PN ABM-516

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AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

DATE: 8/3/88

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18

FROM: AID/SCI, Victoria Ose

SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. C5-221
PR 1-6/88

Attachment

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Improvement of Salt Tolerance in Tomato (C5-221)

A progress report submitted to AID for the period January-June 1988

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Rec'd In SCI: AUG

We continue our work on the improvement of salt tolerance in tomato. It includes: 1) Transfer of genes from wild salt-tolerant tomato species to the cultivated species by conventional breeding. 2) Selection for salt tolerance in organ, tissue and cell culture. 3) Use of mycorhizal inoculation to enhance salt tolerance in tomato. 4) Physiology of the salt tolerance.

Work done in Israel

1. Transfer of genes

The crosses between Floradade (FD) and vigorous and fertile plants of the BC_1F_2 and $F_2(F_1 \times F_1)$ generations *, which grew in Hoagland solution w/o added salt, were performed. Some of the seeds from these crosses will be sown soon and the resulting plants will be selfed. The remaining seeds will be sent to the Philippines.

2. Selection in organ, tissue and cell culture

In Israel more emphasis is placed on Marikit.

a. Calli and suspension cultures prepared from the leaves of Marikit only are used. Some of the experiments (g) include Improved Pop.

b. Calli and cells are exposed to different salt (NaCl) concentrations (50, 100, 150, 200, 250, 300, 350, 400 mM) in order to identify the lethal one.

c. Following the short-term method (progress report July-December 1987) non selected calli will be exposed to almost lethal salt concentration. Plants will be

* CV x Lpa or Lc1401 F_1 (CV x Lc1401) x F_1(CV x Lpa)

x —— F_1(x) $F_2(F_1 \times F_1)$

BC_1

F_2

CV = Marikit (M) or Improved Pop (Ip)

Lpa= *Lycopersicon pennellii* ; Lc1401 = *L. cheesmanii*

regenerated from cells remaining alive. Regeneration of plantlets from calli may be successful in MS medium containing 2% sucrose, 1% mannitol, 2 mg/L Zeatine and 0.1 mg/L IAA.

d. Following the long-term method (progress report July-December 1987) we started the exposure of suspension cells to increasing concentrations of NaCl or mannitol. Salt-adapted cells will be obtained directly on salt or by using the long-term two-step procedure (progress report July-December 1987). In potato we were able to obtain cells adapted to high (200 mM and above) NaCl concentration only by using the latter procedure. The salt-adapted cells will be used as feeder cells (following feeder cells procedure).

e. Non selected cells of tomato will be exposed to almost lethal salt concentration in the presence of salt-adapted cells of potato (which are already available in our lab) which will be used as feeders.

f. Calli are being exposed to various concentrations of the proline analogue hydroxyproline.

g. Detached shoot apices and leaf pieces of the two cultivars (M and Ip) were exposed to various NaCl concentrations (50, 75, 100, 125, 150 mM). Plants were developed from apices of both cultivars in media containing 125 or 150 mM NaCl. Plantlets were regenerated from leaf pieces in media containing 75 mM NaCl. Plants developed in these experiments are grown. These plants will be selfed and salt tolerance will be determined in their progeny.

h. Development of callus from pollen grains - anthers of FD are being exposed to media containing different hormonal combinations and to different temperatures (following Powell and Uhrig, 1987).

References

Powell, W. and H. Uhrig. 1987. Anther culture of Solanum genotypes. Plant, Cell, Tissue and Organ Culture 11: 13-24.

WORK DONE IN THE PHILIPPINES

I. Research Activities:

A. Breeding and selection

1. A preliminary field experiment was conducted using eighteen cultivars, hybrids and segregating progenies of Marikit/Improved Pope with *L. penellii* (Lpa) and *L. cheesmanii* (Lc). Seedlings were subjected to salinity stress using common salt (at an electrical conductivity of 10 dS/m) and selected plants were transplanted in a post-rice paddy field in Minalin, Pampanga, in January, 1988. The salinity of the river used for irrigation ranged from 4.6 to 22.5 dS/m at the time of testing. Among the entries, only Marikit, L-114, Peto 81 and F_2 (Marikit x Lc) survived but they produced only few and small fruits.

