PN-AAE-785
56040

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

A progress report submitted to AID for the period July - December 1987

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

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

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

Rec'd in SCI: FEB 17 1988

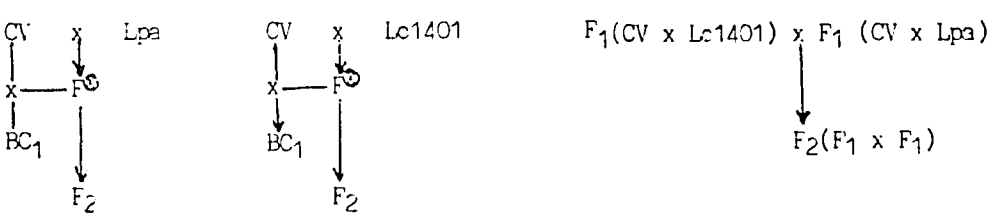
As emphasized in our previous report, we are concentrating on one crop, the tomato. The study includes: 1) Transfer of genes from wild salt-tolerant tomato species to the cultivated species by conventional breeding. 2. Selection for salt tolerance in cell and tissue culture. 3. Physiological research.

Work done in Israel

1. Transfer of genes

The wild salt-tolerant species include *Lycopersicon pennellii* accession Atico (Lpa) and *L. cheesmanii* ecotype 1401 (Lc 1401). The cultivars studied include Marikit (M) and Improved Pop (IP) from the Philippines and Floradade (FD) which was recommended (mainly because of its fruit qualities) and obtained from Dr. E. Kopilevitch from the Faculty of Agriculture, The Hebrew University, Rehovot. The addition of the third cultivar (FD) to our work was decided by the PI and Dr. E.T. Rasco during his visit to Israel. We decided that FD will be used as the recurrent parent in the crosses with BC_1, F_2, and $F_2(F_1xF_1)$ in Israel, and M and IP as the recurrent parents in the Philippines.

Additional seeds of F_1, F_2 and BC_1 generations from the crosses between the cultivars (CV) M or IP and the wild species have been prepared in Israel. More of these seeds were sent to the Philippines. The crosses included the following:



At present, crosses are being made between FD and vigorous and fertile plants of the BC_1, F_2 and F_2 (F_1 x F_1) generations which grow in Hoagland

solution together with 150 mM NaCl.

2. Selection for salt tolerance in tissue and cell culture

As mentioned in the previous report, in Israel more emphasis will be placed on tissue and cell culture of Merikit and in the Philippines on Improved Pop.

A. Calli and suspension cultures were prepared from leaves of the two cultivars M and IP, based on an extensive experimentation which included the exposure of leaf pieces to Murashige and Skoog medium with various hormonal combinations (2,4-D/kinetin; NAA/kinetin; 2-4-D/IAA/kinetin). The best combination for callus induction was found to be 0.5 mg/l kinetin and 5.0 mg/l NAA.

From the extensive data obtained for various species, five protocols were recommended for production of salt-resistant calli, cells or whole plants (Tal, 1988):

1) Long-term (based on reports from many species - references appear in Tal, 1988).

a. Production and establishment of cultured cells (callus and/or cell suspension) from any part of the plant. Cells with high regeneration capability are preferred.

b. Exposure of the cells to a range of salt concentrations for the determination of the highest concentration allowing cell survival and growth. The salt used is that which best characterizes field conditions.

c. Subculturing at the original concentration of salt until growth is uniform and at a reasonable rate. Then subculturing at the same concentration or at gradually elevated concentration with or without previous mutagenic treatment. Determination of stability of salt resistance, usually, by at least 3 transfers in salt-free medium (optional). Selection for salt resistance cell suspension is done in either liquid

medium or by transferring the cells into solid saline medium.

d. Regeneration of plants from selected cells in an appropriate medium with and without salt. Transfer of part of the cells to a regeneration medium after each subculture is recommended.

e. Determination of salt resistance in R_0 plants and also in cultured cells derived from them.

f. Production of R_1 and R_2 plants and determination of their resistance to salt under greenhouse and, if possible, under field conditions after their hardening.

2. Long-term two-step (After Harms and Oertli 1985)

a. As step a in protocol No. 1.

b. Exposure of the cells to a range of concentrations of a nonpenetrable and nontoxic osmoticum.

c. Subculturing of mutagenized or non-mutagenized cells at the original concentration of osmoticum until growth is uniform. Subculturing is continued at the original concentration or at gradually elevated concentrations.

d. Osmotically adapted cells are inoculated into saline media of the same osmolality. Subculturing at the same concentration or at gradually elevated concentrations.

Other operations may follow steps d-f of protocol No. 1.

3. Short-term (After McHughen and Swartz 1984)

a. As step a in protocol No. 1.

b. As step b in protocol No. 1.

c. Exposure (only once) of callus to the highest salt concentration allowing survival of some of the cells. The medium is hormone-free.

d. Transfer only alive cell aggregates to regeneration medium.

Other operations may follow steps d-f of protocol No. 1.

4. Use of salt-adapted feeder cells (suggested by HR Lerner, personal communication)

a. As step a in protocol No. 1.

b. Preparation of cells adapted to high salt level by gradual and long-term exposure to salt, following step a-c in protocol No. 1 or step a-d in protocol No. 2.

c. Treating the cells to be selected for salt resistance with a mutagen..

d. Inoculation of solid medium containing high salt level with feeder cells and overlaying them with the mutagenized cells, the two cell layers being separated by filter paper.

Other operations may follow steps d-f of protocol No. 1.

5. Use of proline analogues (After van Swaaij et al. 1986)

a. As step a in protocol No. 1.

b. Exposure of mutagenized or non-mutagenized cells to different concentrations of the proline analogues hydroxyproline and azetidine-2-carboxilic acid. Subculturing of resistant colonies at the original concentrations of the analogues.

c. Transfer of the resistant cells to regeneration medium.

d. Exposure of regenerants to the analogues for the selection of the analogue-resistant plants.

e. Testing the latter plants for salt resistance (and proline production) by exposure to elevated salt concentrations.

f. As step f in protocol No. 1.

Protocols 3,4 and 5 will be used for the production of salt-resistant plants. We plan to use protocols 1 and 2 for the production of salt-adapted suspension cells. The resistance in cells produced through these protocols is considered as the result of mainly physiological adaptation or

epigenetic change(s) and less of mutational events. These cells will be used as feeder cells in protocol No. 4. Preliminary experiments will be done using salt-adapted cells of potato which are already available in our laboratory3) as feeder cells.

