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Improvement of Salt Tolerance in Tomato (C5-221)

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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 at Los Banos,
College, Laguna 3720, Philippines

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The improvement of salt tolerance in tomato includes mainly: 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.

WORK DONE IN ISRAEL

1. Transfer of genes

Many seeds were obtained by selfing the progeny of the crosses between Floradade (FD) and the BC_1, F_2 and F_2 (F_1 x F_1) generations*. A great part of these seeds was sent for experiments in the Philippines. Some of the remaining seeds were planted in Hoagland solution containing 100 mM NaCl in Israel. Their growth is being recorded using the Plastochron Index (PI) following Erickson, 1960 and Coleman and Gregson (1976). Plants having PI value above a chosen threshhold will be selected for further selfing. Part of the seeds produced from this selfing will be planted in salanized Hoagland solution in the green house in Israel for an additional cycle of selection and selfing. The remaining seeds will be sent for experiments in the Philippines.

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* BC_1

CV x Lpa or Lc
↓ ↓
x F*
↓ ↓
BC_1 F_2

F_2 (F_1 x F_1)

F_1 (CV x Lc) x F_1 (CV x Lpa)
↓
F_2 (F_1 x F_1)

CV = Markit (M) or Improved Pope (IP)

Lpa = Lycopersicon pennellii Atico; Lc = L. cheesmanii 1401

2. Selection in organ, tissue and cell culture (produced from Marikit)

a. Plants are continuously regenerated from leaf explants which are exposed to saline or non saline (control) media. These plants which are grown in salinized or non salinized (control) Hoagland solution, are selfed. Seedlings obtained from these selfed seeds will be exposed to 100 mM NaCl solution and their growth will be recorded based on the Plastochron Index.

b. We continue the following experiments:

b_1. Short-term exposure of calli to sublethal concentration of salt.

b_2. Preparation of salt-adapted cells to be used later as feeder cells.

b_3. Regeneration experiments in which we try to find (among several alternative compositions) the appropriate medium.

More detailed description of the experiments and results will be given in the next report (31/12/1989).

References

Erickson, R.O. 1960, Amer. J. Bot. 47: 350.

Coleman, W.K. and R.T. Greyson, 1976. Can. J. Bot. 54: 2421.

Research activities in the Philippines

1. Screening of lines, accessions and segregating populations for salinity tolerance

Continuous screening experiments using the soil culture technique were conducted to assess the performance of tomato to salt stress at the seedling stage. Seeds were sown in sterilized medium composed of 2:1/2:1/2:1/2 mixture of soil, coir dust, chicken manure and sand. A week after sowing, seedlings were pricked in seedboxes (45 x 30 x 7 cm^3) filled with the same sterilized soil mixture. After seven days of establishment, seedlings were salinized using common salt

(EC = 15 dS/m) for two weeks. Visual stress rating of 1 - 5 (1 = Resistant; 5 = Susceptible) and percentage selection were done both by the breeder and the physiologist.

Nine screening batches were accomplished during the period covered. Two hundred forty two (242) entries consisting of 53 new accessions, 104 segregating F_3 and F_4 populations and backcross populations, 4 cultivars and 61 line selections from previous trials were screened using Marikit and Improved Pope as check varieties. The total number of seedlings screened was 26, 951; of which only 1709 were found to be tolerant, giving a 6.3% selection. Selections showed slight to moderate chlorosis, tip burning and defoliation.

Selected seedlings were either planted in the field or in pots for further evaluation. Pot evaluation of 339 seedlings led to the selection of 92 individual plants which could tolerate salt stress and at the same time, possessed the following horticultural merits: early, prolific and with apparent resistance to foliage diseases. Hybridization among these selections and the commonly-grown varieties Improved Pope and Marikit was done to further improve on plants adaptability and fruit traits as well as tolerance to salinity. Continuous back-crossing of selected advance progenies derived from crosses of Marikit and Improved Pope with Lpa and Lc to the cultivated parent was also done.

2. Field experiment

Field experiments were conducted in a paddy rice field in Baroro, La Union from January to May 1989. The site is adjacent to a river which serves as the source of irrigation water, the salinity level of which ranged from 10.7 to 24.0 dS/m. Also, soil E.C. ranged from 0.15 to 10.6 dS/m during the time of testing. Two trials were conducted. Trial I is composed of 289 selected seedlings from the

greenhouse screening test in Los Banos while 60 seedling selections from the screening test conducted in La Union comprised Trial II. Based on overall field performance (vigor, reaction to diseases and insect pests and apparent yield)), 24 lines were identified to be promising. Selections from backcross and segregating populations were also grouped into different categories based on the observed differences in terms of fruit characteristics (i.e. color, size and shape). One major problem encountered however, was the damage caused by roaming animals such as goats and carabaos of the nearby areas.

