critical levels in localized areas within the plots. The highest salinity levels were generally at the bottom end of the flood irrigation gradient and were severe enough to completely halt maturation of some lines. Table 16 shows that variations in soil salinity were from EC 1.4 ms/cm to 12.1 ms/cm. Previous research has indicated that Hegari suffers some damage at approximately 6 ms/cm.

Table 16. Soil data from Yuma Test Site.

Sample	EC ms/cm	Stage of Growth When Sample taken
West 1/2 composite	7.6	Vegetative
East 1/2 composite	4.7	Vegetative
Plot 1335	5.1	Heading
Plot 2022	1.9	Heading
Plot 1125	6.0	Heading
Plot 1411	1.4	Heading
Plot 605	12.1	Heading
Northeast 1/4 composite	1.6	Heading

Lines Tested. Both the R_2 and R_3 experiments were set-up to screen regenerated germplasm for drought tolerance utilizing the same experimental design. The R_2 lines were selected for testing from the TCCP sorghum germplasm on two criteria. The primary basis was to re-test the R_1 lines that survived stress conditions in Yuma in 1987. The second group of lines included in the R_2 test were from the 1986 Texas increase which had not been previously screened on stress. Space, personnel, and the need for at least three replications per line limited the total number of R_2 lines to less than one hundred.

Based on these criteria, ninety-five lines were chosen for inclusion. All lines were originally derived from Hegari cultured from 4 to 13 passages. Approximately 43% of the lines were from NaCl-selected cultures, with the balance from non-stressed cultures. Table 17 summarizes the origin of the R_2 lines included in this experiment. Most

of the stressed material is regenerated from the higher number of *in vitro* passages. This makes comparison of stress level by passage difficult within this experiment.

Table 17. Number of R_2 lines tested at Yuma from specific *in vitro* treatments.

In vitro Stress level, g/l NaCl	4	5	6	7	10	11	12	13
15								3
9						13		25
0	12	3	8	15	8		8	

Number of Passages *in vitro*

The R_3 lines included in this study were primarily Hegari lines which survived last year's study in Yuma. Also included were three R_3 Hegari lines from acid soil evaluation in Georgia and six RTx-430 lines which survived acid soil stress and drought during 1987 Georgia and Yuma trials. Approximately 80% of the Hegari lines were originally derived from *in vitro* salt-stressed cultures, with the remaining lines originating from non-stressed cultures. The six RTx-430 lines were derived from cultures maintained for three passages under no *in vitro* stress. Table 18 summarizes the number of R_3 lines from specific *in vitro* treatment combinations.

Table 18. Number of R_3 lines from specific *in vitro* treatments.

In vitro Stress level, g/l NaCl	3	6	9	10	11
12			21	38	
9					19
0	6	3		13	

Number of Passages *in vitro*

Two site visits were made to Yuma this year in order to collect data on these experiments. The first visit was on June 15, approximately 50 days after planting. Normally the sorghum would be near the boot stage after 50 days, but poor planting conditions and drought had significantly slowed development. The sorghum ranged in growth stage from four to ten leaves, with six leaves being the average. All plots were scored for stand, vegetative vigor, and vegetative drought tolerance. Ratings were based on a 1 to 5 scale with 1 representing the best plants in the field and 5 the poorest plants. The performance of parental check lines was not considered when establishing the rating scale so that all plots were rated "blind."

The second site visit took place August 15-18 and coincided with anthesis for most of the material. By this time the plots had been under very severe drought conditions and considerable heat, insect, and salt stress as well. All plots were rated for vigor, height, and earliness at this stage. Reproductive vigor ratings were based on the same scale used during the first visit and took into consideration the state of the

vegetative portions of the plants, as well as the number and size of panicles. Height ratings were scored on a 1 to 5 scale that was an average of the entire plot height. A rating of 1 for height was given to plants over 2 meters, 3 was given to plots that averaged 1.3 meters, and 5 was scored on plots which were less than .75 meters. Height data were taken because it is a character which is very elastic in the Hegari cultivar and relates to the level of drought or salt stress. Cultivar RTx-430 does not fluctuate in height to the extent that Hegari does. Maturity or earliness is a third trait which is conditioned by environmental stress. Drought and salinity can slow down the maturation process, and under extreme levels will halt plant growth altogether. Hegari will normally reach anthesis within 60 to 70 days after planting. At 112 days after planting, the plots were rated for earliness and it was noted that the majority of lines had just reached anthesis. Earliness was therefore considered another good measure of stress tolerance in our regenerated material. It was rated on a 1 to 5 scale as follows: 1 = hard dough, 2 = soft dough, 3 = anthesis, 4 = boot, and 5 = vegetative.

Results. Due to the large number of lines evaluated, and the lack of personnel available, only the lines which were to be advanced for further testing were bagged to ensure self-pollination. This necessitated choosing the best 5-10% of R_2 and R_3 lines during the second visit. Choices were made on the basis of data taken on both visits, but before any statistical analysis could be conducted. Therefore, it was also necessary to use subjective criterion and a visual assessment of which lines were maturing normally in the high stress areas in order to select the top 10%. In most cases, selections were made in favor of the lines which performed the best on the bottom end of the field. Both

salinity and drought stress were much more severe in this area and precluded normal maturation in many cases. All three blocks were used for the statistical analysis of the entire data set and this led to some differences between top rated lines statistically and top rated lines visually. At the time of this report, all selected sorghum lines have been harvested, but the grain has not yet been received in Fort Collins. Total grain weights and 100 seed weights will be calculated after all heads are threshed. This data will be included in the analysis and ranking of regenerated sorghum lines.

R_2 Test Results. The R_2 Hegari material screened on drought conditions showed a great deal of variation for tolerance in the field. Some lines appeared to be stress-tolerant while others were barely alive. Statistical analysis of the data using SAS software showed that there were highly significant differences between regenerated lines. There were also large differences between the check line and certain regenerated lines. Table 19 shows that stand, vegetative vigor, and maturity were highly significant. Regenerated lines 864012-22, 862218-24, 862594-37, 2509-36 were significantly superior to the Hegari check for these traits.

Table 19. Statistical data from R_2 Analysis of Variances

Independent variable	Dependent variable	df	Prob>F	C.V.	mean of all lines	mean of check
R_2 Hegari lines	Stand	95	.0001	18.89	3.09	3.74
R_2 Hegari lines	Veg Vig	95	.0119	21.35	3.25	3.56
R_2 Hegari lines	Repro Vig	95	.074	21.81	3.43	3.54
R_2 Hegari lines	Maturity	95	.0039	23.32	3.49	3.77
R_2 Hegari lines	Height	95	.2228	23.70	2.76	2.51
Bagged R_2 lines	Stand	7	.0024	17.21	3.50	
Bagged R_2 lines	Veg Vig	7	.0026	16.57	3.42	
Bagged R_2 lines	Repro Vig	7	.0226	28.09	3.23	
Bagged R_2 lines	Maturity	7	.0001	23.47	3.36	
Bagged R_2 lines	Height	7	.5818	28.49	2.65	

The four above-mentioned lines looked very good in the field under stress conditions, as did several others. A total of seven R_2 lines were selected to be re-evaluated on stress in next year's study. These lines were bagged to ensure self-pollination and were the only lines in the entire R_2 population to be harvested. Table 20 lists these lines and their *in vitro* background. Material stressed on 9 g/l NaCl made up 40% of the total R_2 population, while 57% of the selected lines were from this stress level; however, the two top rated lines were 864012-22 and 862218-24, both from 0 g/l NaCl. The analysis of passage number indicated that length of time in culture was not generally significant in this study. Further study using the line source irrigation gradient would be very useful in identifying the most drought-tolerant material from this population.

Table 20. R_2 lines selected for further study

Line Number	*in vitro* Stress Level	Passage Time
864012-22	0 g/l NaCl	4(0)
862168-22	0	4(0)
862218-24	0	12(0)
862436-32	9	11(5)
862509-36	9	13(7)
862594-36	9	13(7)
862594-37	9	13(7)

R_3 Test Results. The R_3 drought screening yielded ten lines which were superior to the parental checks. These ten included eight Hegari lines and two RTx-430 lines. Five or more heads were bagged out of each replication for all ten lines. These heads will be bulked and advanced to R_4 as lines. Table 21 lists the selected lines and their *in vitro* background.

Table 21. R_3 lines selected for confirmation.

Line Number	*In vitro* Stress Level	Passage Time	Parent
862075-46-1	0 g/l NaCl	3(0)	RTx430
862075-44-7	0	3(0)	RTx430
862021-4-1	0	6(0)	Hegari
862442-7-21	9	11(5)	Hegari
862443-1-21	9	11(5)	Hegari
862640-5-25	12	9(3)	Hegari
862640-10-23	12	9(3)	Hegari
862644-2-22	12	9(3)	Hegari
862649-6-25	12	10(4)	Hegari
862650-2-21	12	10(4)	Hegari

Half of the selected lines originated from an in vitro stress level of 12 g/l NaCl. Statistical analysis of the data on vegetative vigor shows that R_3 lines from all in vitro stress levels were significantly superior to the Hegari check. The linear contrasts shown in Table 22 indicate that significant progress has been on the parental check through tissue culture. All of the R_3 lines have been through at least one cycle of selection on stress, so this is not unexpected; however, the performance of a few lines was exceptional. Hegari R_3 lines 862021-4-1 and 862442-7-21 rated very highly, both visually and statistically. Sister lines of these selections have shown acid tolerance and insect tolerance in Georgia.

