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Improvement of Salt Tolerance in Tomato (C5-221)

A progress report submitted to AID for the period July-December 1988

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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

a. Part of the seeds obtained from the crosses between Floradade (FD) and fertile plants of the BC_1, F_2 and F_2 (F_1 x F_1) generations* were sown and planted in Hoagland solution in the greenhouse. Because of unexpected increase in the population of the white flies during the summer of 1988 all plants were contaminated with the tomato yellow leaf curl virus (TYLCV). The seedlings obtained from the remaining seeds were protected by covering them with agryl P17 and by spraying daily with 0.1% cymbush 10. Consequently, the plants obtained are healthy. Maximum production of seeds in these plants (which segregate, among other characteristics, for the ratio length of style/length of anthers) is ensured by selfing them manually at least once a week. Part of these selfed seeds will be planted in Hoagland solution + NaCl in the greenhouse in Israel. The remaining seeds will be sent for experiments in the Philippines.

b. Crosses are performed between the tomato cultivar Rodade (RD), which is resistant to bacterial wilt, and plants of the BC_1, F_2 and F_2 (F_1 x F_1) generations*.

* CV x Lpa or Lc 1401 F_1 (CV x Lc1401) x F_1(CV x Lpa)

x —— F_1(x) F_2(F_1 x F_1)

BC_1 F_2

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

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

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

a. Plants regenerated from leaf explants and detached shoot apices (while being exposed to saline media) were selfed and their progeny were planted in Hoagland solution + 100 mM NaCl. The growth of the seedlings was recorded using the method of Coleman and Gregson (1976). This experiment was not completed due to infection by TYLCV. New plants are regenerated under identical conditions and the experiment will be repeated. The media used in these experiments include the constituents of Murashige and Shoog (MS) with 2 mg/L BAP and 0.2 mg / L IAA to obtain plantlets, and with 0.2 mg/L NAA for rooting.

b. Calli were exposed to different NaCl concentrations (up to 400 mM). The upper concentrations of NaCl which still allow growth of some callus are 150-200 mM. Following the short-term method of selection (progress report July-December 1987), calli are being exposed to these almost lethal concentrations. The media used in these experiments include the constituents of MS with 5 mg/L NAA and 0.5 mg/L kinetin.

c. We had some difficulties in maintaining the suspension cells of tomato. It seems that after solving the problems, we will be able to continue the experiments as planned (sections 2d and e in the previous report).

d. We continue the regeneration experiments. For that, calli are subcultured on MS media containing 2 mg/L BAP and 0.2 mg/L IAA or 2 mg/L zeatine and 0.2 mg/L IAA together with 1% mannitol and 2% sucrose.

3. Mycorrhiza

We started to check for the existence of mycorrhiza which is salt tolerant or has the ability to interact specifically with plants which are exposed to high levels of salt. For that, roots of Suaeda asphaltica (Boiss.) and soil were collected in the desert eastern to Arad. Mycorrhizal spores were found in the soil. Maximum conductivity and pH were found to be 36

dS/m and 8.5, respectively. The mycorrhiza used already in the Philippines is from another source.

4. Development of calli from anthers of cultivar FD

Calli were developed from anthers (taken from 2-3 mm buds) cultured on W5 medium (Feng and Ouyang, 1988) with 2 mg/L 2,4-D and 0.5 mg/L kinetin. These calli will be checked for ploidy level.

5. Two research associates from the Philippines (V.P. Roxas and P.J. Santos) visited our laboratory for 3 months (August, September and October).

References

Coleman, W.K. and R.T. Greyson. Can. J. Bot. 54: 2421-2428 (1976).

Feng, G.H. and J.W. Quyang. Plant Organ, Tissue and Cell Culture 12: 3-12 (1988).