B. We already determined the medium composition for the production of plantlets from leaf pieces and from detached shoot apices. We plan to induce plantlet formation directly in saline media.

3. Physiological research

During the summer of 1986 we plan to determine the following variables in the parental plants and in lines derived from the various crosses:

1. Daily fluctuations of osmotic adjustment (in shoot apex and the leaf) as recommended by Danon (1984).

2. Na^+, K^+ and Cl^- accumulation in different parts (shoot apex, leaf, stem and root) of the plant. According to Dr. Pasternak (Personal communication) low level of K^+ in the shoot seems to be a limiting factor for fruit setting in tomato plants exposed to saline conditions. It is worthwhile to remember that L. pennellii plants seem to have high efficiency of K^+ utilization (Taleisnik-Gertel et al., 1986).

3. Leaf succulence.

4. Proline production in the shoot apex and leaf.

5. Isozymic variation (in the root and leaf) of six enzymes including Phosphoglucomutase (PGM), Glutamate oxaloacetate transminase (GOT), Phosphoglucose isomerase (PGI), Isocitrate dehydrogenase (IDH), Glucose-6-phosphate Dehydrogenase (G-6-PDH) and Peroxidase (PER). These enzymes are already being determined in the parental species under control and saline conditions.

References

Danon, A. 1984. Salinity effect on Lycopersicon and Solanum . M.Sc. thesis, The Hebrew University of Jerusalem, Israel (in Hebrew).

Harms, C.T. and J.J. Oertli, 1985. The use of osmotically adapted cell cultures to study salt tolerance in vitro. J. Plant Physiol. 120: 29-38.

McHughen, A. and M. Swartz, 1984. A tissue-culture derived salt-tolerant line of flax (Linum usitatissimum). J. Plant Physiol. 117: 109-117.

Tal, M. 1988. Somaclonal variation for salt resistance. In: Biotechnology in Agriculture and Forestry (Y.P.S. Bajaj, ed.), Springer-Verlag, Berlin (in press).

Taleisnik-Gertel, E. and M. Tal, 1988. Potassium utilization and fluxes in wild and salt-tolerant relatives of the cultivated tomato. Physiol. Plant. 67: 415-420.

van Swaaij, A.C., Jacobsen, E., Kiel, J.A.K.W. and W.J. Feenstra, 1986. Selection, characterization and regeneration of hydroxyproline-resistant cell lines of Solanum tuberosum: Tolerance to NaCl and freezing stress. Physiol. Plant 68: 359-366.

Work done in the Philippines

A. Accomplishments

1. Seven tomato genotypes (Marikit (M), Marilag, Mayumi, Improved Pope (IP), PT 3027, Peto 81 and F_1(M x Lpa)) were subjected to salinity by using different dilutions of sea water (tap water alone, 1 sea water : 3 tap water and 1 sea water : 1 tap water). F_1(M x Lpa) was the most vigorous and had higher dry matter accumulation than the other varieties. Among the Philippine cultivars tested, M and IP appeared to be the least affected.

2. Both M and IP were grown in nutrient solution containing 0, 50, 100 and 150 mM and NaCl and $CaCl_2$ (7:1 molar ratio). The two varieties could withstand salinity at about 6.9 dS m^{-1} (50 mM). Increasing salinity levels at about 10 (100 mM) to 18 dS m^{-1} (150 mM) caused detrimental effects on morphophysiological parameters.

3. Pot experiments using again M and IP gave similar response as in the nutrient solution experiment. Different sources of salinity like combination of NaCl and $CaCl_2$ at 7:1 molar ratio, use of NaCl alone and dilution of sea water and tap water at 1:5 ratio did not produce any significant difference on the parameters measured.

4. At IPB, College, Laguna, hybridization work was slow because some genotypes (Arava, Hofit and the interspecific crosses) had low fruit setting and the fruits did not have seeds. The wild species were susceptible to diseases at seedling stage and fruit setting was not yet observed. Hence, these genotypes were brought to the Highland at Benguet State University where higher setting and seeds were obtained.

5. In tissue culture, the decontamination procedure and simple medium for the <u>in vitro</u> germination of tomato seeds of M and IP were established. The medium consist of 1/2 Murashige and Skoog salts plus vitamins, myoinositol and sucrose.

6. Explants for callus establishment using unexpanded and expanded leaves, petioles, roots, stems and shoot apex and different combinations of NAA and kinetin as media supplement were established.

B. Plan of Activities

1. Survey saline areas in the country.

2. Work out a technique for efficient pollination (interspecific crosses).

3. Continue screening for salinity tolerance of varieties, lines and segregating populations under greenhouse conditions.

4. Field confirmatory tests of selected plants.

5. Seed increase of foreign accessions.

6. Backcrossing of salt-tolerant lines to Philippine adapted parents.

7. Maintenance of stock plants cvs. M and IP *in vitro* as source of explants.

8. Establishment of culture requirements for maintenance of callus growth and for efficient plant regeneration.

9. *In vitro* screening at three stages (a) callus induction (b) callus maintenance and (c) plant regeneration stage.

C. Problems Encountered:

1. Delayed release of funds, hence, implementation of the different activities is very slow.

2. There is a problem of growing the wild type of tomatoes because of their susceptibility to diseases at seedling stage. These wild types will be used as one of the resistant checks in most physiological studies and will also be utilized for hybridization work.

3. The Israel cultivars and wild types of tomatoes have very low heat tolerance. They produced only flowers but no fruits under UPLB conditions (low elevation). They had to be grown in the cooler environments of the highlands of Benguet.

4. Another setback is the limited amount of seeds received from Israel.

Projects I and III. Physiology and Breeding

A. Response of different genotypes of tomato to three levels of salinity

Originally, thirteen tomato genotypes were sown for this experiment, but only seven, including an interspecific F_1 population, were examined for salt tolerance under greenhouse conditions. The genotypes tested were chosen to represent various genetic backgrounds of the cultivated (fresh

market and processing types) and wild tomato types. One month old tomato seedlings were transplanted in pots (12") filled with soil collected from Lemery, Batangas. They were watered everyday (for 10 days) with ordinary tap water before subjecting to salinity. Treatment imposition lasted for only one month after which the plants were watered again with ordinary tap water up to harvest. The following salinity treatments were imposed:

1. ordinary tap water, EC 0.75 dS m^{-1} (control)
2. 25% sea water, EC 18.0 dS m^{-1}
3. 50% sea water, EC 26.0 dS m^{-1}

The following data were gathered: a) height of the plants at harvest; b) root and shoot dry weight; c) symptoms exhibited by the plants.