Table 22. Linear Contrasts of R_3 Hegari lines for the variable: Vegetative Vigor*

CONTRAST				
X_1	X_2	$\bar{X}_1$	$\bar{X}_2$	P>F
Hegari Check vs.	Nonstressed in vitro	3.613	3.352	.1587
Hegari Check vs.	Stressed in vitro	3.613	3.101	.0002
Nonstressed vs stressed		3.352	3.101	.0804
Hegari vs. all lines from 12g/l		3.613	3.195	.0024
Hegari vs. all lines from 10 passages		3.613	3.194	.0032

* Lower number indicates superior vegetative vigor score.

The most outstanding and interesting line in the entire drought test this year was RTx-430 selection, 862075-46-1. This line ranked number one for stand and vegetative vigor. It was statistically superior to RTx-430 check for vegetative vigor, reproductive vigor, and height. Visually, it

appeared taller and more vigorous than the check. Panicles were also larger on this selection than on any other regenerate of the check line. From these data and our visual assessment, it appears that this line has significantly enhanced tolerance to drought and saline conditions. Originally, this line was derived from RTx-430 callus which was cultured for three passages under no in vitro stress. During the 1986 field trial on an acidic soil in Griffin, Georgia, line 862075 was first selected as tolerant to stress conditions. Last year it was re-selected as the top yielding line on drought and saline soils in Yuma. This line shows outstanding potential for stress tolerance and will be re-evaluated and characterized in multiple locations next year.

The research in Yuma this year yielded some very promising results on the effect of tissue culture on stress tolerance. Several potentially useful genotypes are expected out of next year's confirmation studies. While this data does not allow the conclusion that one specific tissue culture treatment is most effective, it does indicate that significant improvement has been made on the original parent line for the combined stresses at Yuma.

Evaluation of Sodic-Saline Tolerance in Rice (Pakistan)

Abstract. The Nuclear Institute for Agriculture and Biology (NIAB) located in Faisalabad, Pakistan, evaluated 76 R_1 and 56 R_2 lines of rice for tolerance to sodic-saline soils. Regenerated lines derived from 5 cultivars—IR-36, Mahsuri, Pokkali, Calrose, and Giza-159—were evaluated. Of these cultivars, IR-36, Pokkali, and Mahsuri survived to set seed, while Calrose and Giza-159 did not. IR-36 regenerated lines had the highest yield; these lines will be evaluated for seedling tolerance to sodic-saline conditions in 1989.

Field Conditions. In July 1987, 76 R_1 and 56 R_2 lines derived from the cultivars Calrose, Giza-159, IR-36, Mahsuri, and Pokkali and their respective checks were planted on a sodic-saline soil at NIAB in collaboration with Dr. Kauser A. Malik. The objectives of this study were: 1) to determine which cultivars would survive on sodic-saline soils and 2) to identify superior lines from cultivars which survived. A total of 200 plots were planted with R_1 lines represented by one plot each (due to minimal seed quantities) and R_2 lines represented by two plots each. All lines were evaluated for germination, seedling survival, number of heads, number of tillers, height, and panicle yield.

Results. Out of the 132 lines evaluated at NIAB, 13 regenerated lines of Pokkali, Mahsuri, and IR-36 survived and set seed, while regenerated lines of Giza-159 and Calrose did not survive. Yield as panicle weight per plant was higher for the regenerates of IR-36 as compared to the IR-36 check. The regenerants of Mahsuri and Pokkali yielded the same or less than their respective checks (Table 23). From these three cultivars that set seed, 12% of the R_1 generation and 30% of the R_2 generation survived. Selected lines of IR-36 were planted in

August 1988 and will be confirmed for tolerance to sodic-saline conditions.

Table 23. Average height, tillers per plant and panicle weight of surviving lines from the NIAB sodic-saline field trial and their culture histories.

LINE#	TOTAL PASSAGES	STRESS LEVEL g/l NACL	AVG. HEIGHT cm	TILLERS /PLANT	PANICLE WT/PLANT g.
IR-36					
check	0	0	47.4	32.2	16.1
306026	4	6	51.9	29.0	42.5
306029	5	6	54.8	24.8	35.0
306052	12	6	58.2	26.4	30.0
300049	14	0	56.2	26.0	40.0
IR-36 regenerant means			55.3	26.6	36.9
MAHSURI					
check	0	0	62.8	34.6	26.6
403129	4	3	67.6	26.6	20.1
401001	4	1	58.6	16.6	17.5
408001	3	8	72.5	30.0	27.6
408001	3	8	70.3	26.6	20.2
408002	3	8	70.6	40.6	26.8
400012	8	0	63.0	30.6	17.2
MAHSURI regenerant means			67.1	28.5	21.6
POKKALI					
check	0	0	128.4	15.0	50.0
506056	14	6	107.0	15.9	45.0
509006	2	9	121.8	15.0	50.0
506059	5	6	47.4	32.2	22.4
506059	5	6	47.2	37.2	28.6
500034	4	0	116.4	6.4	30.0
500034	4	0	123.3	17.0	25.0
POKKALI regenerant means			93.9	20.6	33.5

Evaluation of Salt Tolerance in Rice (Philippines)

Abstract. In January 1988, 371 R_2 regenerated rice lines derived from IR-36, Mahsuri, and Pokkali were tested for saline stress tolerance at Pampanga, Philippines. Four treatments plus a control were used to test these lines. Treatments 2 and 4 had severe stress in which no plants survived; 327 plants survived from treatments 1, 3, and 5. Surviving lines from this study will be evaluated in 1989 at Pampanga to confirm their salt tolerance.

Field Conditions. In January 1988, 371 R_1 regenerated rice lines from IR-36, Mahsuri, and Pokkali cultivars were planted in a saline-prone, irrigated lowland rice trial. This research was conducted in collaboration with Drs. Jose E. Hernandez and Eduardo P. Paningbatan of the University of the Philippines. The experimental area was divided into five treatments corresponding to different salinity levels (Table 24). Salinity levels of river water used for irrigation increased steadily during the trial due to dry-season intrusion of salt water. Electrical conductivity and chloride content of saturated soil extract, paddy water, and river water were monitored periodically. Desired salinity levels were to be maintained by pumping fresh ground water from a near-by well. Due to delays in obtaining funds, the pump was not purchased. Consequently, irrigation was discontinued when salinity of the river water exceeded critical levels.

Results. Treatment 1 was intended as a non-saline control, irrigated with fresh ground water. Due to the unavailability of fresh ground water for irrigation, it was irrigated with saline river water, and therefore; treatment 1 was not effective as a control. Electrical conductivity levels for treatment 1 averaged 6.3 ms/cm for the month of March (Table 24) which resulted in an effective salinity screening

treatment. All other treatments exceeded the critical value for plant survival (electrical conductivity 8 ms/cm).

Most plants died due to excessively high salinity during irrigation and to drought after irrigation was discontinued. Surviving plants were found in three treatments. Treatments 1, 3, and 5 had 298, 8, and 21 surviving plants respectively (maximum possibility of 12,500 plants/treatment). Line numbers from the best surviving plants in Treatment 1 and from all surviving plants in Treatment 3 and 5 are shown in Table 25. These lines will be evaluated in 1989 at Pampanga to confirm their salt tolerance.

Table 24. Means of electrical conductivity (EC)* of saturated soil extract for January, February, and March 1988, for 5 treatments at Pampanga, Philippines.

TRT.#	TREATMEMT DESCRIPTION	JAN	FEB	MARCH
		Mean EC (ms/cm)		
1	control	2.8	4.5	6.3
2	5ms/cm from transplanting to harvest	5.5	6.4	11.2
3	2ms/cm 2 mths. after transplanting 10 ms/cm from transplanting to harvest	3.8	4.6	8.5
4	5ms/cm 2 mths. after transplanting 10ms/cm from transplanting to harvest	4.4	5.8	12.8
5	river salinity level	3.8	6.0	10.7

* electrical conductivity (EC) readings in millisiemen/cm (ms/cm).

critical level= 8 ms/cm.

Table 25. Top R_2 regenerated rice lines from treatments 1, 3, and 5 with corresponding passage and stress *in vitro* levels tested at Pampanga, Philippines.

LINE#	TOTAL PASSAGES	STRESS LEVEL (NaCl g/l)
IR-36 check	-	-
300010	5	0
300033	5	0
300035	13	0
300042	9	0
300044	8	0
300083	13	0
303092	6	3
303146	5	3
303152	5	3
303155	5	3
303162	5	3
303163	5	3
303170	5	3
303173	5	3
306022	4	6
306059	15	6
306060	15	6
308001	4	8
309001	5	9
Pokkali check	-	-
500019	3	0
500046	4	0
500052	4	0
509006	2	9

Evaluation of Regenerants from Rice Suspension Culture for Morphological Variations

Abstract. Somaclones derived from suspension cultures showed variation in height, vigor, heading, presence of awns, pubescence, type of ligule, and the presence of auricles. The somaclones were shorter in height and less vigorous than the checks. Only one line had the presence of awns. Five lines had auricles with the 2-cleft type ligules. The somaclones also had late heading and seed set.