Research activities in the Philippines

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

A series of experiments were conducted to assess the performance of tomato to salt stress at the early growth stage using the soil culture technique. One month old seedlings were subjected to salinity stress using common salt at an electrical conductivity of 15 dS/m. Visual stress rating of 1-5 (1 = Resistant; 5 = Susceptible) and % selection were noted. Percentage selection after salt stress during the first and second batches were 13.7 and 7.5, respectively (Progress Report, Jan.-June 1988): 9.7 for the third and 10.9 for the fourth batch. The total number of plants screened were 1,034 (Batch I), 1,588 (Batch II), 2,540 (Batch III) and 5,465 (Batch IV) for a total of 9,039 plants. In all trials, Marikit (M), Edkauri and segregating populations from the cross Marikit x L. cheesmanii 1401 (Lc) showed consistent superior performance. Individual plant selections were planted in pots and

evaluated for their horticultural merits. Percentage selection after evaluation was 4.6, 2.5, 2.4 and 2.8 for batches I-IV, respectively. Due to high night temperature during the March-October period, low fruit setting was observed. Virus infection also caused difficulties in assessing the horticultural merits of the potted plants.

Selected plants from segregating populations of M x L. pennellii (Lpa) and Improved Pope (IP) x Lpa were taller, very vigorous and showed resistance to leaf diseases. They flowered profusely but very low fruit setting was noted. The leaves were more of the Lpa type. On the other hand, those of M x Lc and IP x Lc were shorter. The leaf shape varied from L. esculentum type to intermediate between Lc and L. esculentum. They also showed resistance to leaf diseases particularly leaf spot (Stemphyllium lycopersici) and in contrast to those with Lpa background, high fruit setting was observed among plants.

2. Evaluation of different strains of IP and M for their reaction to salt stress.

Nine strains of M and nine of IP were screened for salt tolerance using the soil culture technique. High % selection was found in M strains # 10 (25.0), # 9 (15.79), # 3 (13.79), # 1 (13.38) and # 5 (12.85) while IP strains # 8, # 6, # 7 and # 4 got 21.06, 18.27, 15.08 and 10,59, respectively. Percent selection for M ranged from 6.36 to 25.00 and 3.35 to 21.06 for IP. IP was more variable than M in fruit characteristics. Superior strains will be used in future experiments.

3. Hybridization among selected plants.

Crossing among selected salt tolerant plants was done including backcrossing of resistant progenies derived from crosses of M and IP with Lpa and Lc to the cultivated parents. High night temperature had reduced fruit setting of most plants, especially those with Lpa background, hence, these plants were used only as pollen source.

Seed production of the wild species was done in the highlands where temperature is more favorable for fruit setting. However, Lpa could not set fruit even under conditions in the highlands.

4. Grafting.

Arching technique showed 89% success with the progenies of the cross between Lc x cultivated as stock and the local cultivated M as scion.

5. Mycorrhiza experiments

Results of two trials conducted showed that inoculation with mycorrhiza had no significant effect on root length, shoot dry weight and succulence of 6 tomato varieties. Irrespective of variety and mycorrhiza, salt treatment caused a significant decrease in shoot length and total dry weight.

6. Germination Test

Seeds of M, IP and UC-204 were germinated in Petri dishes containing solutions of 0, 25, 50, 75 and 100 mM NaCl. Three trials were conducted: I and II at room temperature and III at a growth cabinet with a day/night temperature of 20°C/30°C.

Percent germination was reduced only at 75 and 100 mM NaCl but speed of germination was gradually reduced at increasing concentrations of NaCl. Germination under controlled temperature showed no varietal difference but at room temperature, IP performed better than M and UC-204. Apparently, germination under salt stress is not correlated to salinity tolerance at the seedling stage.

7. Characterizing the response of selected tomato entries to different salt levels.

Five entries, namely M, Lc and their hybrids, were treated with NaCl ranging from 0 to 150 mM. In terms of morphological characters, Lc appeared to be tolerant to saline solutions than M but their F_1's were inferior to their parents.

In another trial, M, Edkauri, IP, UC-204, and F_1 (M x Lpa) were characterized using the same salt concentration in terms of shoot length, root length, shoot weight, root weight, root-shoot ratio, succulence, and visual stress rating. All parameters measured were significantly affected by the treatment. Based on visual stress rating two weeks after treatment, varietal differences were observed. UC-204 showed poor vigor and early signs of salt stress characterized by leaf chlorosis of the mature leaves progressing to younger leaves. M and Edkauri were tolerant to salt stress maintaining vigor and green leaves throughout the duration of the salt treatment. IP and F_1 (M x Lpa) showed intermediate reaction between M, Edkauri, and UC-204. Without the addition of supplemental NaCl solution after the initial application no significant varietal diferences were noted after one month.

