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Improvement of Salt Tolerance in Tomato (C5-221)

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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 (referred to as BC_1S_1) were obtained by selfing the progeny of the crosses between the tomato cultivar Floradade (FD) and plants of the BC_1, F_2 and $F_2(F_1 \times F_1)$ generations*. A great part of these seeds was sent for screening in the Philippines. Some of the remaining seeds were germinated and transferred to Hoagland solution containing 100 mM NaCl, in Israel. Their growth was recorded at the beginning using the Plastochron Index. Finally we decided to select plants based only on their phenotypic appearance: those which remained relatively small or were damaged by the salt (including chlorosis, wilting and drying of leaves) were discarded. Leaves were taken from representative seedlings for determination of the content of Na^+, K^+ and Cl^- (Table 1).

Table 1. Content [mmol (g dry wt)$^{-1}$] of Na^+, K^+ and Cl^- in leaves of BC_1S_1 seedlings exposed to 100 mM NaCl.

Appearance of plant	Ion		
	Na^+	K^+	Cl^-
Normal green	2.4	1.5	1.1
Yellow	11.6	0.7	4.3
Mixed-green and dry	3.7	1.2	2.3

The level of the three ions, Na^+, K^+ and Cl^-, in the leaves of the normal green appearing plants was similar to that found previously in the cultivated tomato under similar conditions in

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*CV x Lpa or Lc F_1 (CV x Lc) x F_1 (CV x Lpa) CV=Marikit (M) or Improved Pope (IP)

↓ ↓ ↓

x —— F_1* $F_2(F_1 \times F_1)$

↓ ↓

BC_1 F_2

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

our lab. The level of both Na^+ and Cl^- was much higher in plants which became yellow and higher also in those with mixed (green and dried) leaves.

Seeds (BC_1S_2) obtained by selfing the salt-treated BC_1S_1 plants were classified according to the size of the fruit from which they were obtained: small-about 6g FW and 23 mm diameter; medium-13g FW and 30 mm; and relatively large-48g FW and 55 mm. Some of the seeds were sent for screening to the Philippines and some of them will be planted in a short time in salinized (100 mM NaCl) Hoagland solution in Israel.

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

a. Plants are continuously regenerated from leaf explants exposed to saline (MS medium containing 2 mg/L benzyl adenine (BA), 0.2 mg/L IAA and 75 mM NaCl) or non saline media. These plants are grown, respectively, in salinized (75 mM NaCl) or non salinized Hoagland solution. Seeds obtained by selfing are sent to the Philippines for screening of salt tolerant plants.

b. Calli developed in MS medium + 4 mg/L NAA and 2 mg/L kinetin (KI2 medium) are transferred to the same medium containing 250 mM NaCl. Small pieces of calli which develop in the saline medium are transferred to the same medium containing 200 mM NaCl for propagation and than to a regeneration media.

c. Regeneration experiments - several media compositions have been tested (Table 2). These were, however, without any success up to now.

Table 2. Composition of regeneration media*

Media	Hormones (mg/L)			Sucrose (%)	Mannitol (%)
	Zeatin	BA	IAA		
MS	2	-	0.2	2	1
"	2	-	0.1	2	1
"	1	-	0.01	1	1
"	1	-	-	2	-
"	2	-	-	2	-
"	-	-	-	2	-
"	-	2	0.2	2	1
MS/10	1	-	-	2	±1
"	2	-	-	2	±1
"	2	-	0.2	2	±1
"	-	-	-	2	±1
"	-	-	-	2	±1
MS	2	-	0.01	2% glycerol	±1

* In some of these experiments calli were developed previous to their transfer to the regeneration medium in KI2 medium + 3 % mannitol.

d. Suspension cultures - various media compositions have been tested (Table 3). The cells became dark in all media tested up to now.

Table 3. Composition of suspension media which were or are tested*

Medium	Hormones (mg/L)			Sucrose (%)	Mannitol (%)
	Kinetin	NAA	2,4D		
MS	0.25	5	-	3	-
"	0.25	2	-	3	±2
"	0.25	-	2	3	±2
"	0.5	-	4	3	±2
"	2.00	4	-	3	±2

* Some of these media will be tested after developing the calli in KI2 medium + 2% mannitol.