Lines Used. Regenerated plants derived from suspension cultures of rice (Oryza sativa L. cv Taipei 309) were grown to maturity in the greenhouse. The R_1 seeds harvested by April 1988 were sent to Louisiana State University's (LSU) Rice Research Station in Crowley, LA for observation of somaclonal variation. Dr. M. C. Rush of LSU collaborated in this research. The R_0 plants were regenerated after 4.5, 5, and 6 months in suspension cultures. A total of 65 somaclones (R_1) of suspension cultures and five checks were planted on May 11, 1988. The seeds were planted with a single five-foot row for each entry with rows spaced 10 inches apart. Characters such as height, presence of awns, pubescence, heading, type of ligules, presence of auricles, and vigor were observed on August 23, 1988. Height was evaluated by measuring three samples from each line. Vigor was rated according to Table 26. Heading was rated by the numbers of heads formed as indicated in Table 27. Observation for seed set was also noted. The rest of the somaclone suspension culture lines will be sent to LSU's Rice Research Station for observation of somaclonal variation in the next growing season.

From 65 somaclones (R_1) of suspension cultures planted at LSU's Rice research Station, 33 lines were derived from 4.5-month-old suspension culture. Thirty-two lines germinated and were planted in the field. Three lines derived from five-month-old suspension cultures were planted and 27 out of 29 lines derived from six-month-old suspension cultures germinated and were planted.

The somaclones were shorter, less vigorous and later in maturity (Table 28). There were variations in the presence of awns, pubescence, and the type of ligules formed. Twenty-two lines of the somaclones had less pubescence than the control. One line had awns and five lines had a

2-cleft type of ligule with the presence of auricles while the rest of the lines including checks had acute type of ligule with no auricles. On August 23, heading was observed to be earlier in the controls than the somaclones. All of the controls had set seed at that time; nine somaclones were starting to set seed.

Table 26. Scoring chart for vigor.

SCORE	CHARACTERS
1	few tillers formed
2	some tillers formed but small in size
3	some tillers formed and large in size
4	many tillers formed but small in size
5	many tillers formed and large in size

Table 27. Scoring chart for heading.

SCORE	CHARACTERS
1	1-3 heads formed
2	4-6 heads formed
3	more than 6 heads formed

Table 28. Observations on height, vigor, and heading.

OBSERVATION	CONTROL[5]*	4.5 MONTHS**[32]*	5 MONTHS**[3]*	6 MONTHS**[27]*
AVERAGE HEIGHT (CM)	113.3	101.7	107.4	104.6
AVERAGE VIGOR	4.6	2.8	4	3.6
HEADING	3	2.2	3	2.3

* NUMBER OF PLANTS
** DURATION IN SUSPENSION CULTURE

Evaluation of Acid Soil Tolerance in Rice

Abstract. Seventy-five R_1 and R_2 regenerated rice lines were tested on acid soils on the eastern plains of Colombia. Lines were rated for blast and acid tolerance and for vigor and stand. Of the five cultivars tested (Calrose, Giza-159, IR-36, Mahsuri, and Pokkali) Pokkali lines performed the best. Calrose line number 106001-1 rated the highest for vigor. The Calrose check and other regenerated Calrose lines had low vigor ratings and many were killed by blast and acid conditions.

Field Conditions. A total of 75 regenerated rice lines derived from the cultivars Calrose, Giza-159, IR-36, Mahsuri, and Pokkali were evaluated for tolerance to acid soils at La Libertad, Colombia. The objectives of this study were: 1) to determine which cultivars would survive acid soil conditions in Colombia, and 2) to identify superior lines from these cultivars. Dr. Surapong Sarkarung of CIAT (Centro Internacional de Agricultura Tropical) collaborated with TCCP in conducting this research. Dr. Sarkarung's research was designed to

develop germplasm with tolerance to both acid soils and to blast (Pyricularia oryzae), a fungal pathogen.

TCCP's lines were tested in the eastern savannahs of Colombia (La Libertad) on an acidic soil of 4.3 pH with 84% aluminum saturation. The experiment was laid out with two replications of 200 plots and with each plot represented by two, five-meter-long rows. Two acid and blast-tolerant checks and one acid and blast-susceptible check were planted every 12 rows of regenerated material. Alleyways were planted with a blast-susceptible cultivar. Plots were visually rated for acid and blast tolerance by Dr. Sarkarung. TCCP personnel rated the plots for general vigor and stand on August 2, 1988. Vigor was visually rated using a 1 to 5 scale with 1 = plants dead, 2 = plants stunted with severe nutritional deficiencies, 3 = some stunting and nutritional deficiencies, 4 = slight stunting and nutritional deficiencies, and 5 = plants healthy.

Results. Vigor ratings taken 75 days after planting should reflect the ability of the plants to withstand both blast and acid tolerance since vigor measures the general health of the plants. Lines derived from Pokkali had the highest mean vigor (3.1), followed by Mahsuri (2.7), Giza-159 (2.6), IR-36 (2.3), and Calrose (1.6).

Vigor ratings among lines within the five cultivars differed significantly ($p<.001$). There were three Pokkali lines, one Calrose, and one Giza-159 line which rated above 3.0 for mean vigor (Table 29). Most Calrose lines were killed by blast and acid soil conditions during early plant development and had low vigor ratings (1.6); however, one Calrose line, 106001-1, rated 4.0 which was the highest mean vigor rating among all lines. Giza-159 rated low for vigor (2.1). There was one exceptional Giza-159 line (206099-1) which rated higher than its check for general vigor. IR-36 lines rated low for vigor (2.3) and appeared to

be poorly adapted for these types of soils. Mahsuri lines were below average (2.6) for vigor; Mahsuri is too late in maturity to be an important cultivar for acid soils, according to Dr. Sarkarung. Pokkali lines rated above average (3.1) for vigor. Three Pokkali lines rated better than their check for vigor.

The best lines from this year's experiment along with new Giza-159 and Pokkali R_1 lines will be evaluated next year for tolerance to blast and acid soil conditions in Colombia.

Table 29. Mean vigor ratings of the top five lines and their checks in Colombia.

Cultivar	Line#	Total Passages	Stress g/l NaCl	Vigor Means*
Calrose	106001-1	7	6	4.00
Pokkali	506036-1	7	6	3.75
Giza-159	206099-1	7	6	3.50
Pokkali	506038-1	7	6	3.50
Pokkali	506056-1	7	6	3.25
Giza-159	check	-	-	3.00
Pokkali	check	-	-	3.00
Calrose	check	-	-	1.00

* Vigor means are based on 4 reps. 1=dead 5=healthy

Evaluation of Regenerated Millet Lines for Tolerance to Sodic-Saline Soils

Abstract. A total of 59 lines of regenerated pearl millet are presently being evaluated at the Nuclear Institute for Agriculture and

Biology (NIAB) located in Faisalabad, Pakistan. These lines are being evaluated for tolerance to sodic-saline conditions.

The objective of this study was to test 47 R_1 lines and 12 R_2 regenerated millet lines derived from HMP-559 and Senegal Bulk germplasm for tolerance to sodic-saline soils. These lines were planted at Faisalabad, Pakistan. The R_1 and R_2 populations were regenerated on NaCl as the in vitro stressing agent. Lines were planted in the third week of August, 1988 in a randomized complete block design. These lines will be evaluated for percent stand, number of plants harvested, and seed yield. This experiment will be harvested in December 1988 and the data analyzed.

Pigeonpea R_1 Salinity Seedling Screen

A total of 21 regenerated pigeon pea lines derived from 5, 7.5, and 10 g/l NaCl in vitro stress were screened for tolerance to salinity stress at the seedling stage. This study was conducted in collaboration with the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) located in Hyderabad, India. The experiment was conducted in ICRISAT's greenhouse and was a split-plot design with main-plots = salinity levels (0, 2, 4, 6, 8 ds/m), and sub-plots = regenerated lines. There were three replications. Lines were evaluated for percent survival, shoot dry weight, and root dry weight at 55 days after planting.

The interaction of lines with salinity levels for all traits measured was highly significant indicating differences in salinity tolerance among lines. Percent survival of many of the tissue culture-derived lines was slightly better than the tolerant checks, but the difference was not statistically significant. Eleven of the 21 lines had higher percent survival than the tolerant checks. Shoot and root dry

weight were not significantly different; however, performance was better than tolerant checks for shoot and root dry weight for 14 and 10 lines, respectively.

Greenhouse Evaluation of Regenerated Wheat Lines for Somaclonal Variations

Abstract. Wheat (Triticum aestivum L.) plants were regenerated from callus cultures of three spring wheat cultivars (Glennson, Pavon and PAK-16171) selected on medium with 0, 10, 15 and 20% (w/v) PEG-4000 for 90 days. Somaclonal variation was examined in the R_1 generation of nine different somaclones and the three non-tissue cultured parent lines. Characteristics examined included days to 50% flowering, flag leaf length, width and area, plant height, spike length, number of grains per spike, and days to maturity. Significant variations were found in all the characteristics measured. The most remarkable differences were observed in the flag leaf length and area which were three to four times greater in somaclones 71B88R1, 71C88R1, and 71D88R1 than the parent lines and two to three times greater in somaclones PA88R1 and PB88R1. The somaclones produced longer spikes and yielded more grains per spike than the parent lines.

