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AGENCY FOR INTERNATIONAL DEVELOPMENT
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MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18
FROM: AID/SCI, Victoria Ose *VO*
SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following

AID/SCI Progress Report No. C5-221
period Oct 86 - June 87

Attachment

2 up

Improvement of Salt Tolerance in Tomato and Potato (C5-221)

progress

A report submitted to AID for the period October 1986-June 1987

Submitted by Prof. M. Tal* (PI) and Drs. D.A. del Rosario, E.T. Rasco
and A.B. Zamora** (Collaborators)

* Department of Biology, Ben Gurion University of the Negev,
Beer Sheva, Israel

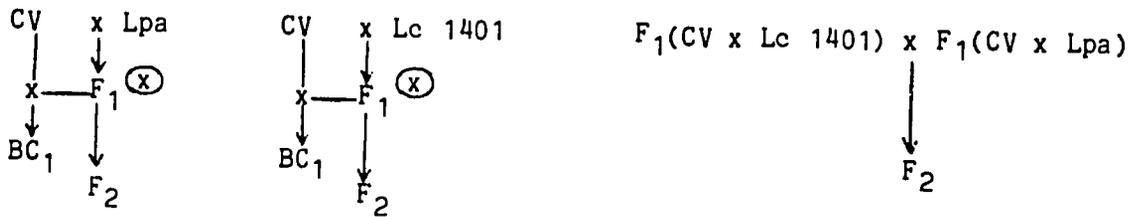
** Institute of Plant Breeding, University of the Philippines of Los
Banos, College, Laguna 3720, Philippines.

The visit of the PI to the University of the Philippines (February 1987) which was organized by Dr. del Dafrosa, included a three-day trip to experimental and farming areas representing some of the aspects and problems of the agriculture in the Philippines. It also included meetings with several scientists from the University or the International Rice Research Institute, and thorough discussions with the counterparts. Based on these discussions we agreed on the following:

- A. As recommended by the reviewers of the proposal, we will concentrate on one crop, the tomato. The study will include transfer of genes from wild salt-tolerant species by conventional breeding and selection for salt tolerance in cell and tissue cultures. We will attack the subject from as many angles as possible.
- B. The agricultural areas affected by salinity in the Philippines can be divided into two main types: a. areas which are flooded seasonally by the sea tides, and b. the inland farming areas which are influenced by underground brackish water. We decided to concentrate on the improvement of salt tolerance for the former conditions. Salt composition and level in the experiments will depend on the average values obtained from measurements taken in a representative area in the Philippines.
- C. The cultivars to be studied will be: Marikit (M) and Improved Pop (IP).
- D. The studies will be carried on whole plants as well as on isolated organs, tissues and cells.
 1. Studies on whole plants
 - a. Breeding - Seeds of F₁, F₂ and BC₁ generations from the crosses between the two cultivars (CV) and the wild salt tolerant species Lycopersicon pennellii (Lpa) and L. cheesmanii (Lc 1401) will be prepared in Israel. Breeding and selection will be continued in the Philippines under controlled

(greenhouse) and field conditions.

Main crosses -



b. Tentative physiological studies

- Determination of the threshold (a), slope (b) and Y_0 (salt concentration for which growth is zero), based on the change in dry weight under various salt concentrations - it will be performed in young plants (2 to 4 leaf stage), which represent the most susceptible stage. According to Pasternak et al. (1976), growth at that stage is positively correlated to yield in later stages.
- Determination of daily changes of sugars (mainly X) by measuring $X = \Psi_{\pi n} - \Psi_{\pi m}$ in leaves and in shoot apices - $\Psi_{\pi n}$ and $\Psi_{\pi m}$ are refractometer readings for sap extracted from organ frozen at noon time and early morning, respectively. According to Danon (1984), X represents best the daily fluctuation of osmotic adjustment.
- Accumulation of ions in different parts of the plant
- Leaf succulence
- Stomatal opening and CO₂ fixation
- Determination of the isozymic variation of various enzymes which play a central role in controlling the rates of important metabolic pathways

These variables will be studied in the parental and F₁ plants. The variables proved relevant to salt tolerance will also be determined in genetically homogenous lines obtained through breeding and selection. Fruit characteristics will be studied in the latter.

