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Improvement of Salt Tolerance in Tomato

Principal Investigator : Moshe Tal

Department of Biology, Ben Gurion University of the Negev, P.O. Box 653, Beer Sheva 84 105, Israel

Principal Co-Investigator : Dafrosa A. Del Rosario

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

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I. Executive Summary

The collaborative research project between the Philippines and Israel on the improvement of salt tolerance in the tomato paved the way for the development of improved tomato varieties with tolerance to salinity. Following an interdisciplinary team approach, both conventional (transfer of genes through crossing from wild salt-tolerant tomato species to the cultivated one) and nonconventional (*in vitro* selection in organ and tissue culture) breeding methods were pursued.

Greenhouse screening of 71,359 seedlings comprised segregating populations produced in the Philippines and Israel as well as several cultivars and accessions of the cultivated species. The screening technique included one-month-old seedlings grown in pots in soil and irrigated with saline water (EC = 15 dS/m). Segregating populations included: (a) progeny of the wide crosses involving the two wild species *Lycopersicon pennellii* acc. atico (Lpa) and *L. cheesmanii* ecot. 1401 (Lc) as the salt-tolerant parents in the breeding program. Backcrossing was performed to the cultivated (processing types) parents Marikit (M) and Improved Pope (IP), as well as the commercial varieties Floradade (in both countries) and Walter (only in the Philippines). The latter two were included for the improvement of horticultural merits. Advanced lines (F_6, F_7, F_8) including brackcross progeny (BC_2-F_4, BC_3-F_4, BC_4-F_3) were produced by the pedigree selection method in the Philippines. Backcross progeny, selfed for four consecutive generations (BC_1S_1 to BC_1S_4) were produced in Israel. The latter were grown in Hoagland solution and were selected for salt tolerance by adding (to seedlings) 100mM NaCl each generation. (b) plants regenerated from tissue and organ cultures. These plants were regenerated (R_0). from Calli of IP which were transfered through repeated cycles of (0.30 and 0.35) NaCl treatments in the Philippines, or were obtained by selfing (R_1) regenerants (R_0) which were produced from leaf explants of M in Israel.

Among the three wild species Lpa, Lc and *L. peruvianum*,, Lpa was found as the most tolerant, producing only slight tip burnings. Among the cultivars, relatively high level of tolerance was observed in cv. Edkauri as well as in the local cvs. M and IP. Marikit exhibited a slight edge in salinity tolerance over IP. The influence of the tolerant parents (especially Lpa) was apperant in the advanced generations of the main crosses (F_2, F_3, F_4) made in the Philippines in terms of salt tolerance, leaf type, growth habit and fruit size. Plants similar to the cultivated types in growth habit, leaf type and other characteristics were selected from advanced progeny (F_4, F_5) of the main crosses. A high degree of salt tolerance was maintained in the selected plants of the BC_2 and BC_3 generations which were almost the same in appearance with cv. M. Five entries, which performed better than cv. M in their reaction to salt stress, were screened from selfed backcross progenies involving Fd and M as the recurrent parents. The observation that temperature has a marked effect on the reaction of plants to salinity was further confirmed by the results of the tests of the three wild species Lpa, Lc and *L. peruvianum*, in the

lowland (los Baños) and highland (Baguio): slower development of symptoms was noted in the highland as compared with the lowland.

Field testing in two saline areas [Feb.-May 1989 in Baroro (La Union) and Nov.-Apr. 1990 in Puerto Galera (Mindoro Oriental)] confirmed the high level of resistance of the greenhouse selected plants. Plants were irrigated with river water (where salinity level during the season changed from 10.7 to 24.0 dS/m) in the former and with a mixture of sea and ordinary water (1:3, EC=14-15 dS/m) in the latter. Twenty-four promising lines were identified in Baroro based on overall field performance. These included F_3 and F_4 of the advanced lines from the wide crosses and their backcross progeny. Promising selections, consisting of backcrosses and advanced progenies of the wide crosses F_5 (M x Lc and M x Lpa), were identified also in Puerto Galera. In most of them an increase of the soluble solid content of the fruit was noted. Final field evalution of promising material, including selected seedlings derived from the *in vitro* selections performed in both countries, is currently on-going in Puerto Galera.

Efforts in the *in vitro* aspect of the project have been geared mainly to the development of protocols for: A. the development of callus, selection of salt tolerant calli, regeneration of plants from calli and anther culture in IP. Murashige and Skoog (MS) medium + 0.5 ppm kinetin and 2.0 ppm NAA was found as the optimum for callus induction, and MS + 4.0 ppm kinetin and 4.0 ppm IAA for calus (creamy and compact) maintenance. Near-kill concentrations(0.30 and 0.35mM) of NaCl were used for the routine *in vitro* selection at the callus level. Regenerant plants (R_0) were obtained from both salt-treated and untreated calli. Shoot regeneration from the former calli was favored by adding coconut water (100 - 200 ml/L) together with sucrose (20g/L) to the medium (MS + 2 ppm BA and 2 ppm IAA). Among the somaclones screeened, 51 performed better than the seed-derived plants. B. the development of callus, cell suspension and regeneration from calli (the latter two without success) and from leaf explants in M. Regenerated plants (R_0) were obtained only from leaf explants [salt (75 mM) -treated and untreated, in MS + 2 ppm BA and 0.2 ppm IAA]. These plants were grown, respectively, in salinized and nonsalinized Hoagland solutions and seeds (R_1) were produced by selfing. The R_1 plants of both groups were found to have slightly higher salinity than the seed-derived M plants.

Additional preliminary experiments, which require further elucidation, included: A. the potential of grafting as an immediate mean to improve salt tolerance of tomato. Plants in which the susceptible cv. Maigaya (MG) was used as either stock or scion together with M (a relatively salt-tolerant cultivar) remained green and healthy in salinized Hoagland solution. Sodium level was drastically reduced in the roots of both grafting combinations as compared with MG/MG plants and in the shoot of MG/M (M as scion) as compared with M/MG plants. On the other hand, plants in which MG was used as either stock or scion with IP (less tolerant than M) had yellowish leaves.

B. the potential of mycorrhizal infection as another immediate mean to improve salt tolerance: (a) three vesicular-arbuscular mycorrhiza species which were used in the Philippine had no significant effect on the salt tolerance of infected plants; (b) mycorrhiza was collected in salty desert soils in Israel. Low level of infection of tomato roots was noticed. C. a comparison of: (a) *in vitro* germination of pollen grains of cv. M and the salt-tolerant species Lpa; and (b) the effect of salt stress applied during pollen development (in M and Lpa plants) on fruit set, fruit weight and number of seeds per fruit in pollinated control M plant. Pollen of Lpa was found as more tolerant to salt. Greater fruits and more seeds per fruit were produced in M plants pollinated with the 'salt-treated' pollen. D. experiments to assess the performance of selected tomato cultivars to salt stress at different growth stages have shown that there is no relationship between tolerance at germination and at later growth stages. E. callus induction (61%) from anthers of IP was achieved by using the Gresshoff and Doy's medium containing 2ppm 2,4-D + 2ppm IAA + 1.5 ppm kinetin.

Results of ion accumulation studies in the prental species as well as in various segregating progenies support the suggestion that the regulation of Na level plays an important role in the salt tolerance of tomato.

So far, the project has contributed to tomato research in the Philippines by developing lines with variable potential as well as genetic stocks for salinity tolerance which can be used in future breeding efforts. A number of these selections can be used in order to make the vast hectarage of idle saline soils of the country, productive. Physiological studies provided benchmark information on the "complexity" of salt tolerance. Workable protocols for callus culture, plant regeneration and *in vitro* selection for salinity tolerance were also developed and served as complementary tools in the overall program.

II. Research Objectives

General

Improvement of salt tolerance in tomato in the Philippines.

Specific

a. Screening for salt tolerance in local and foreign cultivars.
b. Transfer of genes from salt-tolerant wild related species.
c. Production of salt-tolerant regenerants.
d. Physiological analyses.

III. Rationale/Justification of the project

The increasing demands of the expanding population for food and energy necessitate the exploitation of arid and semiarid lands which comprise

about 40% of the world land surface (Fischer and Turner, 1978). Such areas are characterized by high salinity in both the soil and water. It has been estimated that 30 to 50% of the 230 x 10^6 ha currently under irrigation in the world is affected by salinity (Maas and Hoffman, 1978; Epstein et al., 1980). Soil salinity is a big limiting factor of food production also in the humid tropics, including the Philippines. The country has about 400,800 hectares of coastal saline soils of which 125,000 hectares are either idle or with poor productivity (Guerero, 1977). The presence of these areas greatly hampers the increase of agricultural production of the country. The use of crops that are salt-tolerant can be an economical solution to this long-standing problem. In view of the economical importance of tomato (worldwide and in the Philippines where it is ranked among the top ten vegetable crops), and realizing the need to expand crop production in saline areas, salinity improvement has been performed in this crop.