Materials and Methods.

Tissue Culture and in vitro selection. Callus cultures were initiated from mature embryos of three wheat (Triticum aestivum L.) genotypes: Pavon, Glennson and PAK-16171. Mature embryos were germinated on callus induction medium of L & S basal salts, vitamins, 2.0 mg/l 2,4-D and 2% sucrose. The calli originating from the germinating embryos were subcultured at 30-day intervals.

After the fifth passage, when sufficient amounts of E calli were produced, known amounts of the calli were subjected to PEG-4000-induced water stress of 0, 10, 15, 20 and 25% (w/v). Calli were subcultured on the PEG medium for 90 days with a 30-day subculture interval. The E calli that survived after 90 days in stress were put on L & S basal salts and vitamins; the 2,4-D was replaced by 0.1 mg/l IAA and 0.5 mg/l BA for plant regeneration.

Screening and Selection of Potted plant in Soil. Screening was performed on R_1 plants in the greenhouse in the spring of 1988. Seeds were planted in February in individual pots in a mixture of fine peat consisting of perlite (Sunshine Mix of Fission Western Corporation), supplemented with fertilizers ($CaNO_3$ and NH_4NO_3), and grown in the greenhouse. To maintain the greenhouse temperature at 22 $\pm$ 3° C, supplemental heating was provided. The experimental design was a randomized complete block with twelve treatments consisting of three parents and nine R_1 populations with four replications. The plants were evaluated for the following characters: (1) Days to 50% flowering (number of days from planting to the exsertion of the 50% heading; (2) length, width and area of the flag leaf; and (3) plant height (main tiller from the soil level to the tip of the awns). After harvesting the following parameters were measured: (1) spike length; (2) number of grains per spike; (3) number of spikelets per spike; (4) number of grains per spikelet; (5) 100 grain weight; and (6) days to maturity (visual observations were recorded when all the spikes in a plant turned yellow).

Data from the different somaclones and the parent population were statistically analyzed to determine differences among the population. Most of the statistical analyses were done using the SAS statistical procedures employing the General Linear Model. Duncan's Multiple Range Test was applied to separate the means.

Results and Discussion. All nine R_1 somaclones differed from the three parent lines in most of the characteristics recorded (Table 30). A feature of potential importance was the increase in length, and area of the flag leaf. Only three of the nine somaclones (GA88R1, GB88R1, GC88R1) had less or similar flag leaf length, width, and area than the parent line (Table 30), while all other somaclones differed significantly from the parent lines (Figure 22). Two of the nine somaclones (PA88R1, PB88R1) had about twice the flag leaf area of the parent line while three of the nine (71B88R1, 71C88R1, 71D88R1) had about three to four times more flag leaf area than the parent line.

Table 30. Somaclonal variation among nine R_1 somaclones as compared to their parent lines of wheat (Triticum aestivum L. cv Glennson, Pavon, and PAK-16171.

Lines	CHARACTERS				
	Days to 50% Flowering	Plant Height (CM)	Flag Leaf		Number of grains per spike
			Length (cm)	Area (cm)	
GN88 (Parent)	73A *	77.67A	18.07C	17.66CD	48.00CD
$GA88R_1$	59F	72.50B	16.85C	15.78CD	55.50BC
$GB88R_1$	60C	78.28AB	13.88CD	14.36D	50.50C
$GC88R_1$	61D	76.50AB	17.63C	20.24C	62.75AB
$GD88R_1$	68B	72.00B	22.08B	17.91CD	65.50A
PN88 (Parent)	58G	72.25B	17.83C	16.89CD	33.25E
$PA88R_1$	57H	72.25B	26.78A	28.71B	44.75D
$PB88R_1$	57H	75.00B	27.75A	27.99B	49.50CD
PK-16171 (Parent)	61C	82.75A	10.48E	9.24E	34.25E
$71B88R_1$	63C	76.75B	24.75AB	36.13A	41.25DE
$71C88R_1$	57H	72.25AB	26.35A	40.89A	40.75DE
$71D88R_1$	57H	73.00B	22.04B	30.56B	40.75DE

* Means in column followed by the same letter do not differ significantly at 5% level of probability according to Duncan's New Multiple Range Test.

Variation in spike length was significantly different from the parent lines in somaclones of Glennson and PAK-16171 but did not differ in the somaclones of Pavon. The number of grains per spike were also higher in the somaclones with increased flag leaf area. The parental mean

was 37.7 ± 3 grains per spike while in the somaclones the means ranged from 36.0 ± 3.0 to 65.5 ± 1.26. Somaclones GC88R1 and GD88R1 had 62.75 and 65.5 grains per spike, respectively, compared to the parent line which produced 48.0 grains per spike. Hundred grain weight did not differ from the parent line in the somaclones of Pavon but significant variations were observed from the parent lines in the somaclones of Glennson weighing 3.83 and 4.52 gms and PAK-16171 weighing 3.48 and 4.49 gms, respectively.

Evaluation for days to maturity (by visual observation when most of the spikes in a plant turned yellow) was recorded. Since variations were present in the days to 50% flowering, variation in days to maturity was apparent. The plants of somaclones of Glennson and PAK-16171 matured five to six days earlier than the parent lines. These results demonstrate a relatively high frequency of variation in many of the individual characters measured in the R_1 plants with respect to the traits which were evaluated.

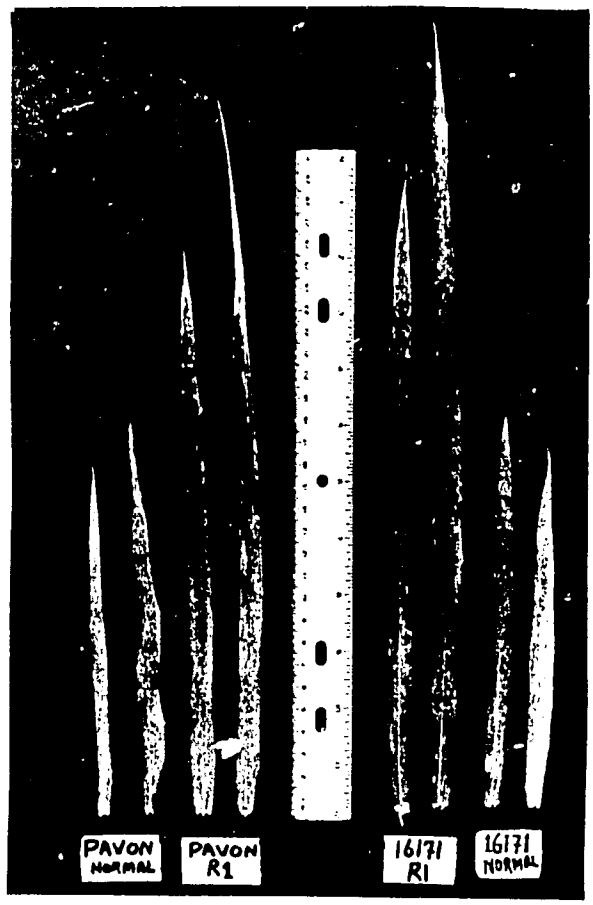


Figure 22. Variation in flag leaf length in R_1 lines of Pavon and PAK-16171.

Fall Armyworm Evaluation

Abstract. This report was extracted from reports sent to the TCCP by Dr. David Isenhour, University of Georgia. In 1986, nine lines were selected as having less feeding damage due to fall armyworm. In 1987, heads selected from these nine lines were evaluated under artificial infestation and showed little feeding damage. One line (2442), which showed tolerance in 1987, was tested in 1988 and again showed good tolerance.

Field Conditions. A study was performed to evaluate resistance to the fall armyworm (FAW) at Tifton, Georgia. The Tifton site is at the Gibbs farm of the Coastal Plain Experiment Station, a unit of the University of Georgia.

Seeds were planted on May 10, 1988 at Tifton and, at the whore stage, artificially infested with FAW neonates at a rate of 30 per plant. Visual rating of leaf-feeding damage was taken at 7 and 14 days post-infestation.

Entries number 12 (1821 cm) and number 11 (DC E57) which have a low level of resistance to FAW leaf-feeding, served as resistant checks. Entries number 9 (non-regenerated Hegari) and number 10 (Huerin) served as susceptible checks.

The study was arranged in a randomized block design. Individual plots consist of a single row three meters in length. The study at Plains was replicated 14 times and at Tifton, 15 times.

Results and Discussion. Table 31 presents mean damage ratings for FAW leaf-feeding for the screening of R_3 sorghum plants from selections of line 2442 made in 1987 at Tifton, Georgia. One entry, number 6, differed significantly from the non-regenerated Hegari at seven days after infestation. This line did not differ significantly from the

resistant checks or the susceptible check Huerin. The low damage rating for 1921 cm and especially DK E67+, are due more to growth characteristics than actual resistance mechanisms. The most vigorous of the entries screened was DK E57+. This line, which was in the late whorl stage at the time of the infestation, actually "out-grew" the FAW infestation as panicle development was initiated. The FAW larvae rely on the whorl as a protective site. Once this stage of the plant has passed, those larvae on the plant are exposed to the weather and natural enemies, thus FAW numbers drop drastically. The resistance in 1921 cm is also due in part to the growth characteristic of this line, but not totally.