2. Experiments with organ, tissue and cell culture

Selection for salt tolerance in calli and suspension culture will include: development of calli and suspension cultures from plants of the two cultivars; direct and gradual exposure of the cultures (preadapted and nonadapted to mannitol) to various salt concentrations or to proline analogs; selection of cells tolerant to salt and proline analogs; regeneration of whole plants (R_0 and R_1) from salt-tolerant cells and determination of their tolerance. In Israel more emphasis will be placed on Marikit and in the Philippines on Improved Pop. Anther culture - Based on previous experience in tomato (Sink and Reynolds, 1985) we will try to develop a technique for production of homozygous diploid plants (after chromosomal doubling) from pollen grains of the two cultivars. This technique may be used to shorten the number of generations required during breeding.

Work done in Israel

1. Whole plants

a. Breeding - seeds of F_1 (M x Lpa), F_1 (M x Lc 1401), F_1 (IP x Lpa), and F_1 (IP x Lc 1401) were prepared and sent to the Philippines. Seeds of BC_1 [F_1 (M x Lpa) x M], BC_1 [F_1 (M x Lc 1402) x M], BC_1 [F_1 (IP x Lpa) x IP], BC_1 [F_1 (IP x Lc 1401) x IP], F_2 [F_1 (M x Lc 1401) x F_1 (M x Lpa)], F_2 [F_1 (IP x Lc 1401) x F_1 (IP x Lpa)], and F_2 from the selfings of all the F_1 plants are being prepared. Seeds of various Israeli and other cultivars proved relatively salt tolerant in several laboratories were also sent to the Philippines.

b. Physiological studies - Isozymic variation is being determined in leaves and shoot apices of the parental species and F_1 plants under control and saline conditions.

c. Symbiosis with salt-tolerant mycorrhiza - Preliminary experiments are conducted with the cooperation of Dr. J. Krikun of the Volcani Center, Israel, in order to examine the effect of mycorrhiza collected in saline habitats on the response of tomato plants to salt, following the work of Pond et al. (1987).

2. Tissue and cell cultures

a. Calli were prepared from leaves of the two cultivars Marikit and Improved Pop. Cell suspension culture is being prepared. The exposure to salt and mannitol will be done following our extensive experience in the selection of salt-tolerant cells in potato cultures (unpublished yet). From the extensive data obtained for various species (summarized by Tal, 1987), it seems that the only reliable criterion for distinguishing between genetic and epigenetic changes in cell culture is the expression of new characteristics in R_0 plants and their sexual inheritance by R_1 plants. The difficulty encountered in the regeneration of healthy and fertile plants constitutes one of the main obstacles to the production of salt-tolerant plants from cells in culture. In order to overcome this problem we will produce R_0 and R_1 and test their salt-tolerance repeatedly during the progress of the selection in culture. We will also try to regenerate plantlets directly from leaf pieces and develop plantlets from isolated shoot apices, both in saline media.

b. We are determining the appropriate mineral and hormone composition which will allow the regeneration of plantlets from leaf discs and the production of plantlets from shoot meristems.

References

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Work done in the Philippines

Originally it was planned to characterize different saline areas in the Philippines based on chemical and physical characteristics of the soil and kind of crops grown, with special emphasis on vegetable crops. Due to limited funds and man power, only two areas were visited, namely: Pangasinan and Batangas.

The electrical conductivity of sea water collected from Pangasinan and Batangas was found to be 43 and 45 mmho/cm, respectively. Sea water and soil

samples were collected from Lemery, Batangas for preliminary experiments at UPLB. It was decided to collect the samples from that place primarily because of its proximity to UPLB and due to the fact that the farmers living in the vicinity of the seashore are growing tomatoes, onions, eggplants and other crops.

1. Whole plants

- a. Response of Improved Pope to increasing salt concentration.

Pot Experiment

Procedure: Nine-day-old tomato seedlings were transplanted in 8" pots filled with ordinary clay loam soil. The plants were watered with ordinary tap water for five days after which salinity treatments commenced. The different treatment combinations used for watering the plants are shown in Table 1.

Treatments 2 to 8 were watered every day for 4 days, after which watering was done every other day. Treatment 1 was done 4 days later than the rest of the treatments. The experiment design was completely randomized design with five replications per treatment. Throughout the duration of the experiment the recorded temperatures and relative humidity in the greenhouse were 24.4 - 32.8°C and 42.5 - 85.0%, respectively. The experiment was terminated 20 days after the beginning of the first treatment. Plant height and dry weight were recorded weekly.