Tomato is one of the genera in which extensive physiological and genetic studies of salt tolerance have been performed (Tal, 1971; Rush and Epstein, 1976; Dehan and Tal, 1978; Rush and Epstein, 1981; Tal and Shannon, 1983; Sacher and Staples, 1985; Jones, 1986; Shannon et al., 1987). The wild relatives of the cultivated tomato which evolved in dry habitats in South America may be used as a source of genes for improving the salt tolerance of the cultivated species (Epstein et al., 1980; Tal, 1984; Tal, 1985). *L. pennellii* and *L. cheesmanii* seem to be good candidates for this purpose. Attempts at selection for salt tolerances based on the integrated plant responses to salinity demonstrated that an effective breeding program for salt tolerance can be developed for the tomato. A detailed example of such a breeding program designed to transfer salt tolerance from the wild species *L. cheesmanii* into the cultivated tomato has been reported by Rush and Epstein (1981). A simple breeding scheme, which included a cross between the cultivated tomato and the wild salt tolerance species *L. pennellii*, one backcross generation, a few generations of selfings, and selections in a field irrigated with brackish water, yielded very promising results regarding both salt tolerance and fruit qualities (Zamir, personal comm.).

The use of organ, tissue and cell cultures to select for salt tolerance has been developed in recent years. Existing results (reviewed by Tal, 1990) suggest that continued efforts in this area could lead to the improvement of crop tolerance to salinity. Selection for salt tolerance in undifferentiated cell cultures was rather successful. The list includes many crops among which are tobacco, citrus, alfalfa, tomato, sorghum and rice. The progress in this area is, however, slow due mainly to the difficulty of regenerating normal and fertile tolerant plants. Nevertheless, successful and promising results i.e. the regeneration of normal and fertile salt-tolerant plants, were reported recently.

By following an interdisciplinary approach in this project, salt-tolerant tomato lines have been produced. These lines are expected to be used by the farmers to increase land utilization and food production in the saline areas of the Philippines.

IV. Innovative Aspects

(1) Various approaches have been used to improve the salinity tolerance of tomato : a) screening for salt tolerance within the cultivated species (Ph = Philippines); b) crosses between wild related tolerant species and the cultivated tomato (I = Israel and Ph); c) selection for salt tolerance in callus and organ cultures (Ph and I).

(2) Additional approaches have been tested : a) graftings (Ph); b) mycorrhizal inoculation (Ph and I); c) selection for salt tolerance in pollen grains (I). A rapid, reliable and inexpensive screening technique (soil cultures) was developed (Ph) to enable the screening of large number of entries for tolerance to saline conditions. Protocols for callus culture, plant regeneration, anther culture and *in vitro* selection were developed to assist the improvement program.

V. Methods and Results

Methods

Breeding and screening: Plants screened included segregating populations, new accessions and various cultivars. The segregating populations comprised progeny of the wide crosses involving *Lycopersicon pennellii* acc. atico (Lpa) and *L. cheesmanii* ecot. 1401 (LC) as the salt-tolerant parents in the breeding program. Backcrossing was performed to the cultivated parents *Marikit* (M) and *Improved Pope* (IP) as well as to the commercial varieties *Floradade* (FD - in both countries) and *Walter* (W - only in PH). The latter two were included for the improvement of horticultural merits. Figure 1 includes the scheme of crosses and figures 2 the scheme of pedigree selection used in the Philippines. The crosses and selections made in Israel are described in Table 1. The plants (about 900 each generation) of the selfed (S_1 to S_4) generations were grown in the greenhouse in salinized (100 mM NaCl) aerated Hoagland solution. Salt was added gradually (25 mM per day) to seedlings containing 3-4 leaves. Hand pollination (to improve the success of selfing) was performed only during the S_1 and S_2 generations. In later generations (S_3 and S_4) selfing was spontaneous. Seeds from all four generations were sent for evaluation to the Philippines.

Mass screening for salinity tolerance in tomato was carried out in the Philippines through the use of the soil culture technique. One-month-old tomato seedlings grown in soil (local soil: coir dust: hog manure: sand/2:0.5:0.5:0.5) were irrigated with saline water (mg/L, Na-290; Cl-5180; Ca-9; Mg-74; EC=15 dS/m). Visual stress rating of 1-5 (1 = Resistant; 5 = Susceptible) and % selection were noted. Final plant selection was done both by the breeder and the physiologist. Selected plants were grown either in pots or in the field for evaluation of their horticultural merits. Preliminary field evaluations have been conducted in the saline area in Sto. Domingo, Minalin and Pampanga. Further field testings were conducted in Baroro (La Union) and Puerto Galera (Mindoro Oriental).

Tissue culture and *in vitro* selection : Based on the agreement between the investigators, tissue culture activity in the Philippines was concentrated on IP, while that involving cv. M was worked out in Israel.

Experiments with cv. IP - A. Development of Protocols for somatic tissues. (a) Tomato seeds were decontaminated with 20% bleach and germinated *in vitro*. After 5 days the seedlings were detopped and the shoots were subcultured onto a plant maintenance medium. Shoot cultures were maintained by transfer of the shoots to fresh medium every four weeks. *In vitro* screening was performed at the plant level. Initially, response to salt treatment was evaluated *in vitro*, using shoots obtained from aseptically grown seedlings (in different NaCl concentrations). Subsequent *in vitro* selection experiment involved treating leaf explants in medium containing salt. (b) Among the different plant parts namely root, stem, petiole, leaves (expanded and expanding), induced to callus, the first fully- expanded leaf was found as the best source of leaf explant. Calli were multiplied and maintained by subculturing to KI4 medium [Murashige and Skoog, 1962 + 4 ppm KI, 4 ppm IAA, 10 ppm myoinositol and 2% sucrose]. Near-kill concentrations of NaCl were determined based on > 50% death of cultured calli. Surviving calli were divided and subcultured to media favorable for plant regeneration or to fresh maintenance medium with salt (Fig. 3). Various concentrations of sucrose, coconut water, auxins and cytokinins were evaluated for suitability for regeneration. The somaclones regenerated from salt-treated calli as well as *in vitro* maintained shoots (not passing through callus culture) were inoculated onto MS basal medium supplemented with salt. *In vitro* grown shoots of Lc served as control.

B. Development of protocols for anther culture. Floral buds of IP, 2-3 mm long, (subjected to low temperature (5°C) before excision of anthers) were inoculated onto several callus induction media consisting of the nutrient formulation (DBM) of Gresshof and Doy (1972) and supplemented with different concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D), indoleacetic acid (IAA), kinetin (KI), 2, isopentenylaminopurine (2i-P) and their combinations. The anther-derived calli were subcultured to different media which may yield regeneration.

Experiments with cv. M - a. Callus was developed from leaf explants taken from youngest fully-developed leaf of M plants using MS medium with (mg/L) : 4-NAA, 2-KI. Various media were tested for the regeneration of shoots from callus (Table 2A), and for the production of cell suspension (Table 2B). b. Plants were regenerated directly from leaf explants exposed to nonsaline (MS + 2 mg/L benzyl adenine (BA) 0.2 mg/L IAA) and saline (+ 75 mM NaCl) media. The regenerated plants were grown, respectively, in nonsalinized or salinized (100 mM NaCl) Hoagland solutions. The former was designated as RLO and the latter as RLS. Seeds obtained from selfing these plants were sent to the Philippines for evalution.

Grafting (preliminary) : Grafting was explored as an immediate mean of utilizing resistance to salinity, using the inarching technique. Different graft combinations were used and the ion profiles of the plants monitored to determine the existence of correlation between resistance and ion content of the plants.

Mycorrhizal inoculation (preliminary) - A. Studies on the effect of mycorrhizal inoculation on salt tolerance in tomato were conducted following the findings of Pond et al. (1984) in tomato. Vesicular-arbuscular mycorrhizal species including *Gigaspora margarita, Glomus etunicatum* and *Glomus macrocarpum* (provided by the Biotech. Institute, UPLB, College, Laguna) were used. The reaction of the inoculated three-week-old seedlings, treated or untreated with mycorrhizal suspension was noted and compared. Irrigation with saline water (15 dS/m) started 9 days after inoculation and continued for a period of 15 days.

B. Soil and roots of various annual and perrenial plant species (the genera Atriplex, Zygophyllum, Salsola, Retema) were collected in the desert (about 40 Km east to Beer Sheva, Israel) during January 1988 and December 1989. Salinity was determined in saturated solutions of soil samples. The highest conductivity value found was 43 dS/m, and the highest concentrations of the salt ions were (mg/L): Na-25,000; Cl-12,200. The roots (pieces of one cm length) of sudan grass, which was grown on these soil samples, were mixed (after testing them for the presence of mycorrhiza) with a commercial soil mixture, free of mycorrhiza. A commercial type of mycorrhiza was used as a control. Tomato seeds were germinated in these soils and then transferred to solid media (Vermiculite or Tarface), or nutrient solutions. The irrigation or nutrient solutions included different concentrations of P and different pH values (adjusted daily). The leaves were examined for endogenous P content and the appearance of symptoms characteristic P deficiency. The presence of mycorrhiza was examined in the roots of plants which contained the lowest P content on one hand but did not show deficiency symptoms on the other. This experiment was performed together with Dr. J. Krikum, Volcani Center, Beit Dagan, Israel.