The 14-day damage ratings revealed little difference between all of the 2442 lines and the susceptible checks. This was somewhat disappointing. A degree of resistance to FAW leaf-feeding does appear to exist in this line.

Table 31. Fall armyworm damage ratings for TCCP R_3 sorghum selected in 1987 from entry number 2442 at Tifton, Georgia, 1988.

		FAW Damage Ratings [1/]	
No.	Entry	7 Days	14 Days
1	1-2442	7 abc [2/]	8 a
2	1-2442	6 bcd	9 a
3	1-2442	7 abc	9 a
4	4-2442	8 a	9 a
5	4-2442	6 cde	8 a
6	4-2442	5 def	8 a
7	4-2442	6 bcd	9 a
8	4-2442 CSU-DO	7 ab	8 a
9	Hegari	7 abc	8 a
10	Huerin	6 bcd	8 a
11	DK E57+	4 f	5 b
12	1821 cm	5 ef	5 b

1/ Visual rating scale of FAW damage 0: Resistant > 9: Susceptible. Replications 1-8 were infested 20 June with 20 FAW neonate/plant. Replications 9-15 were infested on 22 June with 30 FAW neonates/plant. Damage ratings were made on 29 June and 6 July.

2/ Means within a column followed by a common letter do not differ significantly (P > 0.05; Duncan's multiple range test).

Greenhouse Activities

The TCCP greenhouse supports the tissue culture program in a number of ways. The major greenhouse functions include: 1) producing R_1 seed from regenerated plantlets, 2) keeping data on regenerates and processing seed for field testing, 3) providing training and technical support for TCCP trainees, 4) providing services and support for graduate students and visiting scientists, and 5) testing and increasing regenerated progeny.

The most significant task, in terms of space and time required, is the establishment, cultivation, and processing of regenerated plant materials. Improvement of establishment efficiency is a continual goal, and requires careful observation and record-keeping. Specific programs for the culture of each major crop have been developed, and are continually modified to improve seed production. Table 32 lists the total number of R_0 plants by crop that have been established in the TCCP greenhouse in the last year.

Table 32. Number of regenerated plantlets established in the TCCP greenhouse, October 1, 1987 - September 30, 1988.

Crop	Number of plantlets established
Rice	1,126
Millet	1,301
Sorghum	486
Spring Wheat	1,995
Winter Wheat	70
Mothbean	287
Tepary bean	50
Misc. legumes	25

Among the efforts to improve production efficiency is the improvement of greenhouse facilities. This year we completed the installation of overhead fog systems in each bay, as well as the installation of timed fluorescent lighting over the photosensitive crops. Other physical improvements included upgrading the cooling system for millet and wheat and replacing some worn double-poly roof covers.

Computerized Inventory System. Last year, a unified record-keeping system for all regenerated plants and their progeny was introduced. Since that time, the system has been debugged, and a large amount of data has been entered on the system. It is now used to facilitate the field testing in a number of ways; most significantly in quickly generating lists of regenerated lines and their backgrounds. This allows for a rapid summary of how many lines are available for testing from any given population or *in vitro* background. Additions to the inventory are entered on a daily basis. Table 33 summarizes the number of lines currently included on the database. Information recorded for each line includes: crop, cultivar, line number, lab experiment number, total passages, type of stress selection, level of stress, passages on stress, harvest date, grams of seed, and field testing information.

Table 33. Current number of entries for each crop and generations as of October 24, 1988.

Crop	# R_1 entries	# R_2 entries	# R_3 entries	# R_4 entries
Rice	2664	471	39	-
Sorghum	2570	3065	2506	18
Millet	673	53	-	-
Spring Wheat	1526	-	-	-
Winter Wheat	98	-	-	-
Legumes	73	-	-	-

R_0 Establishment Efficiency. One of the benefits of more efficient, streamlined programs is the freeing-up of time to compile and analyze more data. In the past there has been much effort put into improving the percentage of R_0 plantlets surviving transfer to the greenhouse, but little time has been spent in collecting data on efficiency. The effect of in vitro treatment on R_0 plant performance has yet to be quantified. This year, records were kept on all plantlets on the days to flowering, days to harvest, number of sterile plants, number of premature deaths, and the number of normal plants. These data have quantified the fact that plants from tissue culture are often variants from the parental material. Commonly occurring variants or mutants include steriles and albinos. Table 34 shows one example of the effect of passage time and stress on R_0 sorghum plantlets. This is not replicated or statistically analyzed data, but it clearly illustrates that the in vitro treatment has an effect on the physiological status of R_0 plantlets.

Table 34. Fate of R_o sorghum plants from Experiment 201 - Hegari Aluminum Tolerance Screening.

Treatment* #	*in vitro* Stress ppm Al	*in vitro* Passages	Sterile Plants	Premature Deaths	Matured Normally
			%	%	%
01	0	12(0)	-	-	100
02	100	12(1)	-	-	100
03	300	12(1)	-	-	100
04	0	13(0)	-	-	100
05	100	13(2)	-	-	100
06	300	13(2)	-	33	66
07	0	14(0)	-	11.1	88.9
08	100	14(3)	-	-	100
09	300	14(3)	25	12.5	62.5
10	0	15(4)	20	30	50
11	100	15(4)	20	20	60
12	300	15(4)	20	60	20

* Number of plantlets established from each treatment was based on regeneration efficiency and varied significantly by treatment.

TRAINING

The TCCP Training Program continues to serve the needs of researchers from developing countries by providing the necessary training for research in biotechnology. In the past year, TCCP has provided the facilities for the training of personnel with a diversity of interests ranging from *in vitro* selection of stress-tolerant crop species to micropropagation of ornamental plants and fruit crops.

The primary goal of the program is technology transfer to developing countries where it can be applied for general crop improvement. As intended, this has been successfully accomplished in the past twelve months. Nine participants have gained skills in the basic and applied concepts of plant tissue culture in the two, six-month courses conducted. One independent worker from Thailand was trained in the application of plant tissue culture for the propagation of fruit crops.

A major part of the training consists of individual research generally on the tissue culture of a crop plant. The fall 1987 trainees investigated the *in vitro* selection for stress-tolerant cereals and legumes. (See 1987 Annual Report.) The participants of the spring 1988 session based their research projects on cereal, root, and tuber crops, and ornamental plants (Table 35). In September 1988, Dr. N. Siddhivarn, an independent investigator intending to use *in vitro* techniques for the propagation of tropical fruit crops, began six weeks of training at TCCP. Much of her hands-on experience was under the supervision of Dr. H. Hughes of the Horticulture Department at Colorado State University, who is also an instructor in the TCCP Training Program.

Due to drastic cuts in the TCCP's AID budget and to the unavailablity of funds or sponsors for the nominees, the fall 1988, six-month course is not being conducted. A short-course of three months

duration, however, is to be held in November 1988. This course is based specifically on the application of in vitro selection techniques for the development of salt-tolerant cereal crops. Three scientists from Pakistan are scheduled to attend.

Regional Training. In October 1988, the Training Coordinator participated in the instruction of a plant tissue culture course held at CATIE in Costa Rica and sponsored in part by the TCCP. This course is ten weeks long and will be held annually, pending continued funding of the TCCP. The instruction of this course is mainly in Spanish and is designed specifically to train Latin American scientists. The TCCP laboratory manual has been translated into Spanish for use in the CATIE course. In the current session, seventeen students are participating. They represent Nicaragua, Guatemala, Honduras, El Salvador, Panama, Colombia and Mexico. All students are basing their individual research on tropical crop species such as cacao, banana, plantain, pineapple and palm species.

Future Considerations. The fall 1988 TCCP Training Program was not conducted due to lack of financial assistance to the candidates. The persistent difficulty in obtaining funds for longer term training raises a need for the reconsideration of the types of training offered through the TCCP training program. A combination of an annual six-month program on the basics and applied aspects of plant tissue culture and one or more short-term (six to twelve weeks) courses per year on advanced or specialized topics could fulfill the needs of technical personnel involved with crop improvement programs.

Table 35.

Researcher	Country	Funding	Title of Project
A. Bouraimis	Greece	Personal	Micropropagation of ornamental indoor plants
R. L. Daniels	Soloman Islands	TCCP	Micropropagation of excised meristems from greenhouse grown yams (Dioscorea alata)
T. Goubatov	Bulgaria	State Committee for Research & Technology (Bulgaria)	Callus induction, plant regeneration and in vitro screening for drought tolerance in winter wheat
S. Ndungu	Kenya	TCCP	Callus induction and plant regeneration from immature embryos and leaf segments of maize
N. Siddhivarn	Thailand	Personal	Microprogation of fruit crops

INTERNATIONAL PLANT BIOTECHNOLOGY NETWORK

Grant year four, 1987-1988, was a time for growth and pursuing effective collaborations for IPBNet. Core collaborations, set firmly in place through work of prior years, provided IPBNet the opportunity to enhance its growth. A number of different areas received attention. Among these were: 1) Continued germplasm and field testing agreements for both domestic and international sites; 2) Strengthening and establishing regional training centers in Central America and Francophone Africa, respectively; 3) Technical assistance and participation by TCCP personnel in international scientific conferences; 4) Continued planning for the third IPBNet Conference which will take place in Nairobi, Kenya, in January 1989; and 5) Distribution of Newsletters #8 and #9, as well as the first IPBNet Directory and International Funding Resource List.