2. Mass screening involving 35 cultivars, 5 hybrids and segregating progenies of Marikit and Improved Pope with Lpa and Lc, was undertaken at the early growth stage. In the first method, which involved growing of seedlings in nutrient solution, 35 plants were selected from 114 plants, based on vigor and degree of chlorosis. The second method involved planting in soil contained in seedboxes; this method allowed screening of several entries at a time. The total number of plants evaluated was 2622 and 261 plants were selected.

In both cases, salt stress of 10-15 dS/m was applied to one-month seedlings.

Visual observations using both techniques showed individual plant differences even within a variety, suggesting that the varieties used were not pure. Hence final selection was based on both individual plant and variety performance.

Evaluation of selected plant is in progress.

Results on both hydrophonic and soil culture techniques were found to be highly correlated (80%). Because of this close association between the two, soil culture technique was employed in the succeeding trials.

3. Hybridization among selected plants was undertaken. Crosses between selected local cultivars with Edkauri, Hofit, and Peto-81 were initiated. Back-crossing of resistant F_2 progenies derived from crosses of Marikit and Improved Pope with Lpa and Lc to the cultivated parents was also done. Seed production and hybridization were done mostly in the highlands, where temperature is more favorable for fruit setting.

B. Tissue culture

1. In earlier experiments, using cv. Improved Pope (Annual Report 1987), two media treatments were selected for callus establishment from leaf explants. Initiation and growth of the primary callus was good but there were problems of callus maintenance in subsequent cultures. Among the media combinations tried, MS + 0.5 mg/l Kin + 2.0 mg/l NAA repeatedly performed best in terms of callus establishment.

Three-week old calli subcultured to the same medium produced very profuse compact type of callus which is cream-green in color and 100% of subcultured calli showed re-growth. The age of the callus was an impotant factor to ensure re-growth.

2. Anther culture of tomato using the method of Gresshoff and Doy (1972) showed that the method was suitable for tomato under local conditions. Contamination of cultured anthers was nil and 36% of the anthers responded to the medium. Callus formed from the pollen grains.

II. Experiments in Progress:

1. Screening for Salinity Tolerance

Screening of 32 entries for tolerance at seedling stage using the soil culture technique is in progress. These entries are composed of crosses and segregating populations, cultivated varieties and accessions from the germplasm collection.

2. Grafting of tomato

Grafting, as a method of immediate utilization of tolerance from hybrids involving Lpa and Lc with that of the local cultivated varieties, is in progress. The method tried was inarching, with the crosses of wild x local cultivated varieties serving as the stock and the local cultivated variety as the scion. The experiment is still being perfected.

3. Mycorrhiza and salt tolerance

Enhancing salt tolerance in tomato through mycorrhizal inoculation is currently being tested. The experiment involves six tomato entries (classified as susceptible, intermediate and tolerant to salt stress) and three types of mycorrhiza (*Glomus etunicatum, glomus macrocarpum* and *Gigaspora margarita*). These were provided by the Institute of Biotechnology (BIOTECH) and inoculation was done in cooperation with BIOTECH scientists. Data on survival, plant vigor and mycorrhizal count will be gathered.

4. Response of Tomato to Salt Stress at Different Growth Stages

Four tomato varieties are subjected to salt stress using 0, 50, 100 and 150 mM NaCl. Irrigation with saline solution is done at 28, 35, 42, and 72 days after sowing and one treatment is being irrigated continuously with saline solution. Data on morphological and physiological responses will be gathered.

5. Possible sites for field testing were identified in Pampanga and La Union.

Water and soil samples from these sites are being measured every month for electrical conductivity.

6. Tissue Culture

A. Aseptic cultures of cv. Improved Pope seedlings are maintained as sources of experimental material.

B. *In vitro* multiplication is being done to increase vegetative duplicates of regenerants for greenhouse testing for salinity tolerance.

C. The method of Gresshoff and Doy (1972) for anther culture will be tried on cv. Improved Pope.

Literature cited

Gresshoff, P.M. and C.H. Doy. 1972. Development and differentiation of haploid Lycopersicon esculentum (Tomato). Planta 107: 161-170.