Selection of pollen grains (preliminary) : Plants of cv. M and the wild salt-tolerant species (Lpa) were grown in control or salinized (100 mM NaCl) Hoagland solutions. Pollen from control and salt-treated plants was tested for % of *in vitro* germination in salinized solid medium which contained 15% sucrose, 150 mg/L H_3BO_3 and 1 % agar. In addition, plants of the cultivar were pollinated by pollen of control and salt-treated plants of both species. Fruit set and number of seeds per fruit were determined.

Results

(Due to space limitation the results represent only a select sample).

Whole plants

Mass screening, in the greenhouse: Screening experiments using the soil culture technique were conducted in Los Baños, Laguna, to assess the performance of tomato to salt stress at the seedling stage. During the period covered, 647 entries comprising 278 segregating populations, 15 regenerants, 49 cultivars, 80 accessions, 225 line selections were screened (Table 3). The check varieties used were Marikit (M), Improved Pope (IP) and also Edkauri. The total number of screened seedlings was 71,359; out of which only 5,992 (8.4%) were found to be tolerant. During screening period, temperature was a key factor in the reaction of plants. The three testing years were marked by a higher percentage selection during the cool months of the year (i.e. December to early March). Moreover, symptom development was observed to be relatively slow or delayed during this time. In contrast, screening during the hot months of the year (i.e. late March-May) showed lower percentage selection and symptom development was observed immediately from the time when the treatment was applied. Symptoms developed very slow in the highland as compared with the lowland, where they were more prominent. These observations were confirmed from the screenings conducted in the highland (Benguet, Mt. Province) and lowland (Los Baños, Laguna) conditions. The experiments included observations on the three wild tomato species, *L. cheesmanii* (Lc), *L. pennellii* (Lpa), and *L. peruvianum* (Lp) and various cultivars (Table 4). In both locations Lpa (which expressed mild tip burning) was found to be the most tolerant among the three wild species. Among some commercial varieties of tomato, the cultivars Edkauri and the local checks M and IP had relatively high level of tolerance. Both M and IP exhibited chlorosis and moderate to severe tip burning with M showing a more tolerant reaction than IP. Moreover, the F_1 progeny had the most superior reaction th salt stress; with Lpa crosses exhibiting a relatively higher tolerance level than those involving Lc. Most of the advanced lines from the wide crosses and their backcross progenies were consistently tolerant.

The phenotypes of the selected F_2 plants were intermediate between their parents. Generally, selections from F_2 and F_3 popultions had semi-indeterminate growth habit. Those involving Lc as the wild parent had small, pubescent and curly leaves, in between that of the cultivar and Lc. The fruits were produced on cymes similar to those of the wild parent, yellow-orange in color, sometimes with black-tinge near the stem scar but longer in size and around two to three cm in diameter, compared with the one cm of the Lc fruit. On the other hand, selections involving Lpa as the wild parent had bigger plants and broad, velvety leaves. The fruits were hairy and somewhat irregularly in shape, remaining green until maturity. Immature fruits had dark blue to blackish tinge near the stem scar. The advanced generations of the main crosses (F_2, F_3 and F_4) were also salt-stress selected and the influence of the tolerant parents (especially Lpa) was apparent in terms of salt tolerance, leaf type, growth habit, and fruit size.

Selected plants from the advanced progenies (F_4, F_5) of the main crosses were similar to the cultivated types in terms of growth habit, leaf type and plant characteristics. Those with Lc as the tolerant parent had relatively bigger (with a mean fruit diameter of 2.9 cm), more uniform fruits than those with Lpa. Mature fruits of these advanced lines were red-orange in color unlike that of the backcross progenies which were yellow in color.

The selected plants of the BC_1, with Lc as the tolerant parent (BC_1-Lc) had determinate habit, with leaves almost similar to those of cv. M . Fruit size was nearly equal to the size of that of the cultivated parents. Mature fruits remained yellow for a relatively longer period of time before turning red. The skin was quite thin which accounted for the softness of the fruits. On the other hand, selected plant involving Lpa as the tolerant parent (BC_1-Lpa) were shorter, with leaves still showing some Lpa characteristics. Fruit shape varied from globe to flat round. Immature fruits were dark green with blue-blackish tinge near the stem scar which disappears as the fruit matures. Like BC_1-Lc, mature fruits were yellow in color and only turned red after a lapse of time. Between the two BC_1's, BC_1-Lc had relatively higher fruit setting than BC_1-Lpa. The BC_1 generation retained a substantial level of salt tolerance based on the moderate symptoms exhibited by the plants.

The selected plants of BC_2 and BC_3 generations were almost the same in appearance with cv. M, though some plants tended to be spread. Some fruits were even larger than the cultivated parent. A high degree of salt tolerence was maintained.

In the screening of 27 backcross progenies involving Floradade and Marikit as the recurrent parents, varying reactions to salt stress was also noted (Table 5 and 6). Five entries, namely 90-6, 90-7, 90-11 and 90-12, performed better than cvs. IP and M (control) in their reaction to salt stress. Additional backcross entries which showed high level of resistance included 90-13, 90-16, 89-032, 89-033, 90-003 and 90-001 which exhibited slight to moderate yellowing and tip burning only. Pot evaluation of these seedlings resulted in the selection of 90 (18.1%) individual plants.
These selections had semi-indeterminate to determinate growth habit and produced small to medium-sized fruits. Some showed apparent resistance to foliage diseases (Table 6).

Field evaluation in saline areas : The entries which were consistantly selected in the screening tests were planted in the saline areas of Baroro, (La Union) from February to May 1989 (Table 7) and in Puerto Galera (Mindoro Oriental) from November 1989 to April 1990. The area in La Union was adjacent to a river which served as the source of irrigation water. During the trial, salinity levels of the water and soil ranged from 10.7 to 24.0 and 0.15 to 10.6 dS/m, respectively. The site in Mindoro Oriental was adjacent to the sea. Plants were irrigated using a mixture of sea and ordinary water (1:3) with electrical conductivity approximating that used in the greenhouse screening tests (salt concentration (ppm):Na - 7325; Cl - 4997; Ca - 91; Mg - 13). During the time of

testing salinity levels of soil and water ranged from 3.4 to 29.1 and 15 to 20 dS/m, respectively.

Twenty four promising lines were identified in Baroro based on overall field performance (Table 7). These include F_3 and F_4 of the advanced lines from the wide crosses, their backcross progenies and some germplasm collection. Selected entries showed vigorous plants of semi-indeterminate to determinate growth habit, apparent resistance to foliage diseases and higher fruit setting. Among the plants evaluated in Puerto Galera, promising selections consisting of backcrosses and advanced progenies of the wide crosses F_5 (Marikit x Lc) and F_5 (Marikit x Lpa) and selected lines of the cultivated species were identified. An increase in yield was obtained in some selections irrigated with salt water (Figure 4). This observation was more evident in BC_2 -(FD x BC_1) where BC_1 = Marikit x F1 (Marikit x Lpa) and F_5 (Marikit x Lpa). Likewise, an increase in the soluble solid content of the fruits was noted in most selections. During this writing, the final field evaluation of promising material, including the selected seedlings derived from the *in vitro* selection systems in the Philippines and Israel, is currently on-going in Puerto Galera (Mindoro Oriental) prior to recommendation either as genetic stocks for salinity tolerance or as improved tomato varieties.

Improvement of salt tolerance by grafting (preliminary): The possibility of utilizing grafting to enhance salinity tolerance in tomato was explored using the inarching technique. Different graft combinations grown in salinized Hoagland's solution and under field conditions showed marked differences in reaction to salt stress. Among the different stock and scion combinations, M/UC-204 and M/95-43 (resistant stock/susceptible scions) gave the highest marketable yield in Baroro (La Union). In contrast, the combination of Maigaya/VF 134-1-2 (highly susceptible stock/susceptible scion) produced the lowest number of fruits. Differences in visual rating were observed in the different graft combinations grown in salinized Hoagland's solution. When M (relatively resistant check) was used either as scion or stock of Maigaya (susceptible check) the plants were green and healthy. But when Maigaya was used as stock or scion with IP (moderately tolerant) the leaves became yellowish green in color. It appeared then that while M root can influence the susceptible cultivar and nullify its expression of susceptibility, the root of the latter did not affect M shoot resistance. These findings will need further elucidation by experiments which will include additional control graftings.