International collaborations have expanded. Active agreements now include CATIE, CIAT, CIMMYT, IAV Hassan II, ICRISAT, NIAB, PCARRD, UPLB, and ViSCA. Two informal arrangements were established with SADCC/ICRISAT and INTSORMIL involving sorghum field evaluations in Africa. North American collaborative research was altered to suit field testing priorities. Currently five U.S. institutions are involved. A summary of current collaborations appears in Table 36.

Table 36. Current collaborative agreements and sub-agreements.

Institution and Country	Activity	IPBNet Collaborator
CATIE, Costa Rica	Regional Training Course taught in Spanish emphasizing crops of regional importance.	Victor Villalobos
CIAT, Colombia	Field testing for aluminum tolerant rice.	Robert Ziegler
	Short-term training of personnel from CIAT Biotechnology Unit.	William Roca
CIMMYT, Mexico	Widecross and field evaluation of wheat. Personnel exchange	A. Mujeeb-Kazi
IAV Hassan II, Morocco	Regional Training Course taught in French emphasizing crops of regional importance.	Mohamed Aaouine M'Hamed Sedrati
ICRISAT, India	Sorghum drought testing through SADCC/ICRISAT program in Zimbabwe	Lee House Ian Robertson
	Exchange of chickpea germplasm and personnel from ICRISAT in a collaborative program to transfer biotechnology. Field testing of pigeonpea.	H. van Rheenen
NIAB, Pakistan	Personnel from NIAB for research producing rice/ kallar grass hybrids. Field testing rice and millet.	S. H. M. Naqvi Kauser Malik Yusuf Zafar
PCARRD, Philippines	Facilitation of sub-grant awards, coordination of collaborative research in the Philippines.	Crisanto Escaño
UPLB, Philippines (sub-grant [2])	Field testing of rice germplasm for salt tolerance.	Jose Hernandez
	In vitro research on heat tolerance in wheat.	Alfinetta Zamora

Table 36 continued.

U.S. Institution	Activity	IPBNet Collaborator
ViSCA, Philippines (sub-grant)	Research on regeneration and stress selection techniques for corn and rice.	Nguyen Thanh-Tuyen
Univ. of Arizona, Yuma Experiment Station	Field evaluation of sorghum for salt tolerance and millet seed increase.	Robert Voight Bill Blackledge
Univ. of Georgia, Griffin Experiment Station	Field evaluation for aluminum-tolerant sorghum germplasm.	Ronny Duncan
Univ. of Georgia, Tifton Experiment Station	Field evaluation of FAW resistance.	David Isenhour
Louisiana State University, Crawley Experiment Station	Rice germplasm increase.	M. C. Rush
Colorado State Univ., Ft. Collins & Cortez Experiment Station	Field Evaluation of tepary, mothbean, pigeonpea.	Mark Brick
Other collaborative efforts pending as of October, 1988 include:		
ICIPE, Kenya	FAW evaluation in sorghum.	Wellington Otieno
ARC/NARP, Egypt	Field evaluation of Giza-159 material Potential training/ research exchange.	Mohamed Momtaz M. S. Balal NARP project with CID

Developments in regional training courses advanced in both Latin America and Africa. The CATIE/TCCP course was re-negotiated in December 1987 allowing for two additional courses to take place in October 1988 and June 1989. Victor Villalobos, Training Course Director, and Rodrigo Tarte, Director General of CATIE, visited TCCP in April, 1988, for

further planning. The TCCP contributed two scholarships at $2,700 each and sent TCCP Training Coordinator, Sunitha Siriwardana, to assist in lectures during the first three weeks of the fall course. The TCCP was also responsible for printing the course brochure in Spanish. Dr. Villalobos will maintain responsibility for course logistics and recruitment of trainees.

A second regional training course was negotiated in August during Kerri Wright's visit to Morocco. This course has been discussed for several years, but the opportunity to begin planning had not yet been presented. Mohamed Aaouine of the Institut Agronomique et Veterinaire Hassan II (IAV Hassan II) and the Director, M'hamed Sedrati, were anxious to consolidate efforts toward a short-term, beginner-level course taught in French, partially condensed from the TCCP's training manual. The course will run five weeks at the IAV Hassan II campus in Agadir. The five instructors are Moroccan and represent IAV Hassan II, Mohamed V University and INRA. This is the first joint effort of its kind in tissue culture for Morocco. Two of the instructors have trained and studied at TCCP/CSU (Table 37). The TCCP will assist by printing a brochure in French and distributing it to FAO worldwide. FAO officials have expressed an interest in participating through various projects in Francophone Africa and will be contacted by the IPBNet Coordinator. The USAID Missions and REDSO/WCA have also expressed interest in participation and will be key in the course's success.

Table 37. Moroccan Tissue Culture Short Course.

The course will focus on:

Crops	Technique
Banana	Adventitious bud proliferation
Strawberries & potatoes	Apex culture for virus elimination
Tomatoes	Callus experiments
Jojoba	Auxiliary bud branches
Citrus	Shoot tip grafting demonstrations
Tobacco	Callus for suspension cultures
Ornamentals	Shoot regeneration from foliar sections
Tomato	Shoot regeneration from callus

Participants will come from Francophone Africa.

Instructors:

M. Aaouine (PhD), Associate Professor and Head of Tissue culture Laboratory, Department of Horticulture, IAV Hassan II. Complexe d'Agadir.

L. D. Walali (PhD), Associate Professor, Department of Horticulture, IAV Hassan II, Rabat.

H. Chlyah (PhD), Professor and Head of Department of Plant Biology, University of Mohamed V, Rabat.

* A. Zaid (PhD), Head of Tissue Culture Laboratory at INRA, Saharan Research Station in Marrakech.

* O. Benlhabib (PhD), Assistant Professor, Department of Agronomy, IAV Hassan II, Rabat.

* (A. Zaid is currently finishing his PhD at CSU, Horticulture Department, focusing on the in vitro acclimatization of date palm derived from tissue culture. O. Benlhabib is at CSU via the Minnesota/USAID Project to complete course work to receive her PhD from IAV Hassan II.)

Another means of technical assistance is available to IPBNet collaborators through exchange of research personnel, visiting Ph.D. scientists, and travel of TCCP personnel. Scientific conferences also provide an opportunity to share research findings and pursue further

collaborations. During grant year four, several visiting scientists continued their research and training at TCCP. They included Sheila Vijayakumar, ICRISAT; Yusuf Zafar, NIAB; Ahmed Medhat Al Naggar, Fulbright Scholar, University of Cairo; and Paul Chavarriaga-Aguirre, CIAT (See research section for details of collaborations).

In March, Oluf Gamborg traveled to Pakistan and India. While in Pakistan he spoke at the "International Conference on In vitro Selection and Propagation of Economic Plants" at the University of Peshawar. Travel and per diem were paid by the National Science Foundation, USA. Visits were also made to with the Pakistan Agricultural Research Council (PARC), the Nuclear Institute of Agriculture and Biology (NIAB), and ICRISAT to follow-up on collaborative research. On the domestic front, further linkages with private industry were also explored this year. Potential collaborations might include training of industry personnel and research exchange. These options are still in the preliminary stages.

Other conferences attended by TCCP personnel:

Date	TCCP staff	Conference	Presentation
11/29 - 12/4/87 Atlanta, Georgia	Reagan Waskom	American Society of Agronomy Meetings	Poster: "NaCl Selection in Wheat Culture"
4/17 - 4/21/88 Washington, D.C.	Murray Nabors Oluf Gamborg	Strengthening Collaborations in Biotechnology: International Research & Private Sector	
6/6 - 6/9/88 Washington D.C.	A.S. Kumar Kerri Wright	PSTC Conference on Biotechnology for Health & Agriculture	1. Increased Salt Tolerance in Grain Legumes Through Tissue Culture Techniques. 2. Applications of Molecular Biology Techniques to Isolate salt tolerance genes. 3. IPBNet presentation: Resources for Biotechnology in Developing Countries.
8/27 - 9/12/88 CIMMYT, Mexico	Gary Hanning	Second International Symposium on Genetic Manipulation in Crops	*In vitro* NaCl selection of rice and the regenerates performance under saline conditions.