Results and Discussion

Based upon visual observations of the plants, the F_1 hybrid between the cultivar M and the wild parent L. pennellii (Lpa) had the most vigorous growth (Table 1). In contrast with other varieties, this interspecific cross showed no yellowing, chlorotic spotting, or tip burning of the leaves. On the other hand, M and IP showed mild tip burning and chlorotic spotting. However, among the Philippine varieties tested, IP and M appeared to be the least affected. The above mentioned symptoms were readily observed in Mayumi a week after treatment imposition.

Although F_1(M x Lpa) was the most floriferous among the entries, it did not produce any fruits inside the greenhouse. It could be due to the high night temperature prevailing during the course of the experiment.

Reduction in plant height under salinity was evident (Table 2). In the control, F_1(M x Lpa) was the tallest and IP was the shortest. However, relatively to the control, F_1(M x Lpa) decreased more than IP under saline conditions.

F_1(M x Lpa) showed the higher value of dry matter accumulation as compared with the rest of the varieties at all treatments (Table 3). The other genotypes did not differ from each other. In all genotypes both shoot and root dry weight were reduced with increasing salinity levels except the root weight of IP under EC of 25 dS m^{-1} (Table 2).

At an EC of 18.0 dSm^{-1}, the F_1 hybrid had the highest root shoot ratio. This value increased under salinity relatively to the control. But at higher salinity, root shoot ratio of this interspecific cross decreased.

B. Growth response of tomatoes to varying salinity in nutrient solution (July to August 1987)

Procedure

Two Philippine tomato cultivars were used: M and IP. Seedlings (2-3 leaf stage) were established one month after sowing in aerated Hoagland's nutrient solution in one liter bottles. One week after establishment, salinization treatment was started.

Salinity was imposed by adding NaCl and $CaCl_2$ (3:1 molar ratio) at the following concentrations: 0, 50, 100 and 150 mM. The salts were added gradually at the rate of 25 mM per day until the desired concentration was attained. The nutrient solution was changed ten days after the last addition of the salt. The experiment lasted for one month after treatment.

The experiment was set up using a two factor-factorial in completely randomized design.

Plant height was recorded weekly. Two weeks after salinity treatment, stomatal resistance, transpiration rate and leaf temperature were determined using a steady state diffusion resistance meter. Likewise, leaf water potential was determined by means of a pressure chamber. Dry matter and root length were determined at harvest.

Results and Discussion

Growth rate of the tomatoes was not significantly affected by salinity during the first week of treatment. However, on the 2nd and 3rd week, height increment was severely affected with increasing salinity, especially at 10.8 and 15.0 dSm^{-1}.

Stunting of growth, chlorosis, leaf cupping and drooping were manifested by the two cultivars grown in nutrient solution from 6.9 to 15.0 dSm^{-1}. Based on these observations, IP appeared slightly more tolerant than M (Table 4).

Increased salinity caused a decrease in dry matter accumulation and root length in all varieties (Table 5). Root shoot ratio was not affected by the treatment.

Between the two cultivars, M accumulated higher shoot and root dry weights and longer roots than IP (Table 6).

Physiological parameters measured included stomatal resistance, transpiration rate, leaf temperature and leaf water potential (Table 7). There was an increase in stomatal resistance with the increase in salt concentration. Correspondingly, transpiration rate and leaf water potential both decreased. Likewise, temperature of the leaves also increased. All these are indications of response to water deficit caused by salinity.

IP maintained a higher stomatal resistance and higher leaf temperature than M (Table 8).

Interaction effect showed that IP maintained the highest stomatal resistance, lowest transpiration rate and leaf water potential and highest leaf temperature at 15 dSm^{-1} (Table 9). At the same electrical conductivity, M had lower stomatal resistance and higher transpiration rate. At lower EC, 6.9 and 10.8, both cultivars exhibited similar response (Table 9).

Based on the results of this experiment, both tomato cultivars could withstand salinity of about 6.9 dSm^{-1}, while increased salinity, 10.8 to 15.0 dSm^{-1}, was detrimental in terms of morphophysiological effects.

C. Response of two tomato cultivars to varying salinities in the soil (July-October 1987)

Procedure

The Philippine tomato cultivars used were M and IP. Tomato seedlings (2-3 leaf stage) were transplanted in clay pots (12") filled with clay loam soil. One week after transplanting (4-5 leaf stage) salinity treatments commenced using tap water with varying salinities.

The different levels of salinity were maintained by using NaCl and $CaCl_2$ (3:1 by weight), NaCl alone and 1:5 sea water-tap water. The following treatments were made:

Treatment	EC (dSm^{-1})
1. Tap water	0.8
2. 10 gl^{-1} (NaCl + $CaCl_2$)	18.5
3. 5 gl^{-1} (NaCl + $CaCl_2$)	10.2
4. 2 gl^{-1} (NaCl + $CaCl_2$)	5.2
5. 5 gl^{-1} (NaCl alone)	9.9
6. 1:5 (sea water:tap water)	11.0

Initially the seedlings were watered with 750 ml solution every other day. As the plants grew older, daily application was done. Treatment lasted for one month, after which the plants were watered with tap water until maturity or harvest.

The experiment was set-up using a two factor-factorial in complete randomized design with four replications.

Results and Discussion

Regardless of salt concentration, there was no significant difference between the response of M and IP in all parameters measured (Table 10).

Increasing concentration of salinity had pronounced effects on dry matter accumulation, stomatal resistance and transpiration rate (Tables 11 and 12), whereas height increament, root shoot ratio and leaf temperature were not affected. Generally, shoot dry weight decreased significantly at EC from 9.9 to 18.5 dSm^{-1}, while root dry weight was significantly decreased only at the highest EC value of 18.5 dSm^{-1}. EC values close to 10 dSm^{-1} did not show significant difference in these parameters.

Stomatal resistance and transpiration rate were significantly affected only at the highest EC of 18.5 dSm^{-1}, that is, there was partial closure of stomates coupled with a reduction in transpiration rate.

Interaction effects of variety and salinity are presented in Tables 13 and 14. Relative to control, growth rate of M was not affected by varying salinities. For IP, it was only 18.5 dSm^{-1} wherein a significant reduction in height increment was obtained.

In M, shoot dry matter was significantly reduced by all treatments while root dry matter was affected only at higher EC (Table 13). In IP, shoot and dry weights were significantly reduced from 10 to 18 dSm^{-1} but not at lower EC of 5.1 dSm^{-1}. At the same level of salinity, 9.9, 10.2 and 11.0 dSm^{-1}, there was no difference in terms of dry matter accumulation.