Results:

Plants treated with pure sea water died seven days after the beginning of watering. Plants given 1:1 sea water-tap water (T2) or 1.11% NaCl (T3) exhibited severe wilting, drying and browning of the tip of the leaves starting from older leaves, and stunted growth (Table 2). In the other treatments, 1:3 sea water-tap water (T4) and 0.67% NaCl (T5), the

plants showed moderate wilting and browning of the leaves while these symptoms were absent in T6 (1:5 sea water-tap water), T7 (0.22% NaCl) and the control. The plants in the last three treatments also appeared vigorous.

Reduction in plant height was observed in all treatments except in those given 0.22% NaCl (T7). Evidently, reduction in dry matter accumulation increased with the increase in salinity (sea water or NaCl).

Nutrient solution culture

Procedure: Nine-day-old tomato seedlings were established in Hoagland's solution for five days from which they were transferred to freshly prepared nutrient solution containing varying levels of NaCl (0.1%, 0.3% and 0.5%) and to 1:1 sea water in nutrient solution. The electrical conductivities of the different treatments are given in Table 3. The nutrient solution had an EC of $3.35 \text{ mmho cm}^{-1}$ and it increased with a corresponding increase in NaCl concentration.

Plant height and dry matter were recorded. The experiment was laid out using a completely randomized design with six replications. The plants were harvested 3 weeks after imposition of treatment.

Results:

Plants subjected to equal portions of sea water and nutrient solution wilted and died two days after the beginning of the treatment. A low level of NaCl in the solution (0.1%) did not affect final plant height and height increment. Significant reduction in plant height was obtained using 0.3% and 0.5% NaCl. On the other hand, dry matter was not significantly affected by varying levels of NaCl (Table 4).

- b. Response of different species and cultivars to two levels of salinity
(In Progress).

Thirteen tomato entries were grown under greenhouse conditions. These entries included Philippine recommended and promising cultivars, cultivars

from Israel, wild species and F₁ crosses involving wild species (Table 5). F₁ (Marikit x Lpa) produced the most vigorous seedlings while cvs Arava S-5 and Edkauri were the least vigorous. Due to limited number of seeds cv Edkauri and F₁ (Marikit x Lc) were represented by only 2 and 4 seedlings, respectively. The prevailing warm conditions in the Philippines presumably caused the relatively poor standing of some of these cultivars. In addition, some seedlings were also infected with damping-off (Pythium sp.).

The wild tomato species, L. pennellii and L. cheesmanii were germinated using two methods:

- 1) sowing the seeds directly in the soil
- 2) immersing the seeds in 10% hypochlorite (20-30 min for Lpa and 40-60 min for Lc). Afterwards they were transferred to Petri dishes from which seedlings were transferred to light textured soil.

In procedure 2, the seedlings were weak and spindly and after a few days they died. High night and day temperature somehow affected seedling growth in addition to infection with damping-off. Seedlings produced using procedure 1 died also due to damping-off. Seeds of these two species were sown again using procedure 1.

Only nine tomato varieties were included in the pot experiment. Seedlings were transplanted to pots (12") filled with soil collected from Lemery, Batangas. Complete fertilizer was applied at transplanting. One week after transplanting, the plants were watered with sea water at different levels. The treatments were as follows:

<u>Treatment</u>	<u>EC (mmho cm⁻¹)</u>
1. 1 sea water: 3 tap water	18
2. 1 sea water: 1 tap water	26
3. Control - tap water only	0.75

Experiment is still in progress. Data on plant height, vigor rating and dry matter accumulation will be gathered.

The same set of seedlings was established in Hoagland solution and then transferred to nutrient solution with three levels of NaCl. However, due to high temperature and damping-off, many seedlings died. The experiment will be repeated.

2. Tissue Culture

a. Sterilization of Seeds

Seeds of the cultivars Marikit and Improved Pope were initially rinsed under running water for three hours prior to soaking in soapy water. The seeds were placed in a plastic mesh to facilitate pretreatment and rinsing steps. After another rinse using sterile distilled water, the seeds were again immersed in 25% v/v commercial bleach (Zonrox, 5.25 a.i.) for 30 min with shaking. Final rinses and subsequent inoculations were all done in the transfer hood.

The medium used for the germination of seeds was that of Murashige and Skoog (MS) with 2.0% sucrose. Test tubes with filter paper support were used to contain 10 ml each of the liquid medium. Cultures were kept under diffused light for 8 h under temperatures ranging from 25 to 35°C.