3. We started again our experiments with pollen grains, including: a) selection for salt tolerance; b) study of the response of pollen taken from the Lpa and M to salt stress.

Work in the Philippines

Background Information:

The project on salinity improvement of tomato was started in 1987 in view of the importance of the crop and the need to expand crop production in saline areas. Major activities include selection and hybridization, field evaluation testing, monitoring salinity levels of saline areas, determining the most sensitive growth stage of tomato, callus maintenance, anther culture, in vitro selection and regeneration of callus. Genetic resistance was obtained from two wild species (Lpa and Lc) which were used in a backcross breeding program. Relatively high levels of salt-tolerance was observed in some commercial varieties.

Highlights of Accomplishments:

I. Breeding and Physiology

The search for resistance of salinity at seedling stage continued with screening of 45,311 seedlings; of which only 2845 (6.7%) were selected. Advanced lines of crosses involving the wild types Lpa and Lc (F4's and F5's), line selections from accessions obtained from the National Plant Genetic Resources Laboratory (NPGRL) and the check variety Marikit consistently exhibited slight to moderate symptoms only under controlled salinity stress of EC=15 dS/m.

Field testing in La Union confirmed the high level of tolerance of 35 advanced lines of crosses involving the wild types Lc and Lpa and 25 previously selected entries. Grafted plants using salt tolerant rootstocks were also able to tolerate salinity stress and produced marketable fruits.

At UPLB, evaluation or horticultural characteristics in pot experiments among salt tolerant plants resulted in 102 individual plant selections which showed relative heat tolerance and apparent resistance to foliage diseases.

Hybridization among selections and the commonly-grown varieties improved Pope and Marikit was also done to further improve on plants adaptability and fruit traits as well as tolerance to salinity.

The observation that temperature has a marked effect on the reaction of plants to salinity was further confirmed in the results of screening tests of three wild tomato species (L. cheesmanii (Lc), L. pennellii (Lpa) and L. cheesmanii (Lc) in the lowland (IPB) and highland (Baguio). Very slow symptom development was noted in the highland compared to that in the lowland. Among the three wild species, Lpa was the most tolerant producing slight tip burning only. Matrikit exhibited a slight edge in salinity tolerance over Improved Pope.

Screening tomatoes at germination and seedling stage showed that no relationship exists between the reaction of the plants to salinity stress during these growth stages. Differential reactions of Marikit, Improved Pope, VF-134-1-2 and 95-43 to increasing salt concentration were noted at a certain growth stage. Treatment of seedling stage (16 DAS) caused significant reduction in root length, root weight, succulence, number of marketable fruits and yield.

An experiment in hydroponics with increasing concentrations at NaCl using Marikit, Lc, and their F1 and F2 progenies showed that increasing level of salinity caused significant reduction in shoot growth and that Na^+ increased in all plant parts without altering the level of distribution. Lc showed the highest tolerance producing heavier and more succulent roots.

In another hydroponic experiment, the ion profiles of tomatoes with respect to Na^+, K^+ and Ca^{2+} at different plant parts were determined one month after growing in nutrient solution containing 0, 25, 50, 100 and 150 mM NaCl. Relative to the response at 0 mM NaCl, the levels of the different ions were altered under all saline conditions. The resistant check, Lycopersicon pennellii had low level or K^+ in stem, high level of Na^+ in root but low in both

petiole and leaf. Ca^{2+} in the root was also low. Of the two resistant cultivars, Marikit and Improve Pope, the former had ion pattern more similar but not absolute to that of the L. pennellii. This tended to indicate the similarity in the mechanism of salt tolerance between the two. Improved Pope had different ion patterns which were quite similar to that of VF 134-1-2, the susceptible cultivar.

II. Tissue Culture

A Protocols for callus culture and plant regeneration

1. A series of experiments were conducted to assess the best protocol for callus establishment and maintenance. Among three protocols for callus establishment verified, MS + 0.5 ppm kinetin (KI) and 2.0 ppm NAA (MS-C) was optimum for callus induction, while MS + 4.0 ppm kinein and 4.0 ppm IAA (KI4) was optimum for maintenance of creamy compact callus. After callus initiation on MS-C for 2 weeks, calli should be transferred to KI4 (MS-C-KI4).