Continued preliminary planning and extensive funding searches took place for the Third International Plant Biotechnology Network (IPBNet) Conference. The Conference will take place January 8-12, 1989 in Nairobi, Kenya. In July, Kerri Wright traveled to several African countries to identify Conference support and participation, and to solidify potential collaborative efforts in field evaluation and technical assistance. Among the countries and institutions visited were:

Institution	Country
ARC/NARP	Cairo, Egypt
UNDP	Nairobi, Kenya
ICIPE	Nairobi, Kenya
ILRAD	Nairobi, Kenya
KARI	Nairobi, Kenya
WARDA	Bouake, Cote d'Ivoire
African Development Bank	Abidjan, Cote d'Ivoire
IAV Hassan II	Agadir & Rabat, Morocco
FAO	Rome, Italy
IBPGR	Rome, Italy
UNDP	New York, USA

Various USAID Missions were also contacted, as well as both REDSO/WCA and REDSO/ESA. Several letters were sent to the Missions to provide updates and request participation. The Africa Bureau was also contacted, and with the assistance of Dr. Joel Cohen and Mr. Richard Newberg, funding was obtained for several additional scientists. The Nairobi Mission has also been particularly helpful in providing financial support for Kenyan participants and facilitating Conference details. The site of the Conference was changed from the Silver Springs Hotel to the New Stanley Hotel after various business dealings were found not acceptable. The change will provide a more conducive atmosphere for the type of Conference the TCCP desires, and was highly recommended by various international associates in Nairobi. The Keynote Speaker will be Donald Plucknett, Scientific Advisor to the CGIAR Secretariat. The plenary speakers are as follows:

Name	Institution
Roger Beachy	Director, Center for Plant Science and Biotechnology, Washington University
John Dodds	Tissue Culture Specialist, CIP
Irwin Chu	President, Twyford Laboratories, Inc.
Ronny Duncan	Professor, Department of Agronomy, University of Georgia
Oluf Gamborg	Associate Director, TCCP
Gary Hanning	Research Coordinator, TCCP
Barry McCarter	Research Station Manager, Pioneer Overseas Corp., Zimbabwe
Victor Villalobos	Head, Tropical Crops Improvement Program, CATIE
Y. L. Nene	Principal Plant Pathologist and Leader - Pulses, ICRISAT

Various publications were distributed by the TCCP during grant year four. In January, the mailing list was revised. The total count for publication and Newsletter distribution numbers 1,066 including: Developing countries, 595 entries, Developed countries, 100 entries, USAID Missions, 146 entries, USA, 225 entries. The first IPBNet Directory with International Funding Resource List was published and distributed in January with Newsletter #8 (see Table 38). The Directory will be revised and enlarged in 1989 to include various indexes for crop and country information. Newsletter #9 was published in July. The computerized literature system, offering free publications upon request, was used extensively by IPBNet members, averaging 10-15 literature search requests per quarter.

Table 38. IPBNet Directory 1988 (50 countries; 271 entries).
International Funding Resource List (52 agencies/institutions).

AFRICA		SOUTHEAST ASIA		EUROPE	
Morocco	6	Thailand	23	Bulgaria	1
Egypt	4	Philippines	14	Hungary	1
Zimbabwe	3	Malaysia	5	Romania	1
Kenya	3	Indonesia	12	Poland	1
Nigeria	5	Sri Lanka	6	Finland	1
Maritius	1	Taiwan	4	France	1
Burundi	1	Vietnam	1	Austria	1
		Singapore	2	U.K.	2
CENTRAL - LATIN AMERICA		**MIDDLE EAST**		**ASIA**	
Brasil	2	Saudi Arabia	1	India	43
Guatemala	5	Syria	1	Pakistan	10
Venezuela	2	Jordan	3	Nepal	1
Mexico	10	Israel	2	Bangladesh	3
Panama	1			P.R. China	5
El Salvador	2				
Nicaragua	1				
Honduras	2	**SOUTH PACIFIC**		**NORTH AMERICA**	
Costa Rica	8				
Colombia	8	Australia	1	U.S.	48
Argentina	1	New Zealand	1	Canada	6
Peru	2	Fiji Islands	3		
Ecuador	1				

Project Review

The AID External Review of the Project took place at Colorado State University February 29 - March 4. The Review Team, led by Mr. Raymond Kitchell, included Drs. Kenneth Frey (Iowa State University), Toshio Murashige (UC, Riverside), and Donald Plucknett (CGIAR Secretariat). Members of the TCCP management staff gave presentations on various aspects of Project operations and accomplishments. TCCP collaborators Drs. James Colbert (CSU), Ronny Duncan (University of Georgia), Dely Gapasin (PCARRD), and A. Mujeeb-Kazi (CIMMYT) gave presentations on cooperative research. Also in attendance were Drs. Judson Harper, Jack

Hautaluoma, James Meiman, John Raich, and Bruce Wunder, all of CSU, and Drs. Joel Cohen and Tejpal Gill of AID/Washington.

Recommendations of the review team were compiled by Mr. Kitchell in a report which was scheduled for completion April 15. The report arrived at the TCCP in July and written comments and clarifications were sent back to AID in August. Although it was not possible for the reviewers to get a complete picture of the TCCP in one short week, the review process provided an opportunity for both AID and TCCP to agree on some necessary changes in Project scope. These changes have been reflected in the 1988/89 Work Plan.

FINANCIAL HIGHLIGHTS

Total expenditures for Grant Year Four were $1,096,761, higher than any other single year since Project inception. Over $100,000 of this amount was spent on the Molecular Biology program, which was separately funded at a level of $200,000 (total) beginning October 1, 1986.

Of the total amount spent, $51,560 was spent from the TCCP training Account, of which $23,713 in tuition revenues were received this fiscal year. The balance was carried over from last fiscal year's receipts. (The training Account is completely separate from the AID account.)

After the External Review in March 1988, Project resources were gradually shifted to the whole plant program. In the next fiscal year, tissue culture will be phased out and emphasis placed on field testing, networking, and training. The molecular biology program has been discontinued due to budget cuts.

It will be noted that the category "operations" has been eliminated. This was done at the request of AID. Management and office effort has been allocated to all Project areas as shown in the Person-Year Distribution.

As of September 30, 1988, a total of $3,445,728 had been spent from the C.A. Even though the C.A. allocates a total of $5 million to the TCCP, at the end of fiscal 1989, at the current average level of spending ($65,000 per month), only $4,224,000 will have been spent. The balance of $776,000 could carry the Project through fiscal 1990 if a no cost extension is approved by AID. Confirmation of an extension, by April 1989, is crucial to the successful continuance of the Project, as core staff must have ample notice that employment will be available for the upcoming year.

Appendix I.

TCCP BUDGET DISTRIBUTION: OCTOBER 1987 - SEPTEMBER 1988

AID BUDGET -- EXPENDITURES

				IN VITRO										
	NETWORK	TRAINING	WHOLE PL	RICE	WHEAT	SORGHUM	LEGUMES	MILLET	CORN	MOL BIO	WIDECROS	PRO/SUS	REVIEW	TOTAL
PERSONNEL	84513	52355	103274	44986	31359	22703	15077	21660	5694	57363	22974	28107	3877	493942
TRAVEL	17302	712	20267	0	0	0	878	0	0	902	0	0	2764	42825
SUPPLIES	14076	0	17201	7493	5223	3781	2511	3608	948	9554	3826	4682	646	73550
OPERATING EXP	10935	6774	13362	5821	4058	2937	1951	2803	737	7422	2972	3637	502	63910
PRINT/COPY	4981	1417	3958	1724	1202	870	578	830	218	2199	881	1077	149	20084
CONSULTING	999	200	0	0	0	0	0	0	0	0	0	0	0	1199
TRAINING STIP	0	2550	0	0	0	0	0	0	0	0	0	0	0	2550
TUITION	0	2181	112	8722	0	0	0	0	0	0	0	5209	0	16224
SUBGRANTS	0	0	8438	7657	12888	0	0	0	12547	0	0	0	0	41530
EQUIPMENT	251	0	0	0	0	0	0	0	0	0	0	0	0	251
INDIR COSTS	52990	24522	66434	27005	21682	12086	8377	11531	3894	30898	11588	14964	3167	289137
TOTAL	186047	90710	233048	103407	76412	42378	29372	40432	24038	108338	42240	57676	11104	1045201

OTHER EXPENDITURES - TRAINING

PERSONNEL	11417
TRAVEL	241
SUPPLIES	1658
OPERATING EXP	2921
PRINT/COPY	297
CONSULTING	1000
STIPENDS	27419
INDIRECT	6607
TOTAL	51560

REVENUES FROM OTHER SOURCES

TRAINING TUITION		23713												23713
GRAD TUIT SUPPORT		380		1520								1608		3508
INTNL PROG SUPPORT	1900													1900
TOTAL														29121

PERSON-YEAR DISTRIBUTION

MGMT/OFFICE	2.00	1.90	1.10	0.40	0.35	0.35	0.30	0.30	0.23	0.65	0.23	0.30	0.26	8.36
RESEARCH	0.00	0.00	2.68	0.67	1.03	0.38	0.33	0.75	0.00	1.00	1.00	0.00	0.00	7.83
TECH SUPPORT	1.25	0.25	2.50	1.00	0.50	0.50	0.50	0.50	0.00	0.50	0.00	0.00	0.00	7.50
GRAD STUDENTS	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.25	0.00	1.00	0.00	2.25
TOTAL	3.25	2.15	6.28	3.07	1.88	1.23	1.13	1.55	0.23	2.40	1.23	1.30	0.26	25.95

Appendix II.