Treatment with 150 mM NaCl caused significant increase of Na in all plant parts. The relative increase was highest in old and new leaves of the scion. Likewise, significant difference in Na accumulation was found among the different graft combinations. High Na content was found in the root of the Maigaya (MG) graft combination (MG/MG). On the other hand, if Marikit was used either as stock (M/MG) or scion (MG/M) with Maigaya, Na accumulation in the root was drastically reduced to 32% and 53%, respectively. In the stem, M/MG accumulated more Na than MG/M. Other graft combinations showed no definite pattern of Na accumulation. No significant differences in K and Ca distribution were observed in the different graft combinations.

Effect of mycorrhiza on salt tolerance (preliminary) : The use of three species of mycorrhiza in the Philippines did not enhance salt tolerance in cvs. M, IP, VF-134-1-2, Maigaya and UC-204.

Low mycorrhizal infection was found in the roots of some of the M plants in Israel. Further experiments are required to determine the appropraite conditions for infection, before mycorrhizal effect on salt tolerance can be tested.

In vitro manipulation

In vitro manipulation of cv. IP : Unlike one-month old calli , leaf explants were more sensitive to salt and died even at low concentrations of NaCl. Survival of calli decreased with increasing salinity. Callus turned brown with prolonged exposure to salt and browning became severe with increasing NaCl concentration. The near-kill concentrations for callus were determined to be at 0.30 and 0.35 M NaCl. These concentrations were utilized for the routine *in vitro* selection at the callus level (Fig 3). Plant regeneration from calli not treated with salt was obtained from MS + 2.0 ppm zeatin riboside. However, salt-treated calli were not induced to regenerate shoots on this medium. Regeneration of shoots from salt-treated calli was very poor using sucrose or coconut water separately. Addition of coconut water (100 to 200 ml/L) and sucrose (20g/l) improved the regeneration ability. Among the cytokinin/IAA combinations which were supplemented to MS medium, 2 ppm BA + 2 ppm IAA was the most favorable for plant regeneration from salt-treated calli (1.6 to 8% of the cultures formed shoots with 10-12 shoots per culture). The capacity of calli to regenerate plants declined with time or culture cycles and was influenced by culture medium. The 51 somaclones screened performed better than the seedling-derived plants. Six somaclones namely, S_1T_2, S_1T_{18}, S_2T_{42}, S_2T_{45}, S_1T_{66} and S_2T_{82} proved to be resistant at 0.30 M salt (Tables 9 & 10). These somaclones showed good leaf appearance, excellent stand with fully developed primary and secondary roots. Performance of these somaclones were comparable to the salt resistant *L. cheesmanii*. On the other hand, the less resistant somaclones were characterized by having less developed secondary root system or short primary roots. Shoots of unselected lines exhibited signs of salt stress ranging from general yellowing, narrowing of leaves, tip burn and death when exposed to NaCl-supplemented media.

Development of protocols for anther culture - Sixty-one percent of anthers which were cold-treated for 24 hr prior to inoculation formed callus on Gresshoff and Doy's formulation + 2 ppm 2,4-D + 2 ppm IAA + 1.5 Ki. Of the callusing anthers, 50% appeared to arise from within the anther. The calli were potentially competent for plant regeneration. To date, shoot regeneration has not been observed on any of the plant regeneration media.

In vitro manipulation of cv. M : Repeated attempts to develop cell suspension and to regenerate plants (Table 2) from calli of M were not successful. However, regeneration directly from leaf explants of M, which were exposed to saline (RLS) or nonsaline (RLO) media, was successful. Selfed progeny obtained from these regenerated plants were screened for salt tolerance in the Philippines. The screening experiment (Tables 5 and 6) included entries 90-17 (selfed progeny of RLO), 90-18 (progeny of RLS) and a check variety of Marikit (produced by selection in the Philippnes). The two entries which were produced from regenerated plants in Israel had slightly higher salinity tolerance level than that of the seed-derived check variety. Possibly by repeating this procedure with leaf explants from RLO and RLS, the level of salt tolerance can be improved further. Such a strategy for improving various aspects of agronomic performance was suggested by Evans (1989). It is necessary to continue selfing and selections and also to collect seed from selected single plants to ensure stable inheritance.

Selection of Pollen Grains (preliminary)

It was indicated that pollen of the wild salt tolerant species Lpa responded (Table 11 - % of *in vitro* germination; Table 12 - % fruit set and no. seeds after pollinating with pollen of control and salt-treated plants) better than that of the cultivated species to salt stress. Such a correlation between the response of the diploid and haploid stages is one of the pre-requisites for the establishment of a successful selection system based on pollen grains. In addition, Marikit (MC) plants pollinated with pollen taken from salt-treated plants (MS or LpaS) produced greater fruits with more seeds per fruit (as compared with MC plants pollinated with pollen from MC or LpaC plants). These findings (which is studied further for confirmation) may have relevance while producing hybrid seeds.

Physiological studies

Ion accumulation : A. Experiments in the Philippines on the accumulation of K, Na and Ca ions in the wild salt-tolerant tomato Lpa and the cultivated varieties M (relatively tolerant) and VF 134-1-2 (susceptible) grown in saline solutions showed as expected (based on the literature) that Na content increased while K content decreased with increasing salinity. Additional results of the level of the various ions in different parts (root, stem, petiole and lamina) of plants grown in solutions containing different NaCl concentrations (50, 100 and 150 mM) are evaluated.

B. The content of salt ions (Na and Cl) and K was determined (about one week after the termination of NaCl addition) in healthy and affected leaves of young plants of the BC_1S_1 generation (exposed for selection to salinized 100 mM NaCl Hoagland solution) which exhibited sensitivity symptoms (wilting and drying leaves) and therefore, were discarded (Table 13). The results

suggest that the sensitive plants were not able to regulate the level of Na, Cl and K, at least in the salt-affected leaves.

Response to salt stress at different growth stages : Studies were conducted to determine the specific growth stage at which tomato is most sensitive to salt stress. In preliminary experiments, germination was totally inhibited at high concentrations of 150 and 200 mM NaCl. Succeeding trials showed that there were significant reductions in percent germination at 75 and 100 mM NaCl and rates of germination gradually decreased with increasing NaCl concentration (Table 8). Comparison of the reaction of tomatoes to salt stress imposed at germination and at seedling stage revealed different types of responses, suggesting the need to screen separately for resistance at germination and at seedling stage. Response at the seedling stage was more or less reflected at later growth stages in terms of yield. In agreement with Pasternak et al, (1979) of the four cultivars tested, namely M, IP, VF 134-1-2 and 95-43, the percent reductions in number and weight of fruits were significantly higher for VF 134-1-2 and 95-43 which were most sensitive also as seedlings. On the other hand, salt stress caused an increase of yield in Marikit.

VI. Impact, Relevance, Technology Transfer and Future Work

The project has shown that classical breeding methods coupled with *in vitro* manipulation can result in a significant improvement in tolerance to salinity, including adaptation, fruit quality and other horticultural merits.

With careful selection of sources of genes for salinity tolerance and consistent and systematic coordinated efforts, tomato lines with improved tolerance to salinity can be developed over a relatively short period of time. Such lines can be used as genetic stocks for further breedingand some of them probably directly for field planting in saline-affected areas of the country to increase food production. Additional potential techniques of enhancing salinity tolerance in tomato, e.g grafting, use of pollen grains for selection and possible mycorrhizal effection (as well as the effect of salt stress applied during pollen development on fruit qualities after pollination) deserve further consideration. More studies on need to be conducted in order to evaluate the potential of these technologies to combat salinity problem. More studies on *in vitro* manipulation, specifically various strategies of production of salt tolerant somaclones will be useful for future attempts.

Equipment purchased by the project will be retained at the Institute. These will be useful in undertaking future studies in physiology and biotechnology, by its research staff and students.

VII. Project Activities/Output

1. List of papers and posters presented in conferences and meetings

Del Rosario, D.A., A.C. Sumague, C.P. Roxas and A.T.S. Bautista, 1990. Response of four tomato cultivars to salt stress. Paper presented at the 6th Federation of Crop Science Societies of the Philippines (FCSSP) Conference, May 16-18, 1990, Naga City, Philippines.

Del Rosario, D.A., A.B. Zamora, E.T. Rasco, Jr., V.P. Roxas, P.J.A. Santos, V.C. Lapitan, A.C. Sumague and M. Tal, 1990. Improvement of salt tolerance in tomato, 1990. Paper and poster presented during the PSTC-CDR tissue culture networking workshop meeting, July 16-20, 1990, Davis, California, USA. Also presented as poster during the First Cellular and Molecular Plant Biology Program Annual Review and Planning Workshop, October 19 and 23, 1990, IPB, UPLB College, Laguna.

Roxas, V.P., E.T. Rasco, Jr., D.A. Del Rosario and A.C. Sumague, 1990. Prospects and progress in breeding for salinity tolerance in the Philippines. Paper presented during the 6th FCSSP Conference, May 16-18, 1990, Naga City, Philippines.