8. Determining the most sensitive growth stage of tomato to salinity treatment.

Four tomato entries namely, M, Edkauri, UC-204 and IP were subjected to salinity stress at 5 different growth stages. Data obtained were inconclusive due to severe virus and nematode infections. A follow-up experiment is in progress.

9. Monitoring salinity levels of saline areas.

From January-August 1988, EC level of a field in La Union ranged from 0.47 to 1.5 dS/m (soil) and 3.7 to 25.8 (river) while that of Pampanga is 5.6 to 21.3 (soil) and 4.6 to 23.0 (river). Monthly monitoring of soil and water samples is being continued.

10. Aseptic maintenance of tomato plants and callus establishment.

Of the growth regulators evaluated for primary callus induction, combination of 0.5 ppm kinetin (kin) and 2.0 ppm NAA repeatedly performed best. Survival and callus formation from leaf explants were not significantly affected by population (5, 7, 9 and 11 leaf explants/flask).

11. Callus maintenance

Callus induced in 2.4-D supplemented medium fared poorly on subsequent culture as compared to calli induced from kin and NAA supplemented medium. The logarithmic phase of growth of callus was from 6 to 21 days. Browning was observed after 15 days. No effect on population was observed on subculture of callus.

12. Anther culture

Callus induction was successfully done using the method of Gresshof and Doy (1972). Mean of callus formation was 36% for mixed varieties and 10.5% for IP.

Another growth was supported with combinations of kin and NAA. Callus formation of microspores were observed on cytological inspection.

13. In-vitro selection

A. Leaf tissue (on callus induction medium)

Only 0.2 M NaCl supplemented media showed callus formation. Death of tissues was observed on other treatments (0.25, 0.30, 0.35 and 0.40 M).

B. Leaf callus

Callus subcultured to fresh media with the same treatments used in A affected survival and growth of callus. Increasing salinity level caused severe browning of callus; 24.8% callus survived at 0.40 M NaCl.

Experiments in Progress

1. Screening for salinity tolerance.

Screening of 35 new accessions at seedling stage using the soil culture technique is currently being done. Most of these entries came from the Asian Vegetable Research and Development Center (AVRDC).

2. Determining the effect of temperature on the reaction of plants to salinity stress.

Seeds of 4 wild tomato species (Lc, Lpa, L. peruvianum and L. hirsutum), M and IP were sown in the highlands (Baguio) and lowland (IPB). Plants' reaction to salt stress will be assessed under these locations.

3. Response of tomato to salt stress at different growth stages.

Two tomato varieties are being 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 continuously being irrigated. Data on morphological and physiological responses will be gathered.

4. Plant regeneration and callus induction, using the method of Gresshof and Doy, is being done.

Literature Cited

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

Plan of activities for 1989:

1. Continue screening of accessions, lines, segregating populations, and backcross progenies for salinity tolerance.
2. Field confirmatory test and horticultural evaluation of selected plants.
3. Continue production of hybrids.
4. Seed production of wild species.
5. Grafting experiments: a) root stock evaluation, b) grafting of M with commercial varieties and evaluation of the performance under field conditions.
6. Continue screening for salt tolerance of IP and M strains.
7. Continue determining the best growth stage of tomato for application of salt stress for selection purposes.

8. Continue monitoring salinity level of saline areas (more areas will be included).

9. Tissue culture techniques:

a) Callus maintenance - effect of pH of media on the growth of tomato leaf callus.

b) Plant regeneration from long term callus cultures from somatic and haploid calli.

c) Establishment of techniques for cell suspension cultures of somatic and haploid calli.

d) Anther culture of callus of IP.

10. In vitro selection:

a) Maintenance of salt-stressed callus.

b) Plant regeneration from callus exposed to NaCl medium.

c) Response of salt-sressed callus at different temperatures.

d) Evaluation of regenerants.