Recent Visitors to the TCCP Facilities

Alberto Acevedo	Instituto Nacional de Technologia Agropecuaria, Argentina
Ahmed Medhat Al Naggar	University of Cairo, Egypt
Abdullah Al-Niemi	University of Mosul, Iraq
Yank Banowitz	Ft. Collins, Colorado, USA
Roger Beachy	Washington University, St. Louis, USA
John Call	Gilroy Foods, USA
Carlos Carpio	PCA-ARC, Philippines
Joel Cohen	USAID/Washington, USA
Ronny Duncan	University of Georgia, USA
Frank Ellison	University of Sydney, Australia
Robert Finch	University of Nottingham, England, U.K.
Kenneth Frey	Iowa State University, USA
Junzo Fujigaki	Tokyo Agricultural University, JAPAN
Dely Gapasin	PCARRD, Philippines
Tejpal Gill	USAID/Washington, USA
Yuri Gleba	Institute of Botany, Kiev, USSR
Tom Gorell	Colorado State University, USA
Hiroki Hamada	Okayama University of Science, Japan
Jack Hautaluoma	Colorado State University, USA
Marcelo Huarte	Instituto Nacional de Tecnologia Agropecuaria, Argentina
Mohamed Ismail	Somalia
Hans Jobert	University of Arizona, USA
Ken Keegstra	University of Wisconsin, USA
John Keenan	University of Wyoming, USA
Wayne Keim	Colorado State University, USA
Iqrqr Khan	University of California, Riverside, USA
P. P. Khanna	Division of Germplasm Conservation, NBPGR, INDIA
Raymond Kitchell	Alexandria, Virginia, USA
Josefina Lantican	Bureau of Agricultural Research, Philippines
Zaida Lentini	Cornell University, USA
H. R. Lerner	Hebrew University, Israel
Judi Li	Huazhong Agricultural University, P.R. China
Kauser Malik	Nuclear Institute for Agriculture and Biology, Pakistan
James Mattoon	Colorado College, USA
Willie L. McCuistion	NARP/ARC Cairo, Egypt
David Mok	Oregon State University, USA
Sama Monde	Rice Research Station, Sierra Leone
A. Mujeeb-Kazi	CIMMYT, Mexico
Toshio Murashige	University of California, Riverside, USA
Musa Mohamed Musa	Agricultural Research Corp., Sudan
Julius Odongo	Uganda
R. S. Paroda	ICAR, India
Suresh Patil	University of Hawaii, USA
Donald Plucknett	CGIAR Secretariat, USA
G. M. Reddy	Osmania University, India
Joy Reese	Denver, Colorado, USA
William Roca	CIAT, Colombia

K. N. Satyapal	United Nations Development Program, New York City, USA
Laxman Sherchand	Ministry of Agricutlure, Nepal
Shambhu L. Shresthe	NARSC, Nepal
Otto Silberstein	Gilroy Foods, USA
Veronica Silversmith	Navajo Agricultural Products Industry, New Mexico, USA
B. P. Singh	National Bureau of Plant Genetic Resources, India
Shree P. Singh	CIAT, Colombia
Guinko Sita	University of Ouagadongon, Burkina Faso
Pompimon Suriyajantratong	Khon Kaen University, Thailand
Steven Tanksley	Cornell University, USA
Rodrigo Tarte	CATIE, Costa Rica
Marion Verzea	Research Institute for Cereals and Industrial Crops, Romania
Victor Villalobos	CATIE, Costa Rica
Deng Wanyin	Genetics Institute, Academia Sinica, P.R. China
Ian Wardlan	CSIRO, Australia
Dale Wirth	BIA, New Mexico, USA
Dirayos Wititsuwannakul	Prince of Songkla University, Thailand
Rapepun Wititsuwannakul	Prince of Songkla University, Thailand
Yongyuth Yuthavong	NCREG, Thailand
Abdelouahhab Zaid	INRA, Morocco

APPENDIX III

TCCP Staff List

Name	Title/Area	Comments
Murray Nabors	Project Director	
Sr. Research Scientist		
Oluf Gamborg	Associate Director	
Research Associates		
Barbara Ashton	Group Leader/Millet	resigned 5/31/88
Gwendolyn Bane	Asst. Manager/Greenhouse	resigned 4/30/88
Fariha Faizi	Group Leader/Wheat	on leave of absence 8/88 - 1/89; resigning 1/31/89
Lori Frederickson	Tech/Rice Hydroponics Office Assistant	resigned 2/2/88
Touba Ghazi-Moore	Group Leader/Cowpea	resigned 10/31/87
Gary Hanning	Research Coordinator	
Glen Hildreth	Lab Manager	80% time beginning 8/1/88; resigning 1/31/89
Julie Ketchum	Operations Director	
Jolanta Menert	Greenhouse Technician	hired 4/11/88
Dan Miller	Researcher/Whole Plant	hired 1/2/88
Carol Ostrom	Tech/Wheat, Rice	75% time
Catherine Rivera	Group Leader/Sorghum, Rice	resigned 6/30/88
Sunitha Siriwardana	Training Coord., Wheat Researcher	
Reagan Waskom	Greenhouse Manager	
Kerri Wright	Network Coordinator	
State Classified		
Lee Olton	Office Manager	
Sue Sidinger	Office Assistant	hired 12/28/87, 75% time

Post Doc		
Suresh Kumar	Pigeonpea, Moth Bean, Tepary Bean, Molecular Biology	resigning 1/15/89
Visiting Scientists		**Country**
Paul Chavarriaga	Common Bean	CIAT, Colombia
Ahmed Al Naggar	Wheat	Fulbright Scholar, Egypt
Sheila Vijayakumar	Chickpea	ICRISAT, India
Yusuf Zafar	Rice/Kallar Grass Hybridization	NIAB, Pakistan
Grad Students		**Funding**
Iteu Hidayat	Potato Protoplast Fusion	Winrock
Isaac John	Molecular Biology and Salt Tolerance	AID/PAK
Ray Ketchum	Protoplasts/Rice and Millet	TCCP
Doug McMurray	Rice/RFLP	Biology Department
Akbar Mohmand	Wheat/Drought	FAO
Anna Novero	Rice/NR	TCCP
Poungpet Poonsapaya	Rice Suspension	TCCP
Sathish Puthigae	Rice/Cond. Factor	TCCP
Gaith Sasi	Triticale	Libyan Government
Nanan Widiyanto	Rice/Blast	World Bank
Student Support		**Part-time %**
Leann Avery	Greenhouse	25
Carol Brumley	Greenhouse	25
Barbara Buchnam	Office	25
Laurie Clauss	Rice	25
Heather Cwach	Greenhouse	25
Dawn Dow	Greenhouse	25

Kimberly Ellis	Greenhouse	25
Doree Dufresne	Office	25
Jan Emming	Greenhouse	25
Lauren Fesler	Millet	25
Hal Gibson	Greenhouse	25
David Hewitt	Greenhouse	25
Michelle Hunt	Hormone Detection/HPLC	25
Jeannette Hut	Hormone Detection/HPLC	25
Julie Hewitt	Millet	25
Christa June	Greenhouse	25
Alanna Kulchak	Sorghum	25
Heather Loomis	Office	25
Kristina Muscarella	Greenhouse	25
Elizabeth Orle	Sorghum	25
Greg Pott	Molecular Biology	25
Scott Price	Greenhouse/Rice	25
Peter Schuerman	Greenhouse/Rice	25
Debra Singleton	Office	25
Nitschka ter Kuile	Widecross/Wheat	100
Robert Tengler	Molecular Biology	25
Jeffrey Tjaden	Pigeonpea/Molecular Biology	25
John Tamulonis	Whole Plant	50
David Timm	Rice	50
Michael Tinker	General Lab	25
Kristin Vanderbilt	Wheat	25
Brian Wood	Greenhouse	25

APPENDIX IV

Publications

Tissue Culture for Crops Project, 1988. IPBNet Newsletter No. 8. J.L.F. Ketchum (ed). Colorado State University, Ft. Collins, Colorado.

Tissue Culture for Crops Project, 1988. IPBNet Newsletter No. 9. J.L.F. Ketchum (ed). Colorado State University, Ft. Collins, Colorado.

Tissue Culture for Crops Project, 1988. IPBNet Directory. K.L. Wright and J.L.F. Ketchum (eds). Colorado State University, Ft. Collins, Colorado.

Reports

Chavarriaga, P., A.S. Kumar, O.L. Gamborg, and W.M. Roca. 1988. Tissue Culture for Genetic Improvement of Bean (Phaseolus spp). Report on collaborative program between TCCP and CIAT.

Hanning, G.E. 1988. R_3 Progeny of RTx 430 regenerated plant demonstrates both acid and drought tolerance. AID Brief.

Hanning, G.E. 1988. Effect of sorbitol on established embryogenic callus of Giza 159. AID Brief.

Hanning, G.E. 1988. Preliminary data from an R_1 rice seedling screen for salt tolerance. AID Brief.

Naggar, A.M.A., T. Goubatov and O.L. Gamborg. 1988. Stress Selection in Wheat Tissue Culture. Report on research at TCCP by Dr. A.M.A. Naggar, Fulbright Scholar.

Tissue Culture for Crops Project, 1988. Response to "Issues" presented to the External Evaluation Team for use in the March, 1988 External Project Review.

Vijayakumar, S., O.L. Gamborg, H.A. van Rheenen, and Y.L. Nene. 1988. Chickpea Tissue Culture Program. Report on collaborative program between TCCP and ICRISAT. 28p.