Santos, P.J.A., A.M. Ocampo, E.M. Ocampo and D.A. Del Rosario, 1990. Changes in the ion profile between wild and cultivated tomatoes grown under saline conditions. Paper presented during the 6th FCSSP Conference, May 16-18, 1990, Naga City, Philippines.

Tal, M. and D.A. Del Rosario, 1990. Improvement of salt tolerance in tomato by conventional breeding and selection in culture. Eucarpia Meeting Malaga, Spain March 6-9, 1990.

Zamora, A.B. and E.A. Perez, 1988. Callus establishment and plant regeneration in tomato (*Lycopersicon esculentum* cv. Improved Pope). Poster presented during the First National Symposium in Plant Tissue Culture in Philippine Ariculure and Forestry, May 26-28, 1988, IPB, UPLB, College, Laguna.

2. Papers published in scientific journal

Del Rosario, D.A., A.C. Sumague, V.P. Roxas and A.T.S. Bautista, 1990. Response to tomato (*Lycopersicon esculentum* Mill) to salt stress. Philippines Agr. 73: 193-198.

3. Training/visits

M. Tal (Principal Investigator) conferred with Drs. D.A. Del Rosario and E.T. Rasco and A.B. Zamora on the conditions and problems unique to the tropics as well as the planning progress and future of the project while visiting (twice) the Philippines during the first two years. The visit included other laboratories in the Institute of Plant Breeding and in IRRI as well as the experimental station at Bagio and the salt-affected experimental plots in the field.

D.A. Del Rosario (Principal Co-Investigator) conferred with Dr. M. Tal on the progress and future plans of the project; also visited different laboratories at BGU and the Volcani Center, Bet Degan, Israel and conferred with other scientists on physiology, genetics and production aspects in saline areas (September 1989).

V.P. Roxas and P.J.A. Santos (University Researchers of the Institute of Plant Breeding) attended a 3-month training on physiology and genetics of salt tolerance at Ben Gurion University of the Negev, Beer Sheva, Israel from August 1 to October 31, 1988.

VIII. Project Productivity

The central target of the project, the enrichment of the germplasm of the cultivated tomato in the Philippines with salt-tolerant genotypes, has been accomplished. These genotypes will be used as genetic stocks for further breeding for salt-tolerace and some of them, probably, for field planting in salt-affected areas. Some of the potential of *in vitro* manipulation for the improvement of salt-tolerance in the tomato has been demonstrated. The study has also been indicated that the effect of grafting on salt tolerance, the effect of 'salt-treated' pollen grains on fruit qualities, the use pollen to select for salt tolerance, (and possibly also the effect of mycorrhizal infection) worth being further investigated.

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Table 1. Crosses performed between tomato cultivars (M-Marikit*; IP - Improved Pope*; FD - Floradade) and wild salt tolerant species *L. pennellii* accession atico (Lpa) and *L. cheesmanii*, ecotype 1401 (Lc).

BC_1S_4 (F_1) = [FD x F_1 (M x Lpa)]$^{\otimes}$

BC_2S_4 (M) = {FD x [M x F_1(M x Lpa)]}$^{\otimes}$

BC_2S_4 (IP) = {FD x [IP x F_1(IP x Lpa)]}$^{\otimes}$

$BC1S_4$ (F1 x F1) = {FD x [F1(M x Lc) x F1 (M x Lpa)]}$^{\otimes}$

$BC1S_4$ (F2) = [FD x F2 (IP x Lpa)]$^{\otimes}$

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* Cultivars from the Philippines

** S_4 signifies the number of selfing (and selection) generations

Table 2. Hormonal composition of some of the media tested for regeneration (2A) and for developing cell suspension (2B) from callus of Marikit.

2A

Media	Hormones (mg/L)				
	Zeatin	BA	IAA	Sucrose (%)	Mannitol (%)
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

2B

Medium	Hormones (mg/L)				
	Kinetin	NAA	2,4D	Sucrose %	Mannitol %
MS	0.25	5	-	3	-
"	0.25	2	-	3	±2
"	0.25	-	2	3	±2
"	0.50	-	4	3	±2
"	2.00	4	-	3	±2

Table 3. Summary of material screened for salt tolerance from January 1988 to December 1990 (greenhouse, Los Baños).

Composition	Year	No. of entries	Total no. of Plants Screened	Selected	%Selections as seedlings
Segregating populations[1]	1988	58	3341	479	15.0
	1989	132	17518	1356	7.7
	1990	88	11011	1667	15.1
Regenerants[2]	1990	15	839	158	18.8
Cultivars[3]	1988	35	3364	275	8.2
	1989	8	1615	19	1.2
	1990	6	565	79	14.0
New accessions[4]	1988	58	1583	194	12.2
	1989	18	5669	472	8.3
	1990	14	1860	194	10.4
Selection from previous screening[5]	1988	59	3101	471	15.2
	1989	75	4452	405	9.1
	1990	91	2519	135	5.4
Check varieties	1988	3	1164	236	20.3
	1989	2	2686	190	7.1
	1990	2	2302	310	13.5
Total number of plants screened	1988 - 12553		1989 - 31940		1990 - 19096
Total number of plants selected	1988 - 1676		1989 - 2442		1990 - 2543
% selection	1988 - 13.3		1989 - 7.6		1990 - 13.3

1 'Segregating populations' included progeny of backcrossing and selfing between local cultivars and two wild salt-tolerant species as well as regenerants produced in Israel.

2 Regenerants include the products of *in vitro* work in both countries.

3 'Cultivars' included processing and fresh market, local and foreign types.

4 'Accessions' were obtained from the germplasm collection at the National Plant Genetic Resources Laboartory.

5 Screenings done before Jan. 1988 on recommended cultivars and progeny of early crosses for evaluation of the screening technique.

Table 4. Reaction of the cultivated tomato (Marikit-M, Improved Pope-IP), its wild relatives *L. pennellii* (Lpa) a and *L. cheesmanii* (Lc) and their progeny (F_1, F_2, F_3) to salt stress (EC = 15 d5/m) (Los Baños, Laguna, November 1988)

Entry	No. of seedlings Screened	Selected	% Selection	Salinity Index[1]	Rating Selection[2]	Remarks[3]
M						mod. chl. and tb
IP						mod. to serve chl. and tb
Lpa						slight tb
Lc						mild chl.
F_1 (M x Lc)	30	10	20.0	1.8	1.5	vigorous, slight Y
F_1 (M x Lpa)	30	11	36.7	1.5	1.2	most vigorous, slight Y
F_1 (IP x Lc)	30	9	30.0	2.0	1.8	slight to mod. Y
F_1 (IP x Lpa)	30	11	36.7	1.8	1.7	slight tb
F_2 (M x Lc)	150	40	26.7	2.8	2.5	mod. Y
F_2 (M x Lpa)	120	30	25.0	2.2	2.0	slight Y
F_2 (IP x Lpa)	120	33	27.5	2.5	2.0	slight Y
F_3 (M x Lc)	317	80	25.2	2.8	2.5	mod. Y
F_3 (M x Lpa)	180	48	26.7	2.7	2.2	slight to mod. Y
F_3 (IP x Lc)	150	34	22.7	3.2	2.8	mod. tb, Y
F_3 (IP x Lpa)	120	31	25.8	2.9	2.6	slight to mod. Y, slight tb

[1]Salinity Index = (Total rating values for all plants/entry) / (Total number of seedlings screened) - 1.0 - 2.0 = Resistant; 2.1 - 2.5 = Moderately Resistant; 2.6 - 3.5 = Moderately susceptible; 3.6 - 4.5 = susceptible

[2]1-5 = increasing order of susceptibility.

[3]Mod. chl. = moderate chlorosis; tb = tip burning; y = yellowing

Table 5. Reaction of the (selfed) backcross progenies (BC_1S_2 and BC_2S_2) of the wide crosses and of the progeny of selfed regenerants (RLO and RLS) to salt stress, EC = 15 dS/m (Los Baños, Laguna, April 1990).