In M, stomatal resistance increased significantly only at 10.2 and 18.5 dSm^{-1} and there was also a corresponding decrease in transpiration rate (Table 14). In IP, increased stomatal resistance and decreased transpiration rate were observed only at the highest EC of 18.5 dSm^{-1}. Leaf temperature was not affected at all by the salinity treatment.

One problem encountered inside the greenhouse was the high night temperature and this was manifested by the low fruit setting ability of both cultivars (Table 13). It can be noted that the highest level of salinity caused the lowest fruit setting in both cultivars. However, it was surprising that fruit setting at about an EC of 9.9 dSm^{-1} was higher than the control. This aspect has to be verified in succeeding experiments.

Conclusions

Some morphophysiological responses of M and IP were not affected at an EC of 5.2 dSm^{-1} inhibitory effects were observed only at about 10 to 18 dSm^{-1}. Shoot dry weight was more affected than root dry weight.

Responses typical to water deficit including increased stomatal resistance and reduced transpiration rates were observed only at high EC of 18.5.

Different sources of salinity like combination of NaCl and $CaCl_2$, NaCl alone and dilution of sea water (1:5) did not differ significantly in their effect on the parameters measured.

Table 1. Horticultural characters of seven tomato entries grown under three salinity levels (including control).

Entry	Vigor Rating[1]	% of Flowers	Fruit Set Rating[2]	Remarks
Marikit	4.0	35	3	Mild tip burning of leaves
Marilag	2.0	28	3	Fruits with blossom end fot (BER)
Mayumi	2.0	29	2	Browning and yellowing of leaves were readily observed upon tratment imposition
Improved Pope	3.3	32	3	Mild to moderate browning and yellowing of leaves
PT 3027	4.0	40	4	Moderate browning and yellowing of leaves
Peto 81	3.0	20	2	Fruits with BER. No seeds were extracted. Moderate yellowing and tip burning of leaves
F_1(M x Lpa)	5.0	112	1	Most floriferous, but without fruits. No yellowing and burning of the tip of the leaves

[1] Vigor Rating: 1 - least vigorous; 5 - most vigorous.

[2] Fruit Set Rating: 1 - 0; 2 - 1-5; 3 - 6-10; 4 - 10-15; 5 - 16-20 fruits.

Table 2. Plant height (cm plant^{-1}) of seven tomato entries grown under three levels of salinity.

Entry	Treatment 1 (0.75 dSm^{-1})	Treatment 2 (18.0 dSm^{-1})	Treatment 3 (26.0 dSm^{-1})
Marikit	122.0 b	94.00 b (23)	82.5 a (32)
Mayumi	108.5 c	71.5 c (34)	58.0 b (47)
Improved Pope	72.0 e	65.5 c (9)	50.5 b (30)
Marilag	100.5 c	71.0 c (29)	64.5 b (36)
PT 3027	87.0 d	71.0 c (18)	54.5 b (37)
Peto 81	101.5 c	67.0 c (34)	63.0 b (38)
F_1(M x Lpa)	150.5 a	114.5 a (24)	86.0 a (43)

Means having the same letter are not significantly different from each other (DMRT 5%). Values in parentheses indicate % reduction.

Table 3. Dry matter accumulation of seven tomato cultivars subjected to salinity treatment.

Entry	Treatment 1 (0.78 dSm^{-1})	Treatment 2 (18.0 dSm^{-1})	Treatment 3 (26.0 dSm^{-1})
	Shoot weight (g $plant^{-1}$)		
Marikit	55.15 b	24.90	15.50 b
Mayumi	45.65 b	27.30	21.60 b
Improved Pope	25.80 d	26.60	16.00 b
Marilag	46.90 b	21.25	21.40 b
PT 3027	42.30 bc	21.05	12.00 b
Peto 81	29.70 cd	17.90	15.25 b
F_1(M x Lpa)	78.55 a	30.25	38.05 a
	Root weight (g $plant^{-1}$)		
Marikit	10.00 bc	3.05 b	4.50 b
Mayumi	13.75 b	3.42 b	3.65 b
Improved Pope	4.28 d	3.62 b	4.85 b
Marilag	9.45 bc	3.38 b	3.18 b
PT 3027	12.98 b	3.85 b	2.38 b
Peto 81	8.38 c	3.15 b	3.18 b
F_1(M x Lpa)	23.32 a	11.30 a	10.13 a
	Root shoot ratio		
Marikit	0.18 bc	0.12 b	0.20 ab
Mayumi	0.20 abc	0.16 b	0.15 b
Improved Pope	0.17 c	0.14 b	0.21 ab
Marilag	0.30 a	0.20 b	0.17 b
PT 3027	0.30 a	0.18 b	0.21 ab
Peto 81	0.28 ab	0.20 b	0.21 ab
F_1(M x Lpa)	0.30 a	0.39 a	0.27 a

Means having the same letter are not significantly different from each other (DMRT 5%).

[illegible] July-August [illegible]

Variety/ Treatment (mM)	Salinity (dSm^{-1})	Height Increment ($cm\ wk^{-1}$) I	II	III	Shoot Dry Weight ($g\ plant^{-1}$)	Root Dry Weight ($g\ plant^{-1}$)	Root Shoot Ratio	Root Length (cm)	Visual Observation
A. Marikit									
0	2.3	4.04 j-m	13.72 c	18.20 ab	5.13 a	0.63 a	0.12 b	25.27 a	Tall, but tip of older leaves were slightly affected with chlorosis. Produced lateral shoots and flowers
50	6.9	3.38 j-m	7.74 fg	12.30 cd	2.62 c	0.53 a	0.20 ab	23.03 ab	Moderately tall. Almost all leaves except shoots were with slight chloro
100	10.8	2.20 lm	4.28 j-m	5.70 g-j	1.69 d	0.27 c	0.16 b	17.37 c	Chlorosis was more apparent. Exhibite leaf cupping and stunting.
150	15.0	2.06 lm	2.22 lm	2.70 klm	1.08 d	0.14 d	0.38 a	12.50 d	Severe stunting an yellowing of leave All leves were dro ing.
B. Improved Pope									
0	2.3	3.84 j-m	10.34 def	20.00 a	3.41 b	0.49a	0.15 b	23.67 ab	Tall with green healthy leves. Lateral shoots and flowers were produced.
50	6.9	3.68 j-m	7.68 fgh	11.90 cde	1.71 d	0.37 b	0.22 ab	21.60 b	Quite tall. Very slight chlorosis on older leaves. Developed flowers.