2. Calli induced from the MS-C, KI4 or MS-C-KI4 media were transferred to plant regeneration media using different growth regulator combinations. Shoot regeneration was affected by the source of callus. Calli induced on KI4 and MS-C-KI4 yielded shoot regeneration while calli induced on MS-C did not produce any regenerant.

Among growth regulators evaluated for shoot regeneration, 1.1 ppm zeatin and 2.0 ppm zeatin riboside gave 40 and 50% regenerants from KI4 induced calli, respectively.

Forty and fifty percent calli cultures formed shoots when subcultured to plain MS medium with 15g and 20g/L sucrose.

B. Anther Culture

1. Induction of callus from anthers of Improved Pope was performed using Gresshoff and Doy's media formulation containing NAA or IAA. Percentage of calli induction was 40% on Gresshoff and Doy's formulation with 2 ppm NAA while 10% on media with 2 ppm IAA.

Indole acetic acid (IAA)-induced calli originated from anther walls; on NAA media calli originated from within the anthers at the ruptured site of the anther.

2. Ploidy level of anther-derived callus was assessed every hour from 8:00 a.m. to 3:00 p.m. No dividing cells were observed.

C. In vitro Selection

1. Experiment on plant regeneration from selected callus lines was conducted using different sucrose or coconut water levels without NaCl. Shoots were more frequently regenerated on coconut water-sucrose supplemented media than the sucrose media. Among the concentrations of coconut water tested, 100 to 200 ml/L favored regeneration. Combinations of coconut water with higher concentrations of sucrose may prove favorable to regeneration.

Shoot regeneration among salt-stressed calli was higher when the calli was induced from KI4 than MS-C-KI4.

2. Salt-stressed calli were also regenerated on media with NaCl. Among the media evaluated at three culture cycles, the combination of 2 ppm BA and 2 ppm IAA performed the best.

With increasing time of exposure to maintenance media, percent shoot regeneration decreased.

3. A preliminary selection on plant level was tried using the unselected lines. All the shoots turned yellow one month after exposure to 0.25 M NaCl. No roots were developed among salt-treated shoots.

4. In vitro screening of selected somaclones was performed. Among the 35 somaclones preliminary screened in vitro, 30 did not show yellowing within the month culture on MS ± 0.05 M NaCl. These resistant lines also produced fully developed roots while the susceptible plants rooted poorly.

The control, L. cheesmanii, showed resistance when cultured on MS media with 0.05 M NaCl. Early rooting in L. cheesmanii was observed as compared to all the somaclones screened in vitro.

Seedling-derived Improved Pope plants were susceptible when cultured to saline media. All the leaves turned yellow and within a month of culture. No rooting was observed.

Of the 30 somaclones resistant at 0.05 M NaCl, 29 further proved to be resistant on 0.10 M NaCl. In vitro screening should be done with plantlets from NaCl-free propagation medium because of adaptation.

5. Combinations of IAA (0.5-4.0 ppm) and Ki (0.5-4.0 ppm) were evaluated to identify growth regulator combinations which would maintain regenerative callus in the presence of salt. Calli browning was reduced with 0.5 or 1.0 ppm IAA during the first month. Absence of calli regrowth during second month may have been influenced by incubation conditions.

Plans for 1990:

1. Screening of lines, accessions and segregating populations of tomato for salinity tolerance.
2. Field confirmatory test of selected plants.
3. Hybridization among selected lines. Backcrossing of salt tolerant lines to Phil. adapted parents.
4. Seed production of 25 advanced lines/previous selections with a target yield of 200 g each.
5. Field evaluation of grafted plants and selected Marikit and improved Pope strains.
6. Study the mechanism of salt tolerance.
7. Anther culture with emphasis on media studies for callus establishment and subsequent plant regeneration.
8. In vitro selection - related activities including maintenance and increase of callus stock, exposure to salt supplemented media and regeneration of plants from salt-treated calli.

9. Evaluation of plant regenerants for salt resistance in vitro.

10. Initiate experiments on incubation conditions (particularly temperature) as these factors relate to in vitro selection.