Entry Code[1]	Description[2]	Total No. of Plts.screened	Overall Rating[3]	No. of Selections	Rating of Selection[3]	Remarks
90-1,19	FD x F_1(M x Lpa)-l	89	4.8	6	3.8	Death tip burning, severe yellowing
90-2	FD x F_1(M x Lpa)-i	140	4.6	15	3.7	Death, severe tip burning
90-3,9, 22,23	FD x [M x F_1 (M x Lpa)]-l	315	3.5	51	3.1	Death, moderate to sever tip burningslight to moderate yellowing
90-4,10	FD x [M x F_1 (M x Lpa)]-i	210	3.9	27	3.4	Death, tip burning
90-5	FD x [M x F_1 (M x Lpa)]-s	175	4.5	18	3.6	Severe yellowing
90-6	FD x [IP x F_1 (IP x Lpa)]-l	35	2.8	9	1.5	Slight yellowing
90-7	FD x [IP x F_1 (IP x Lpa)]-i	175	2.2	38	1.4	Slight tip burning
90-8	FD x [IP x F_1 (IP x Lpa)]-s	105	4.6	12	3.5	Death, tip burning some
90-24	FD x (F_1 x F_1)-l	70	4.9	7	3.7	Death, moderate to severe tip burning plants are very vigorous
90-12	FD x (F_1 x F_1)-i	140	2.0	45	1.5	Slight tip burning
90-13,25	FD x (F_1 x F_1)-s	105	3.2	13	2.7	Death, sever defoliation, slight yellowing
90-11	FD x [M x F_1 (M x Lpa)]-s	140	1.5	30	1.2	Slight yellowing and tip
90-14	FD x F_2 (IP x Lpa)-l	70	4.2	7	2.8	Yellowing, tip burning
90-15	FD x F_2 (IP x Lpa)-i	70	1.8	15	1.6	Slight tip burning only, better than 90-16
90-16	FD x F_2 (IP x Lpa)-s	70	2.0	15	1.8	Tip burning
90-20	FD x [IP x F_1 (IP x Lpa)]-i	35	4.8	2	3.9	Death, severe tip burning
90-21	FD x [IP x F_1 (IP x Lpa)]-s	105	3.9	15	2.8	Severe tip burning
90-001	M x BC_2; BC_2 = M x [M[F1(MxLpa)]	30	2.0	18	1.5	Slight yellowing
90-003	M x BC_2; BC_2 = M x [M[F_1(MxLc)]	35	2.2	20	1.6	Slight yellowing & tip burning
89-032	M x [M x F_1(M x Lpa)]	70	2.9	18	1.6	Moderate yellowing, better than M
89-033	M x [M x F_1(M x Lc)]	70	3.4	15	2.0	Moderate yellowing and tip burning
90-17	Marikit (RLO)	105	2.6	25	2.2	Moderate to severe yellowing
90-18	Marikit (RLS)	210	2.8	23	2.4	Moderate defoliation, yellowing check variety
M	check variety	280	3.2	55	2.8	Moderate to severe yellowing and tip burning
IP	check variety	175	3.8	35	3.4	Severe yellowing, defoliation

Total No. of Seedlings Screened = 2,959
Total No. of Seedlings Selected = 498
Percent Selection = 16,8

[1]Except for entries 90-001, 90-003, 89-032, 89-033 which were produced in the Philippines, the rest of the entries came from the laboratory in Israel. These entries were derived from selfing of selected plants grown in salinized solution (100 mM NaCl in Hoagland solution).

[2]FD = Floradade; M = Marikit; IP = Improved Pope; size of fruits from which the seeds were obtaibed : l = large (about 45 g in weight, 50 mm in diameter); s = small (6 g in weight, 20 mm = in diameter); i = intermediate (14 g in weight, 30 mm in diameter).

[3]1-5 = increasing order of susceptibility.

Table 6. Horticultural characteristics of selected entries screened for salinity tolerance (Los Baños, Laguna, April - July, 1990).

Entry Code[1]	No. of Plts. Selected	Growth Habit[2]	Reaction to Foliage Diseases[3]	Ave. No. of Fruits/Plant	Remarks
90-7	8	Sp±	MS	4	green shoulder (small 5-7 g fruit)
90-8	1	Sp±	MS	4	green shoulder, high round to pope type (5-8 g/fruit)
90-9	1	Sp±	MR	3	oblong, green shoulder (40-45 g/fruit)
90-10	2	Sp±	MR	4	oblong, green shoulder (25-30 g/fruit)
90-11	5	Sp±	MR	3	oblong, some fruits are somewhat oblate, (10-12 g/fruit)
90-13	1	Sp	S	3	small fruits (<5 g/fruit)
90-15	3	Sp (tall)	MS	5	green shoulder (<10 g/fruit)
90-20	8	Sp±	MS	6	green shoulder (15-22 g/fruit)
90-24	1	Sp	S	5	green shoulder (18-25 g/fruit)
89-032	5	Sp	MR	4	slightly green shoulder (10-15 g/fruit)
89-033	7	Sp	MS	6	tends to be spreading, higher fruit than 89-032 (18-23 g/fruit)
90-001	11	Sp	MS	7	has almost the same characteristics of M except for slightly smaller fruits
90-003	8	Sp	MR	5	taller plants than 90-001, better in fruit setting than 90-001
90-17 (RLO)	11	Sp	MR	8	round fruits, prolific, slight cracking
90-18 (RLS)	5	Sp	MR	7	uniform (20-30 g/fruit)
M check	31	Sp	MR	7	uniform, oblate
IP check	23	Sp (tall)	MS	9	uniform fruits

Total No. of Plants Selected	= 90
Percent Selection (Over No. of Seedlings Screened)	= 3.0
Percent Selection (Over No. of Plant Evaluated)	= 18.1

[1]Description as in Table 5.
[2]Sp = determinate (self-pruning); Sp± = semi-indeterminate
[3]MR = moderately resistant ; MS = moderately susceptible; S = susceptible.

Table 7. Horticultural characteristics of selected tomato plants from the field evaluation trial1 (Baroro, La Union, Feb. - May 1989)

Entry Code[2]	Growth Habit[3]	Plant Vigor	Reaction to Foliage Diseases[5]	Leaf Type[6]	Fruit Setting[7]	Yield (G/Plt)	Remarks
Trial I							
F_3(M x Lc)	Sp+	4.5	4.0	Le	5	294	early, prolific, small round, red-orange fruits
F_3(IP x Lc)	Sp+	4.5	4.0	Le/Lc	2	53	oval-shaped, small, orange-clored fruits
F_4(M x Lc)	Sp-	4.5	4.0	Le/Lc	5	277	prolific, red-orange fruits
[BC_1F_3]-1	Sp-	4.5	5.0	Le	2	176	round, red fruits, big-fruited
[BC_1F_3]-2	Sp-	4.5	4.5	Le/Lpa	5	273	very prolific, orange-colored fruits
BC_1	Sp-	4.5	4.0	Le	2	549	early, big, deep-red fruits
Marikit (ck)	Sp-	4.5	4.0	Le	2	457	deep-red, soft fruits
Trial II							
GC-1	Sp-	4.0	4.0	Le	3	506	big, red fruits, good foliage cover
GC-2	Sp-	4.0	4.0	Le	5	1360	deep-red fruits
GC-3	Sp-	4.5	3.5	Le	5	900	deep-red, round fruits
GC-4	Sp-	4.5	4.5	Le	4	800	prolific and deep-red, big fruits
GC-5	Sp-	4.5	3.0	Le	5	600	non-cracking, deep red fruits

No. of plants selected = 24 Percent Selection (over total plants planted) = 8.3

1Average salt composition of irrigation water (ppm) : Na - 3637; Cl - 7028; Ca - 83; Mg - 370

2[BC_1 F_3]-1 = BC_1 → M x F_1 (M x Lpa)1; [BC_1 F_3]-2 = BC_1 →IP x F_1 (IP x Lpa)]; [BC_1 = M x F_3 (M x Lc); GC = germplasm collections.

3Sp± = semi-indeterminate; Sp- = determinate

41 = poor vigor; 5 = very vigorous

51 = susceptible; 5 = resistant

6Le = *L. esculentum* type, Le/Lc = in between Le and *L. cheesmanii*, Le/Lpa = in between Le and *L. pennellii*.

71 = < 10 fruits/plant, 2 = 11-20; 3 = 21-30; 4 = 31-40; 5 = > 41.

Table 8. Effect of varying concentrations of NaCl on germination of tomatoes (average of M,IP,VF 134-1-2 and 95-43)

NaCl (mM)	% Germination			Uniormity of Germination (UGT)[1]		
	I[2]	II	III	1	II	III
0	88.0a[3]	88.9ab	90.9a	1.08d	0.74d	1.85e
25	84.2a	91.1a	88.7a	1.50c	0.84d	2.04d
50	84.0a	90.0a	83.1a	2.22b	1.28c	2.64c
75	41.6b	82.7b	64.7b	2.73a	1.98b	2.94b
100	12.7c	44.0c	29.1c	2.96a	2.58a	3.17a

[1]UGT = $(x_1.1+x_2.2+.....+x_n.n)$ /% germination, when x_1, x_2 and x_n represent the numbers of seeds that germinated at the 1st, 2nd and nth day, respectively . n represents the day after which no more germination occurs.

[2]I, II and III represent different experiments.: I and II tested at room temperature and III at 20°/30°C, night/day, temperatures.

[3]Means within trial followed by the same letter(s) are not significantly different at 5% level by DMRT.

Table 9. Visual stess rating of somaclones of IP with different NaCl concentrations.