Variety/ Treatment	Salinity (dSm^{-1})	Height Increment (cm wk^{-1}) I	II	III	Shoot Dry Weight (g $plant^{-1}$)	Root Dry Weight (g $plant^{-1}$)	Root Shoot Ratio	Root Length (cm)	Visual Observation
100	10.8	1.56 m	5.24 g-k	7.20 g-i	1.43 d	0.26 c	0.18 ab	15.73 c	Slightly stunted. Yellowing of first to third leaves were more distinct.
150	15.0	2.22 lm	3.36 j-m	4.80 i-l	1.14 d	0.13 d	0.12 b	11.87	Matured leaves with brown spots were drooping. Severe stunting.

Means having the same letter are not significantly different from each other (DMRT, 5%)

Table 5. Mean effect of treatments (regardless of variety) on morphological parameters of two tomato varieties to increased salinity in nutrient solution (July-August, 1987).

Treatment	Salinity (dSm^{-1})	Shoot Dry Weight ($g\ plant^{-1}$)	Root Dry Weight ($g\ plant^{-1}$)	Root Shoot Ratio	Root Length (cm)
0	2.3	4.27 a	0.56 a	0.13 a	24.47 a
50	6.9	2.16 b	0.45 b	0.21 a	22.32 b
100	10.8	1.56 c	0.27 c	0.17 a	16.55 c
150	15.0	1.11 c	0.14 d	0.25 a	12.18 d

Means having the same letter are not significantly different from each other (DMRT 5%).

Table 6. Mean effect of variety (regardless of treatment) on morphological parameters of two tomato varieties to increasing salinity in nutrient solution (July-August, 1987).

Variety	Shoot Dry Weight ($g\ plant^{-1}$)	Root Dry Weight ($g\ plant^{-1}$)	Root Shoot Ratio	Root Length (cm)
Marikit	2.63 a	0.39 a	0.22 a	19.54 a
Improved Pope	1.92 b	0.31 b	0.17 a	18.22 b

Means having the same letter are not significantly different from each other (DMRT, 5%).

Table 7. Mean effect of treatment (regardless of variety) on physiological parameters of two tomato varieties to increasing salinity in nutrient solution (July-August 1987).

Treatment	Salinity ($dS\ m^{-1}$)	Stomatal Resistance ($sec\ cm^{-1}$)	Transpiration Rate ($\mu g\ cm^{-2} sec^{-1}$)	Leaf Temperature (°C)	Leaf Water Potential (MPa)
0	2.3	0.85 c	16.36 a	31.49 c	-0.20 a
50	6.9	1.02 c	14.48 b	31.83 bc	-0.30 ab
100	10.6	1.90 b	9.27 c	31.95 ab	-0.34 bc
150	15.0	3.91 a	5.22 d	32.71 a	-0.45 c

Means having the same letter are not significantly different from each other (DMRT, 5%).

Table 8. Mean effect of variety (regardless of treatment) on physiological parameters of two tomato varieties to increasing salinity in nutrient solution (July-August 1987).

Variety	Stomatal Resistence ($sec\ cm^{-1}$)	Transpiration Rate ($\mu g\ cm^{-2} sec^{-1}$)	Leaf Temperature (°C)	Leaf Water Potential (MPa)
Marikit	1.64 a	11.70 a	31.48 a	-0.32 a
Improved Pope	2.20 b	10.96 a	32.50 b	-0.33 a

Means having the same letter are not significantly different from each other (DMRT, 5%).

Table 9. Physiological response of two tomato cultivars to increasing salinity in nutrient solution (July-August 1987)

Variety/Treatment (mM)	Salinity ($dS m^{-1}$)	Stomatal Resistence ($sec\ cm^{-1}$)	Transpiration Rate ($\mu g\ cm^{-2} sec^{-1}$)	Leaf Temperature (°C)	Leaf Water Potential (MPa)
A. Marikit					
0	2.3	0.79 d	16.76 a	31.02 c	-0.21 a
50	6.9	0.99 d	14.53 b	31.46 d	-0.28 ab
100	10.8	1.77 c	8.81 c	31.60 cd	-0.43 bc
150	15.0	3.00 b	6.72 d	31.86 bcd	-0.39 bc
B. Improved Pope					
0	2.3	0.90 d	15.96 ab	31.96 bc	-0.19 a
50	6.9	1.04 d	14.43 b	32.20 b	-0.31 ab
100	10.8	2.02 c	9.74 c	32.30 b	-0.26 ab
150	15.0	4.82 a	3.73 e	32.76 a	-0.51 c

Means having the same letter are not significantly different from each other (DMRT, 5%).

Table 10. General response of two tomato cultivars to salinity (regardless of concentration).

Variety	Stomatal Resistance ($sec\ cm^{-1}$)	Transpiration Rate ($\mu gr\ cm^{-2} sec^{-1}$)	Leaf Temperature (°C)	Height Increment	Shoot Dry Weight	Root Dry Weight	Root Shoot Ratio
Marikit	1.24 a	12.66 a	30.69 a	25.98 a	58.81 a	10.26 a	0.15 a
Improved Pope	1.31 a	13.34 a	30.73 a	30.81 a	58.41 a	9.05 a	0.16 a

Means having the same letter are not significantly different from each other (DMRT, 5%).

Table 11. Morphological response of tomato to increasing salinity.

Treatment (NaCl + CaCl 3:1)	Salinity (dS m^{-1})	Height Increment (cm)	Shoot Dry Weight (g $plant^{-1}$)	Root Dry Weight (g $plant^{-1}$)	Root Shoot Ratio
Tap Water	0.8	30.75 a	87.35 a	12.77 a	0.15 a
10 gl^{-1}	18.5	24.81 a	43.15 d	5.55 b	0.16 a
5 gl^{-1}	10.2	28.94 a	57.20 cd	8.39 ab	0.15 a
3 gl^{-1}	5.2	30.00 a	74.05 ab	12.90 a	0.18 a
5 gl^{-1} (NaCl)	9.9	29.63 a	63.65 bc	8.25 ab	0.13 a
1:9 (sea water: tap water)	11.0	26.29 a	56.26 cd	9.6 ab	0.18 a

Means having the same letter are not significantly different from each other (DMRT, 5%).