Field planting of new accessions screened at seedling stage was also done in Los Banos, the objective of which is to further evaluate the horticultural merits of the plants. Several lines were observed promising based on overall performance and these lines will be used in future screening and hybridization work.

3. Grafting

Preliminary field experiment of grafted plants showed that the method used (in arching technique) could be used as an immediate means of utilizing salinity tolerance in tomato plant. The six combinations planted in La Union with Marikit/Improved Pope as stocks and three processing and one fresh market types as scions were observed to be vigorous and were able to produce marketable fruits.

The physiological mechanisms of grafting as a means of incorporating salinity tolerance will be further elucidated in future studies.

4. Evaluation of selected Improved Pope and Marikit strains

Individual plant selections from Impoved Pope and Marikit strains derived from previous tests were further screened and evaluated. Based on the results obtained, the tolerance of IP_4 and M_9 to salt stress was confirmed. These superior strains identified are now being used as check varieties in the screening trials being conducted and as parents in hybridization works.

5. Comparative response of tomato to salt stress under high and low elevation conditions

Three wild tomato species (L. cheesmanii (Lc), L. pennelli (Lpa) and L. peruvianum (Lp) and the cultivated types Marikit and Improved Pope were screened for salinity tolerance at the seedling stage both under high and low elevation conditions and their reactions were compared. Mean air temperature during the time of testing ranged from 15.8 - 19.2°C (Baguio) and 21.6 - 22.8°C (Los Banos). Results showed that symptom development was apparently slower in the highland than in the lowland. Moreover, the degree was observed to be more severe in latter conditions.

Based on this two-location testing, Lpa was observed to be the most tolerant among the three wild types producing slight tip burning only. Both Marikit and Improved Pope were moderately tolerant to salt stress.

In addition, comparison was also made between the screening trials conducted in Los Banos during the cool and the hot seasons. Generally, plants were observed to show prominent stress symptoms when screened during the latter season. Differences in reaction of salt stress were noted in several entries screened at different periods.

These observations support the findings that temperature has a marked effect on the reaction of plants to salinity.

6. Screening of tomato lines, accessions and segregating populations for salinity tolerance at the germination stage

Germination of tomato was found to be affected by increasing concentrations of NaCl; the critical level of which was between 75 to 100 mM (Technical Report July-December 1989). This finding became the basis for the screening trials conducted at the germination stage. Two screening batches, each with nine and 13

entries were done. Germination was carried out in two levels of salt solution (0.0 and 100 mM NaCl) with electrical conductivities of 2.2 and 10.4 dS/m, respectively. Twenty-five seeds of each entry were arranged in 2-layer filter paper in a petri dish treated with 10 ml of the salt solution.

From a total of 22 entries screened, two entries namely, 13-39-1 and 88-003 were judged to be the best because they showed the least percentage reduction at 31.72 and 35.06%. In contrast to the results obtained at the seedling stage screening, the check varieties Marikit and Improved Pope were observed to be susceptible to salt stress at this early stage of growth. Between the two, Improved Pope performed better than Marikit producing a much lower percentage reduction in both trials.

7. Determinin the most sensitive growth stage of tomato to salinity treatment

Seeds of Marikit and Improved Pope were sown in sterilized soil medium. Seedlings were pricked directly in size 12" pots. Four concenrations of NaCl were used: O (control), 100, 150 and 200 mM with electrical conductivities of 1.7, 11.1, 16.1 and 21.3 dS/m, respectively. Plants were salinized for two weeks at five different growth stages: a) seedling stage (16 DAS), b) early vegetative stage (23 DAS), c) late vegetative stage (30 DAS), d) flowering stage (44 DAS) and e) fruiting stage (51 DAS). In addition, one set of plants was continuously treated with salt solution throughout the course of the experiment. The experiment was laid out in 2 x 4 x 6 factorial in RCBD with three replications.

Increasing salt concentration from 0 to 200 mM NaCl caused reduction in all parameters measured except succulence. At 100 mM NaCl, root length and weight, and root to shoot ratio were significantly reduced, however, shoot length, number of marketable fruits and yield (marketable and total) were only affected at concentrations of 150 and 200 mM NaCl. Marikit performed better than Improved Pope in terms of overall performance.

Plants treated at the seedling stage (16 DAS) showed greater reduction in root length and weight, succulence, number of marketable fruits and marketable yield than those treated at later vegetative stages. There was no significant interaction between variety and growth stages, however, salt level and growth stage interaction showed highly sigificant differences in root and shoot length, shoot weight and total yield.