Somaclones	NaCl (M)					
	0.10	0.15	0.20	0.25	0.30	0.35
S1T2	1.2	1.6	1.6	1.8	1.7	4.2
S1T3	2.2	2.3	1.8	4.0	4.5	4.8
S1T4	1.5	2.0	2.4	1.6	4.8	4.8
S1T5	1.5	2.0	1.2	2.5	4.8	4.8
S1T6	1.2	2.0	2.1	4.3	4.2	4.7
S1T8	2.2	2.2	2.5	4.4	4.8	4.8
S2T9	1.2	1.8	1.5	4.2	4.8	4.5
S2T10	2.0	2.3	1.7	4.2	4.0	4.7
S1T11	1.5	2.1	2.2	4.2	4.5	5.0
S1T12	1.2	1.8	2.3	4.3	4.5	4.7
S1T13	1.2	1.6	2.1	4.5	4.7	4.7
S1T14	2.2	1.7	1.8	4.0	4.2	4.8
S1T16	1.7	1.2	2.1	2.0	4.6	4.7
S1T17	1.7	2.3	1.7	1.9	4.5	4.8
S1T18	1.2	1.5	1.8	2.9	2.1	4.0
S1T19	1.3	2.1	2.3	4.0	4.7	4.7
S1T20	2.0	2.2	1.5	3.9	4.6	4.8
S1T21	1.6	1.8	1.8	3.5	4.2	4.8
S1T22	1.2	2.2	1.8	3.6	4.1	4.5
S1T23	2.1	2.3	2.3	4.2	4.3	4.8
S1T24	1.8	1.6	1.8	4.5	4.2	5.0
S1T25	1.7	1.6	2.0	3.8	4.2	4.7
S1T26	1.8	2.2	2.4	4.6	4.6	5.0
S1T27	1.8	2.1	1.9	4.2	4.7	4.7
S1T28	2.0	2.4	2.4	4.0	4.2	5.0
S1T29	2.0	2.4	1.8	4.0	3.9	4.8
S1T31	2.1	2.4	2.4	1.7	4.3	4.7
S1T32	2.3	2.0	1.6	2.0	4.3	4.7
S1T33	1.7	1.9	1.6	4.0	4.2	5.0
S1T34	1.8	1.8	1.7	4.0	4.2	5.0
S1T35	1.9	1.8	1.7	1.8	4.3	4.3
Control (LC)	1.2	1.5	1.6	1.6	2.0	3.5
Control (IP)	2.3	3.0	3.2	4.5	5.0	5.0

[1]Visual Stress Rating : 1.0 = green leaves, excellent stand, vigorous stems, with fully developed roots (>7); 2.0 = green leaves, slight yellowing of the shoot tips, 3-6 roots; 3.0 = pale green leaves with no shoot growth, with 1-2 roots but no root hairs; 4.0 = 50% yellowing of the leaves, stunted growth, withered shoot tips, no root development; and 5.0 = severe yellowing and withering of the leaves, no root development.

Table 10. Second batch screening (greenhouse) for salt tolerant somaclones of I P with different NaCl concentrations.

Somaclones	NaCl (M)		
	0.25	0.30	0.35
S2T40	3.9[1]	4.8	5.0
S2T42	1.5	2.9	4.1
S2T44	2.5	4.5	5.0
S2T45	2.0	2.4	4.2
S1T48	3.5	5.0	4.9
S1T49	4.0	5.0	5.0
S1T51	4.1	5.0	5.0
S2T53	3.5	3.8	4.9
S1T55	4.0	4.5	5.0
S1T58	4.0	3.8	4.5
S1T66	2.2	2.6	4.5
S2T70	3.1	4.2	5.0
S2T71	3.5	4.7	5.0
S2T72	3.8	4.0	4.8
S2T73	3.0	4.0	4.8
S2T82	2.5	3.0	4.5
Control (Lc)	1.2	2.5	3.7
Control (IP)	3.8	5.0	5.0

[1]Visual Stress Rating is exactly as that in Table 9.

Table 11. *In vitro* germination (%) of pollen grains from control (C) and salinized (S, 100 mM NaCl) plants of the cultivated tomato (M) and the wild salt-tolerant species *L. pennellii* (Lpa). The values represent the average of three independent experiments which gave similar results.

	NaCl concentration (mM)						
Source of pollen	0	10	% from 0	20	% from 0	30	% from 0
MC	47.3	34.3	72.5	19.3	41.0	5.0	10.6
MS	48.0	30.6	63.8	17.0	35.4	6.0	12.5
Lpa C	66.0	49.0	74.2	27.6	41.8	10.3	15.6
Lpa S	61.1	45.6	74.7	28.3	46.3	8.6	14.0

Table 12. Fruit set (A), fruit fresh weight (g) and number of seeds per fruit (B) in tomato plants pollinated with pollen from control (C) and salinized (S- 100 mM NaCl) plants of the cultivated tomato (M) and the wild salt-tolerant species *L. pennellii* (Lpa).

A.

Crosses	Total number of pollinations	Number of fruit set	%
MC x MC	36	26	72.2
MC x MS	50	27	54.0
MC x Lpa C	58	36	62.1
MC x Lpa S	102	77	75.5

B.

Crosses	Fruit wt	No. of seed/fruit
MC x MC	36.3±3.9	16.6±1.5
MC x MS	47.7±3.9	25.6±2.5
MC x LpaC	34.2±5.2	16.5±2.3
MC x LpaS	45.0±5.0	22.4±1.6

Table 13. Content [mmol (g dry wt)$^{-1}$] of Na, K and Cl in leaves of BC_1S_1 seedlings exposed to 100 mM NaCl. The results represent the average of 3-4 replications in one experiment. The differences between ion content of different leaves are significant.

Appearance of leaf	Ion		
	Na	K	Cl
Normal green	2.4	1.5	1.1
Chlorotic	11.6	0.7	4.3
Mostly green + dry patches	3.7	1.2	2.3

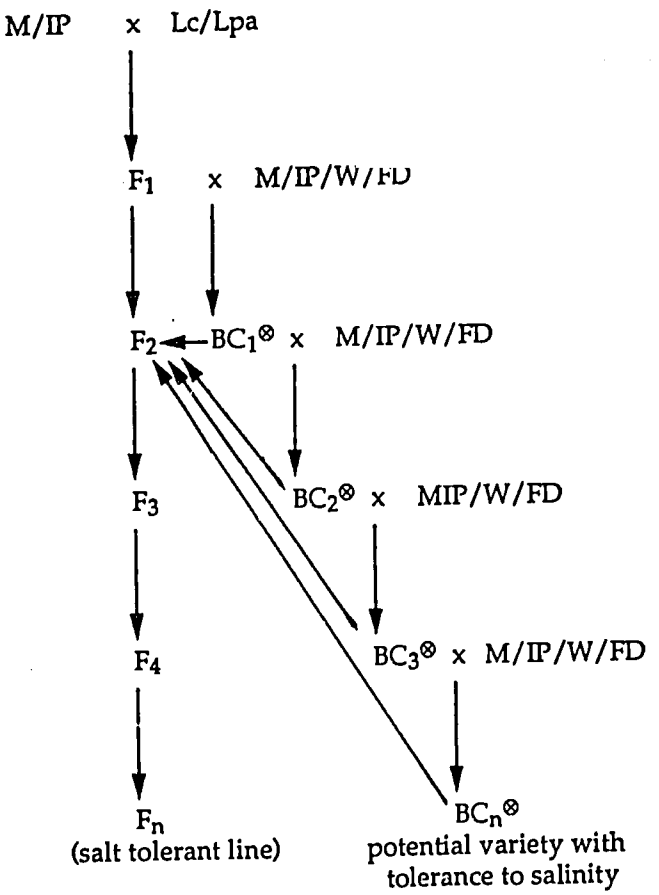

Figure 1. Breeding strategy for the development of salt tolerant tomato lines. Pedigree selection method was used to develop salt tolerant lines that can be used as genetic stocks. Backcrossing to the cultivated parent(s) was done to improve the horticultural merits of the selected progenies. Progeny was screened for tolerance to salinity at an EC = 15 dS/m.

M = Marikit; FD = Floradade; Lc = *L. cheesmanii* eco. 1401;
IP = Improved Pope; W = Walter; Lpa= *L. pennellii* acc. atico.