Table 12. Physiological response of tomato to increasing salinity

Treatment (NaCl + CaCl 3:1)	Salinity ($dS\ m^{-1}$)	Stomatal Resistance ($sec\ cm^{-1}$	Transpiration Rate ($ug\ cm^{-2} sec^{-1}$)	Leaf Temperature (°C)
Tap water	0.8	0.79 b	17.68 a	31.19 a
10 g l^{-1}	18.5	2.31 a	7.43 d	31.25 a
5 g l^{-1}	10.2	1.35 b	11.55 c	29.00 a
2 g l^{-1}	5.2	0.89 b	15.22 abc	30.56 a
5 g l^{-1} (NaCl)	9.9	1.11 b	13.60 abc	31.98 a
1:5 (sea water: tap water)	11.0	1.30 b	12.53 bc	30.03 a

Means having the same letter are not significantly different from each other (DMRT, 5%).

Table 13. Morphological response of two tomato cultivars to varying levels of salinity in the soil (July 27 to October 20, 1987)

Variety/Treatment ($NaCl + CaCl_2$ 3:1)	Salinity ($dS\ m^{-1}$)	Height Increment ($cm\ plant^{-1}$)	Shoot Dry Weight ($g\ plant^{-1}$)	Root Dry Weight ($g\ plant^{-1}$)	Root Shoot Ratio	Fruit Setting (%)
Marikit						
Tap water	0.8	26.5 bcd	100.0 a	13.6 a	0.14 a	9.70
10 $g\ l^{-1}$	18.5	22.5 cd	45.3 de	5.8 d	0.13 a	0.00
5 $g\ l^{-1}$	10.2	28.6 abc	60.5 bcd	8.6 bcd	0.14 a	27.91
2 $g\ l^{-1}$	5.2	27.6 bcd	74.3 b	13.9 a	0.19 a	11.72
5 $g\ l^{-1}$ (NaCl alone)	9.9	28.9 abc	71.8 bc	9.0 abcd	0.13 a	23.76
1:5 (sea water: tap water)	11.0	21.8 d	61.0 bcd	10.7 abc	0.18 a	10.91
Improved Pope						
Tap water	0.8	35.0 a	74.7 b	11.9 ab	0.16 a	29.15
10 $g\ l^{-1}$	18.5	27.1 bcd	41.0 e	6.5 cd	0.17 a	3.13
5 $g\ l^{-1}$	10.2	29.3 ab	53.9 de	8.0 bcd	0.15 a	14.61
2 $g\ l^{-1}$	5.2	32.4 ab	73.8 b	11.9 ab	0.16	9.69
5 $g\ l^{-1}$ (NaCl alone)	9.9	30.4 ab	55.5 cde	7.5 bcd	0.13 a	33.25
1:5 (sea water: tap water)	11.0	30.8 ab	51.5 de	8.5 bcd	0.17 a	4.37

Means having the same letter are not significantly different from each other (DMRT, 5%).

Table 14. Physiological response of two tomato cultivars subjected to varying salinities in the soil (15 DAT) (July 27 to October 20, 1987)

Variety/Treatment ($NaCl:CaCl_2$, 3:1)	Salinity ($ds\ m^{-1}$)	Stomatal Resistance ($sec\ cm^{-1}$)	Transpiration Rate ($ug\ cm^{-2} sec^{-1}$)	Leaf Temperature (°C)
Marikit				
Tap water	0.8	0.80 d	17.00 ab	30.63 ab
10 g l^{-1}	18.5	1.75 b	8.80 de	31.20 a
5 g l^{-1}	10.2	1.52 bc	10.42 cde	30.40 ab
2 g l^{-1}	5.2	0.89 cd	15.20 abc	30.56 ab
5 g l^{-1} (NaCl alone)	9.9	1.16 bcd	12.93 bcd	30.30 ab
1:5 (sea water: tap water)	11.0	1.33 bcd	11.63 cd	30.50 ab
Improved Pope				
Tap water	0.8	0.77 d	18.35 a	31.70 a
10 g l^{-1}	18.5	2.71 a	6.05 e	31.30 a
5 g l^{-1}	10.2	1.17 bcd	12.67 bcd	30.90 a
2 g l^{-1}	5.2	0.89 cd	15.24 ab	30.50 a
5 g l^{-1} (NaCl alone)	9.9	1.07 bcd	14.27 abc	31.60 a
1:5 (sea water: tap water)	11.0	1.26 bcd	13.42 abcd	31.56 a

Mean having the same letter are not significantly different from each other (DMRT, 5%).

Project II : Tissue Culture and *In Vitro* Selection for Salt Resistance in Tomato

Research Activities

A review of literature on establishment of callus in tomato showed that leaf explants were usually employed in tissue culture studies (Table 1) and that a combination of auxins and cytokinins was necessary. These conditions for callus establishment were verified for IP and M. Information regarding tissue culture of these cultivars is not available.

Materials and Methods

A. Callus induction and maintenance.

1) Effect of Kinetin (Ki) and naphthalene acetic acid (NAA) and their combinations on tomato leaves.

Seeds of the tomato variety IP were placed on a piece of gauze cloth, tied-up and washed in running water for 20 min, soaked in 20% chlorox for 20 min, and rinsed thrice in sterile distilled water. Disinfected seeds were inoculated to a nutrient medium containing 1/2 Murashige and Skoogs (MS) salts plus (mg l^{-1}):10 thiamine hydrochloride, 0.5 pyridoxine hydrochloride, 0.5 nicotinic acid; 10 myo-inositol; 2 glycine and 25 g l^{-1} sucrose, pH 5.8. After 5 days, shoot tops of tomato seedlings were cut and subcultured onto fresh medium containing full strength MS salts plus the same concentration of vitamins, myo-inositol, glycine and sucrose used in the aseptic germination. Aseptically grown plants served as sources of explants for the different experiments for callus establishment. Plants were maintained and multiplied by nodal cuttings every four weeks.

Leaf explants measuring about 1 X 1 mm were excised from the first fully expanded leaves with a sterile scalpel and aseptically inoculated onto sterile medium in 125-ml erlenmeyer flasks.

The nutrient medium consisted of MS major and minor salts plus (mg l^{-1}):100 myo-inositol, 10 thiamine hydrochloride, 0.5 pyridoxine hydrochloride, 0.5 nicotinic acid, 2 glycine and 25 gl^{-1} sucrose. Kinetin and NAA at various concentrations were incorporated into the medium (Table 2). Five leaf pieces were inoculated onto each flask and incubated in the culture room of 25 - 27°C at 16-hr photoperiod. Survival, callus formation, type of callus growth, and shoot formation were noted 3 weeks after inoculation.