8. Characterizing the response of Marikit, L. cheesmanii, F_1 M x Lc and F_2 progenies to different levels of salinity

L. cheesmanii, Marikit and their F_1 and F_2 progenies were grown in Hoagland's solution containing the required amount of NaCl to give 0, 50, 100 and 150 mM NaCl. The total amount of salt required was added in three increment every other day. The electrical conductivities of the solution were 2.15 dS/m (0 mM), 7.72 dS/m (50 mM), 13.62 dS/m (100 mM) and 18.22 dS/m (150 mM). The experiment was terminated one month after the salt treatment.

Increasing salt concentration from 0 to 150 mM resulted in reduction in shoot and root length and total dry matter and had no effect on root -shoot ratio and succulence. Reduction was drastic in Marikit but slight and gradual in L. cheesmanii, however the F_1's appeared to be more sensitive than the parents based on the above parameters. Varying rate of reduction was observed in the F_2's and some segregants showed capacity for adaptation to saline environment.

In general, ion concentration was highest in root than in stem and leaf. Under non-saline condition, the F_1's and F_2's contained lesser amount of Na^+ than their parents. With increasing salt concentration, Na^+ content in all entries increased.

Salt had no effect on K^+ uptake of the plant. Varietal differences were observed with Marikit having the highest concentration of K^+ absorbed from 0 to 100 mM NaCl.

L. cheesmanii had the highest Ca^{++} content in all treatments. Its uptake tended to increase at 100 mM. The F_1's and F_2's had lower Ca^{++} content than their parents.

Na^+/K^+, Na^+/Ca^{++} ratios of the F_2's tended to increase with increasing concentration of salt.

9. Callus establishment and maintenance from somatic tissues

Experiments on callus establishment and maintenance from somatic tissues were carried out. Growth curves of explanted leaf tissues were determined and plants were regenerated from tomato leaf calli.

10. Anther culture

Successfully-grown calli explants on DBM supplemented with 2.0 ppm Ki + 0.02 - 0.25 ppm NAA gave 92.5% survival and callus regrowth was 67.5% on the medium supplemented with 0.02 ppm NAA + 2.0 ppm Ki. Callus which were planted onto the media supplemented with 0.25 and 0.1 ppm NAA and 2.9 ppm Ki exhibited 80% survival and 42.5 and 45.0% callus regrowth respectively.

11. Plant regeneration of leaf callus on salt media

Shoots were regenerated from calli grown up to three months on media supplemented with 0.3 M NaCl but up to one month only on 0.35 M NaCl. No shoots were recovered for calli derived from K14 and subjected to the salt medium (0.3 M) for 1 month.

Shoot regeneration was observed on calli derived from K14 exposed to 0.3 M salt for two months and also from MS-C-K14 exposed to 0.3 M NaCl for three months on media with 100 ml/l coconut water - 20 g/li sucrose (10%).

12. Shoot culture on media with salt

One month exposure to 0.25 M NaCl caused shoot yellowing, wrinkling of stems and non-root development as compared to the normal growth observed from the control.

Experiments in progress:

1. Screening for salinity tolerance

Thirty-nine entries totalling to 4987 seedlings are being screened for salinity tolerance using the soil culture technique. These entries are composed of selections from previous screening tests, advanced populations of crosses involving the wild and the cultivated species and selections from the field trials conducted in La Union.

2. Grafting of tomato

Seeds of Improved Pope and Marikit (scions) and one fresh market and three processing types (scion) were sown for grafting purposes. The mechanism of grafting as an immediate means of utilizing salinity tolerance in tomato will be elucidated.

3. Determining the effects of salt stress on two growth stages of tomato

Experiment to determine the effects of salt stress on two growth stages of tomato cv. Marikit is in progress. Two concentrations of salt stress were used: 0.0 and 200 mM NaCl applied at vegetative and flowering stage. Data on morphological as well as physiological responses are being gathered.

Tissue culture techniques:

4. Induction of callus on anthers of tomato and plant regeneration.
5. Cytology of cultured anthers (new and old callus).

In-vitro selection:

6. Establishment and maintenance of tomato callus on media with salt. The media used in this experiment include the constituents of MS with different levels of Ki and IAA.
7. Testing for viability of callus on liquid and solid state in the presence of salt (0, 0.30 and 0.35 M NaCl).
8. Plant regeneration of salt-stressed callus at different levels of sucrose (0, 15, 20, 25, 30, 35, 40 g/l).
9. Assessment of the performance of the regenerants to salt stress in-vitro. Shoots (clump + 2 nodes) are exposed to MS basal medium with 0.2 M NaCl.