Germination was largely dependent on the source of the seeds. While germination of Marikit was good, seeds of Improved Pop did not germinate at all.

b. Multiplication of material

Shoots of cv Marikit are being multiplied using single nodal cuttings on the same medium used for germination. Most shoots that developed were also rooted (90%).

c. Callus induction experiment

Callus induction on media containing different concentrations of various auxins (IAA, NAA and 2,4-D) in combination different concentrations of cytokinins (benzyladenine and kinetin) will be investigated with Improved Pope and Marikit.

Problems:

Due to limited manpower and resources at the Institute of Plant Breeding, implementation of the project is very slow. This is a new project and therefore, it is necessary to conduct studies relating to techniques in the physiological and tissue culture parts. Modifications will be made to suit existing environmental conditions and availability of local materials. Numerous activities can be undertaken with additional research assistants.

The project leaders request that the funds for the project be given in advance to enable full implementation of the activities.

Table 1.

Table 1. Different combinations of sea water, tap water and NaCl and their corresponding electrical conductivities.

Treatments (T)	EC (mmho cm ⁻¹)
1. Pure sea water	45.00
2. 1 sea water: 1 tap water	26.00
3. Tap water + 5 g NaCl (1.11%)	21.80
4. 1 sea water: 3 tap water	18.00
5. Tap water + 3 g NaCl (0.67%)	13.95
6. 1 sea water: 5 tap water	12.15
7. Tap water + 1 g NaCl (0.22%)	5.10
8. Control - tap water only	0.75

Table 2. Effects of the different salt concentrations on tomato cv Improved Pope

Treatment ¹	Height (cm)			Remarks	Dry Weight (g/plant)	% Reduction
	6 DAT ²	13 DAT	20 DAT			
1. Pure sea water (SW)	8.5 ³	2 plants died	all died	Seedling mortality as early as 7 DAT	0	-
2. 1 SW 1 tap water (TW)	8.6	10.7	3 plants died	Stunted growth, severe wilting and yellowing of leaves. Browning of the tip of the leaves	2.20 ⁴	74
3. TW + 5 g NaCl (1.11%)	9.3	12.3	13.0		2.10	75
4. 1 SW: 3 TW	10.3	15.0	16.5		3.27	62
5. TW + 3 g NaCl (0.67%)	0.2	15.5	19.8	Moderate yellowing and wilting of leaves. Browning of the tip of the leaves	5.53	37
6. 1SW: 5 TW	12.0	18.7	22.4		5.83	33
7. TW + 1 g NaCl (0.22%)	12.8	21.3	27.2	Absence of symptoms or hardly unnoticeable wilting. More vigorous plants	6.86	23
8. Control (TW only)	12.4	22.3	25.0		8.71	-

¹ Each pot was watered with 450 ml.

² Days After Treatment imposition.

³ Data obtained from the mean of 5 plants.

⁴ Average of 2 plants only.

Table 3. Electrical conductivity of the different solutions

Treatment	EC: (mmho cm ⁻¹)
1. Control - nutrient solution (N.S.) only	3.35
2. 0.5% NaCl + N.S.	11.70
3. 0.3% NaCl + N.S.	8.55
4. 0.1% NaCl + N.S.	5.20
5. 1/2 sea water + 1/2 N.S.	20.00

Table 4. Performance of Improved Pope seedlings under varying salinity levels

Treatment	Final plant height (cm)	Height increment (cm/wt)	Shoot dry weight (g/plant)	Root dry weight (g/plant)	Root-shoot ratio
1. Control - Nutrient Solution (N.S.)	29.83 a	10.17 a	0.93	0.20	0.22
2. 0.5% NaCl + N.S.	19.67 c	5.50 b	0.79	0.20	0.26
3. 0.3% NaCl + N.S.	23.17 c	8.33 ab	1.03	0.25	0.25
4. 0.1% NaCl + N.S.	29.00 ab	9.33 a	1.19	0.30	0.25

Means within column followed by the same letter are not significantly different at 5% DMRT.

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Table 5. Vigor rating of selected tomato varieties one month after sowing

Variety	Vigor Rating ¹
Peto-81	3.5
Arava S-5	3.0
Hofit	3.0
Edkauri	2.5 (2 plants only)
F ₁ (Marikit x Lpa)	5.0
F ₁ (Marikit x Lc)	4.8 (4 plants only)
<u>L. pennellii</u> acc. Atico	-
<u>L. cheesmanii</u> ecotype 1401	-
PT 3027	3.0
Marikit	4.5
Marilag	4.0
Improved Pope	4.3
Mayumi	4.0

¹ 5 = most vigorous and 1 = least vigorous.