2) Effect of 2,4-D on the callus formation of tomato leaves

Explants and basal medium were similar to those described in section 1. Culture media supplemented with various concentrations of 2,4-D and combinations of Ki and NAA were compared for callus establishment (Table 3). Five leaf pieces were inoculated onto each flask. There were five replicate flasks per treatment which were inoculated at 25 - 27°C at 16-hr photoperiod. Survival, callus formation and callus type were observed 3 weeks after inoculation.

3) Callus maintenance and/or regeneration of plantlets.

Different hormones were incorporated to MS medium to determine an effective media for long term callus maintenance of callus induced by 2,4-D. The following treatments were taken from the different protocols. Source of explants were pieces of calli from section 2.

a. MS

b. MS + 0.5 mgl^{-1} 2,4-D

c. MS + 0.5 mgl^{-1} Ki + 2 mgl^{-1} NAA

d. MS + 2 mgl^{-1} Ki + 0.5 mgl^{-1} NAA

e. MS + 4 mgl^{-1} Ki + 4 mgl^{-1} IAA

f. MS + 0.5 mgl^{-1} 2,4-D + 1 mgl^{-1} BAP

g. MS + 1 mgl^{-1} NAA + 2 mgl^{-1} BAP

Four callus pieces were inoculated onto each flask. There were five replicate flasks per treatment. Callus, shoot and root formation as well as the relative growth of callus were noted. Growing of callus was rated 1 to 6 with higher numbers connoting very profuse callus.

B. Induction of callus from different parts of the plant.

Tissue explants from roots, stem, petioles, expanded and unexpanded leaves and shoot apices were excised from aseptically grown seedlings of tomato cv. IR. Five explants were inoculated onto MS medium with 0.5 mgl^{-1} Ki and 2 mgl^{-1} NAA contained in flasks. The five replicate flasks were incubated at 25 - 27°C at 16-hr photoperiod. Survival, callus formation, callus type, shoot and root formation were noted 3 weeks after inoculation.

C. Preliminary evaluation of <u>in vitro</u> seedling shoots on various salt supplemented media.

Shoots of IR having only the clump of unexpanded leaves were inoculated onto a solid Murashige and Skoogs medium supplemented with 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35 M NaCl. The control was not supplemented with NaCl. There were three shoot per replicate flask and three replicates per treatment. Cultures were maintained at 25 - 27°C and 16-hr photoperiod. Growth and quality of the shoots were noted three weeks after inoculation.

Results and Discussion

A. Establishment of media for callus induction and maintenance

1) Effect of Ki and NAA and their combinations on tomato leaves

Calli were induced in all levels of Ki and NAA (Table 3) but greater amounts were produced in specific combinations of Ki and NAA.

Supplementing NAA alone in the medium has shown a high percent age of survival (100%) and percent callus induction (100%) but with only slight degree of callus development. No significant differences were shown by each of the three levels of NAA (0.5, 1 and 2 ppm). A slight degree (1.4 - 2.6)

of callus was formed, being a watersoaked type and slightly brown. At 0.5 ppm Ki, a 100% survival was obtained but with only 20% callus formation. Percent callus was increased to 100% at concentration 1 and 2 ppm but decreased to 50% when the concentration was increased to 4 ppm. Degree of callus formed was very slight as compared to explants inoculated on NAA supplemented media. Furthermore, callus was loose, friable and white.

Combinations of Ki and NAA produced callus of good type with regenerable potential. Such calli were nodular compact and green or cream in color. Percent survival and callus formation was optimal at 0.5 ppm Ki and 2 ppm NAA with a rating of 6 for the degree of callusing (very profuse callus). Combinations 0.5, 1 and 2 ppm NAA with 2 ppm Ki gave a 100% callus formation although percent survival (80%) and degree of callus (3 - 5) was lower as compared to those in combinations with 0.5 Ki. Degree of callusing was 5.5 (profuse) at 1 ppm Ki plus 0.5 ppm NAA, but only 3 - 4 (callusing all over the explant and slightly profuse) at 1 and 2 ppm NAA plus 1.0 Ki or 0.5, 1 and 2 ppm NAA plus 2 ppm Ki. Survival was reduced to 40% at combinations 0.5 and 1 ppm NAA plus 4 ppm Ki. At 4 ppm Ki in combination with 2 ppm NAA, only 40% of explants survived but all of them formed calli which were hard and compact and creamy and greenish in color.

Shoot formation was observed at 0.5 ppm NAA plus 1, 2 and 4 ppm Ki.

2) Effect of 2,4-D on the callus induction of tomato leaves.

Callus establishment

Callus was produced in all media supplemented with 2,4-D (Table 4). Percent survival was high (92%, 80% and 94%) at 0.5, 1 and 2 ppm 2,4-D, respectively. Survival decreased to 72% when 2,4-D increased to 4 ppm. Degree of callus formation was highest at 0.5 ppm 2,4-D (3.2). The type of callus on media supplemented with 2,4-D varied from nodular compact to loose while color ranged from cream to gray. Degree of callus formation using 2,4-D was

similar to that formed using 0.5 ppm Ki plus 2 ppm NAA, but the type of callus on 2,4-D supplemented media was non-regenerable. More compact green and cream calli were noted on media with Ki and NAA.

b. Induction of callus from the different plant parts of the plant.

Tomato explants from the stem, petiole, mature and immature leaves and shoot apices produce calli which were nodular and compact, and creamy and greenish in color. Degree of callusing (4.4) was highest in explants obtained from the stem. The other explants showed similar degrees of callusing (3 - 3.3) except for the root (1.1) (Table 5). Shoots were formed on callus from immature leaves explants taken from the stem, and shoot apices. Roots were also produced on stem explants.

C. Preliminary evaluation of <u>in vitro</u> seedling shoots on various salt supplemented media

The shoots on the control media were green, while those on NaCl-supplemented media showed signs of salt stress ranging from general yellowing, curling and narrowing of leaves, stem bulging, tip burnings and death (Table 6). Severity of symptoms increased with the concentration of NaCl. The lowest concentration of NaCl, 0.05 M, proved to be stimulatory to growth although plants also showed general yellowing. This experiment will be repeated on liquid medium to enable us to measure the conductivity of the medium as influenced by NaCl addition.

The concentrations of NaCl which will be used in subsequent experiments of <u>in vitro</u> selection will be based on the results of the experiment on liquid medium.

On-going Activities

1) Studies on callus maintenance and regeneration.

2) Multiplication of regenerated plants for <u>in vitro</u> evaluation of salt resistance.