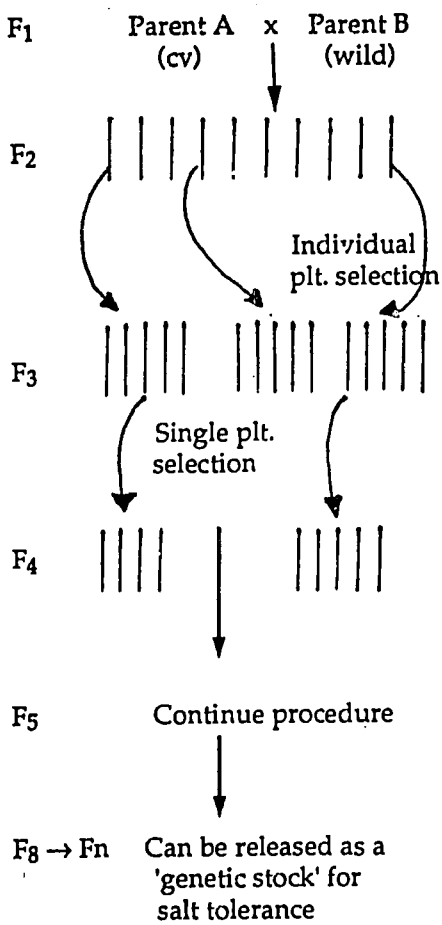

Fig. 2A. Pedigree selection method for salt tolerance

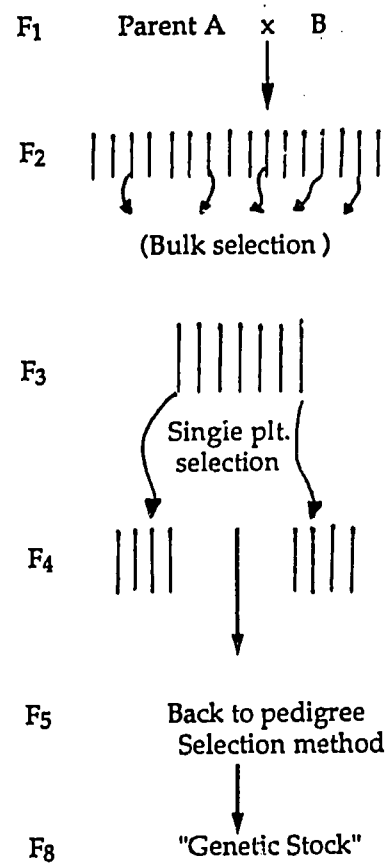

Fig. 2B. Modified pedigree method (pedigree alternate with bulk method).

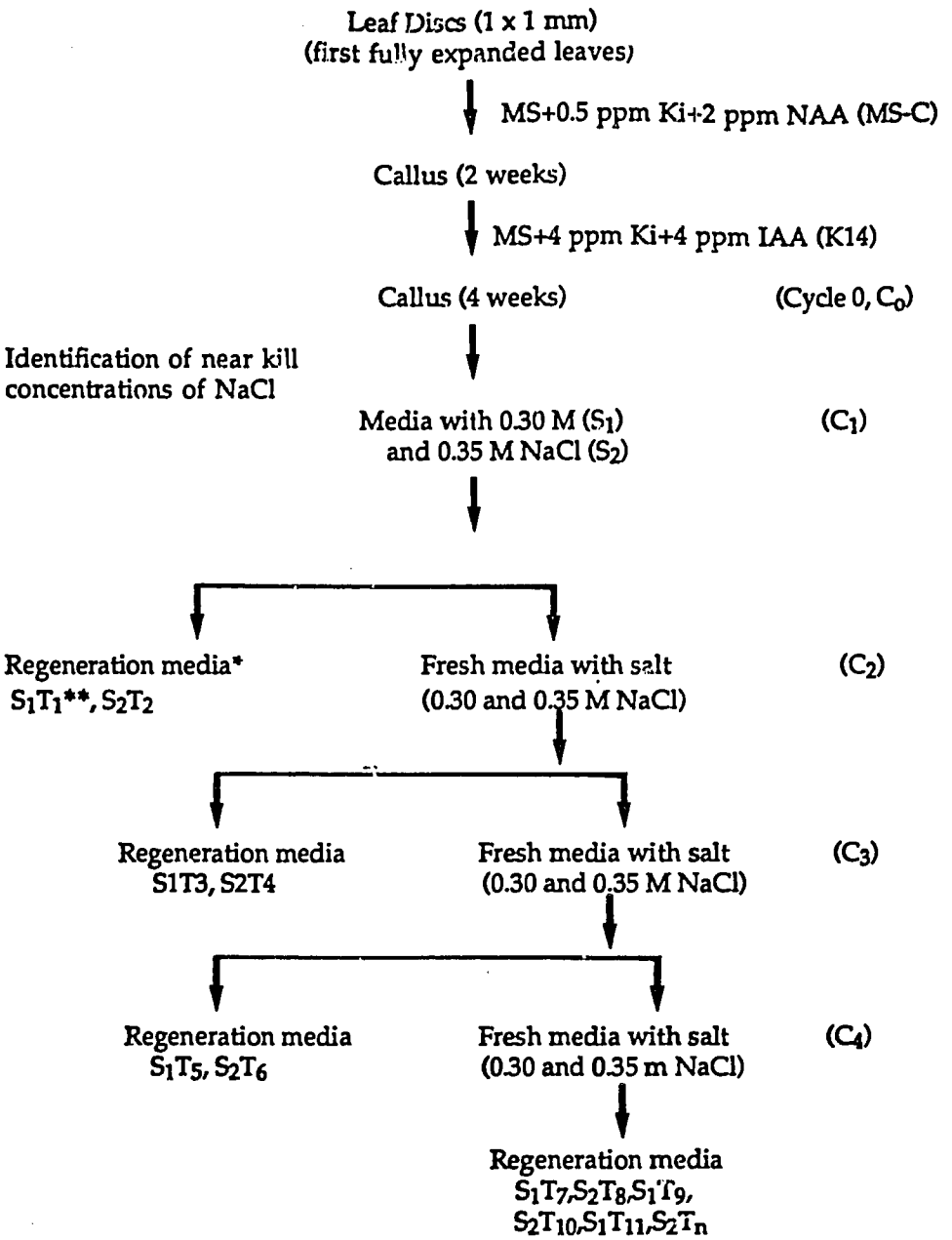

Figure 3. *In vitro* selection system (callus) for NaCl tolerance

* - best regeneration media: MS + 2 ppm BA + 2 ppm IAA

** - Tn - number (n) of somaclones produced

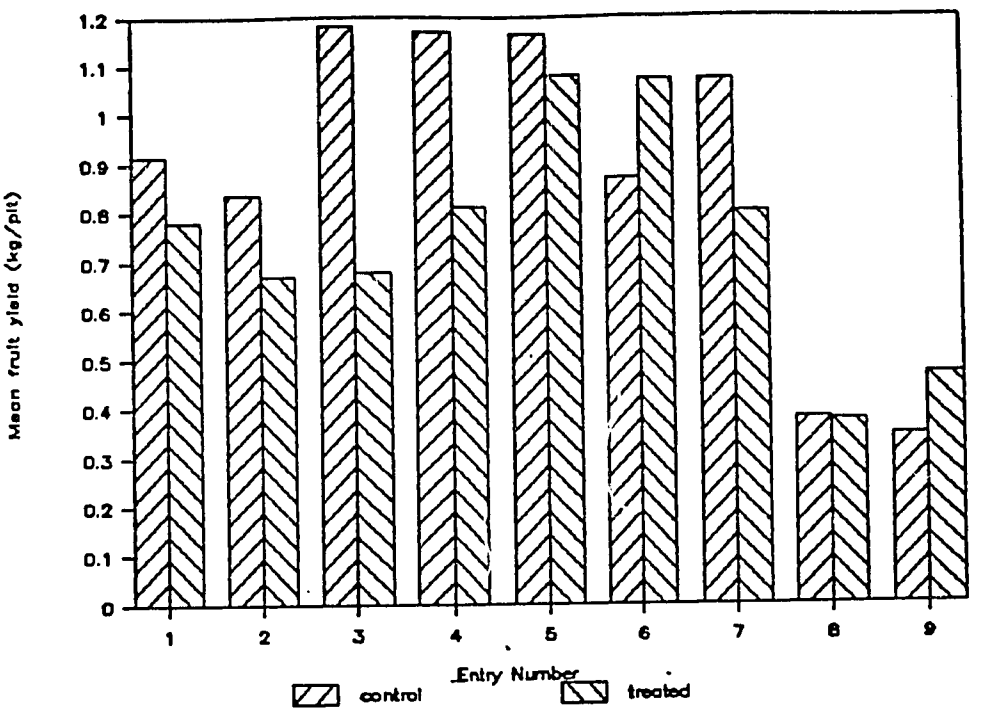

Figure 4. Mean fruit yield (g/plant) of the selections planted in Puerto Galera (Mindoro Oriental, Nov. 1989 - April 1990).

1 - Marikit (GT)*
2 - Marikit Select
3 - Improved Pope
4 - Improved Pope (NV)**
5 - Germplasm Collection # 2 (B-499-1)
6 - BC_2 — FD x [M x F_1 (M x Lpa)]
7 - BC_2 — FD x [M x F_1 (M x Lc)]
8 - F_5 (M x Lc)
9 - F_5 (M x Lpa)

Control - irrigated with tap water, EC = 0.4 dS/m
Treated - irrigated with a mixture of sea and tap water, EC = 15 dS/m
* Selection from the germination test (Tabel 8-150mM) and also from the seedling screening test.
** Collected in the province of Nueva Viscara.