Table 1. Cultural requirements for callus establishment for tomato

Citation	Explant	Culture media	Culture requirements	Remarks
Padmanabhan et al. 1973	leaves (6 X 8 mm)	MS + Ki + IAA	room temperature (22°C±5°C) 12 hr photoperiod 3 - 4 wks subculture	Root initiation (2 ppm IAA + 2 ppm Ki) Shoot formation (4 ppm IAA + 4 ppm Ki)
Behki and Lesley, 1976	leaves (5 disc of 5 mm diameter)	MS + NAA + BA	22°C, 16 hr photoperiod	Shoot initiation (1 X 10^{-5}M BA + 5 X 10^{-7}M NAA) Root initiation (1 X 10^{-6}M BA + 2.5 X 10^{-6}M NAA)
Behki and Lesley, 1979	leaves (5 disc of 5 mm diameter)	MS + 1 ppm 2,4-D	22°C, 16 hr photoperiod 4 - 6 wks subculture	White friable non-regenerable callus
		MS + 5 ppm IAA + 1 ppm BAP		Profuse root formation
		MS + 2-5 ppm NAA Hard callus MS + NAA + BAP		
		MS + 0.5 ppm 2,4-D + 1 ppm BAP		Significant callus growth (white and friable) Callus of friable texture, lighter in color and retains its regenerable shoots consistently when transferred to morphogenesis inducing media
Kartha et al, 1975	leaves (disc of 5 mm diameter)	MS + 10 µM BA + IAA	26°C and 70% RH 16 hr photoperiod (3000 lx)	Multiple shoot differentiation
		MS + Ki + IAA or NAA		0 shoot formation
		MS + 0.1 µM Zeatin + 10 µM IAA		Shoot and root differentiation
		MS + 1 µM Zeatin + 0.1 µM IAA		Shoot and root differentiation
		BA or Z at 5 µM		Complete plant regeneration from callus

Table 2. Summary of media treatments.

Treatments	Basal Medium	Growth Hormones	
		Ki	NAA
T_1	MS	0	0
T_2	MS	0.5	0
T_3	MS	1	0
T_4	MS	2	0
T_5	MS	4	0
T_6	MS	0	0.5
T_7	MS	0	1
T_8	MS	0	2
T_9	MS	0.5	0.5
T_{10}	MS	0.5	1
T_{11}	MS	0.5	2
T_{12}	MS	1	0.5
T_{13}	MS	1	1
T_{14}	MS	1	2
T_{15}	MS	2	0.5
T_{16}	MS	2	1
T_{17}	MS	2	2
T_{18}	MS	4	0.5
T_{19}	MS	4	1
T_{20}	MS	4	2

Table 3. Effect of Ki and NAA on callus induction in tomato leaf explants *in vitro*

Growth Hormones Ki	NAA	Percent Survival	Percent Callus	Degree of Callus[a]	Callus Type	Shoot
0	0	100	0	0	-	
0.5	0	100	20	0.2	white friable	0
1	0	40	100	1	white friable	0
2	0	100	100	1	sl. brown, loose	0
4	0	40	50	1	sl. brown, loose	0
0	0.5	100	100	1.4	sl. brown, water-soaked	0
0	1	100	100	2.6	sl. brown, water-soaked	0
0	2	80	100	1.75	sl. brown, water-soaked	0
0.5	0.5	100	100	4	white and green compact	0
0.5	1	100	100	4	cream and compact	0
0.5	2	100	100	6	cream and green nodular compact	0
1	0.5	80	100	5.5	cream compact	3
1	1	100	100	3.4	green and brown compact	0
1	2	100	100	3.4	cream and brown compact	0
2	0.5	80	100	3.75	cream nodular compact	1
2	1	80	100	4	brown hard compact	
2	2	80	100	4.25	slight brown and compact	0
4	0.5	40	67	5.5	compact cream	2
4	1	40	50	5	compact green	0
4	2	40	100	5	cream hard and compact	0

a 1 - very slight callus
2 - calli on more than half of the explant
3 - callus all over the explant
4 - slightly profuse callus
5 - profuse callus
6 - very profuse callus

Table 4. Effect of 2,4-D and combination of Ki and NAA on the induction of callus of tomato leaves *in vitro*.

Treatment	Percent Survival	Percent Callus	Degree of Callus formation[a]	Callus Type
Control (MS only)	100	-	-	-
MS + 0.5 mg l^{-1} 2,4-D	92	100	3.18	Few watersoaked and some are compact, cream
MS + mg l^{-1} 2,4-D	80	100	2.96	Watersoaked and compact, cream and slight brown
MS + 2 mg l^{-1} 2,4-D	84	100	3.02	Watersoaked and friable white and cream
MS + 4 mg l^{-1} 2,4-D	72	100	2.84	Nodular compact, some are watersoaked, cream and opaque
MS + 0.5 mg l^{-1} Ki + 2 mg l^{-1} NAA	76	100	3.56	Nodular cream compact
MS + 2 mg l^{-1} Ki + 0.5 mg l^{-1} NAA	32	100	1.66	Cream compact

a 1 - very slight callus
2 - callus more than half of the explant
3 - callus all over the explant
4 - slightly profuse callus
5 - profuse callus
6 - very profuse callus

Table 5. Callus induction and morphogenetic response of the different plant parts of tomato

Explant Source	Percent Survival	Percent Callus	Degree of Callus formation[a]	Callus Type	Shoot (average)	Root (average)
Root	80	100	1.12	ws	-	-
Stem[b]	100	100	4.40	Compact cream & green	0.05	0.05
Petiole	85	100	3.32	Nodular compact cream & brown	-	-
Leaf	80	100	3.21	Nodular compact cream	-	-
Leaves near the shoot	100	100	2.13	Compact cream	0.062	-
Shoot apex	50	100	3.00	Compact cream & green	1.64	-

a 0 - no callus
1 - very slight callus
2 - callus more than half of the explant
3 - callus all over the explant
4 - slightly profuse callus
5 - profuse callus
6 - very profuse callus

b Taken from internode tissues between first 2 expanded leaves

Table 6. Qualitative assessment of shoot tops of tomato cv. IP to NaCl supplemented media

Treatment (NaCl in M)	Percent shoots showing salt stress symptoms				
	Stem bulging	Yellowing	Leaf curling/ narrowing	Tip burn	Death
0	-	-	-	-	-
0.05	-	100.0	33.3	33.3	-
0.10	100.0	100.0	33.3	33.3	-
0.15	100.0	100.0	66.7	33.3	-
0.20	100.0	100.0	100.0	55.3	-
0.25	100.0	100.0	100.0	88.6	-
0.30	100.0	100.0	100.0	100.0	-
0.35	100.0	100.0	100.0	100.0	55.6