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The Response to NaCl of Excised Fully Differentiated and Differentiating
Tissues of the Cultivated Tomato Lycopersicon esculentum and its
wild relatives L. peruvianum and Solanum pennellii

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ABSTRACT

The responses to NaCl of cultured leaf discs and leaflets derived from fully differentiated leaves and of shoot apices excised from the cultivated tomato Lycopersicon esculentum and its wild salt-tolerant relatives L. peruvianum and Solanum pennellii were compared.

The results suggest that the tolerance of the whole plant to salt depends largely on the tolerance of plant organs containing meristematic tissues rather than on that already differentiated. This suggestion is based on the positive correlation found between the response to salt of shoot apices and the whole plant, i.e. both whole plants and apices of the wild species were more resistant to salt than those of the cultivated species. No difference was found between the species with respect to the responses of the fully differentiated parts.

The ion balance (K^+/Na^+ and Cl^-/Na^+) in detached leaves and apices exposed to salt was found to be different from that in the same parts while attached to the salt-treated plant. This difference may be due to the severance of the excised parts from the major sites controlling the balance of ions in the whole plant.

Key words: Tomato species, Salt tolerance, Shoot apex.

INTRODUCTION

The salt-tolerant relatives of the cultivated tomato, Lycopersicon peruvianum and Solanum pennellii, were suggested as a potential source of germplasm for salt resistance (Tal, 1971; Dehan and Tal, 1978). Under saline conditions their relative growth rate was found to be superior to that of the cultivated species, and their leaves to accumulate more Na^+ and Cl^- and less K^+ (Tal, 1971; Dehan and Tal, 1978;). Dehan and Tal (1978) suggested that a better osmotic adjustment may be responsible for the better performance of the wild species under salinity.

Calli derived from either leaf, stem, or root of L. peruvianum and S. pennellii grew better in salinized medium and accumulated more Na^+ and Cl^- than those derived from the cultivated tomato in salinized medium (Tal et al, 1978). The plating efficiency in saline medium of protoplasts isolated from leaves of L. peruvianum was higher than that of protoplasts of the cultivated tomato (Rosen and Tal, 1981). The positive correlation found between the response to salt of calli and dividing protoplasts, which are relatively undifferentiated, and that of whole plants led to the suggestion that the better osmotic adjustment which characterizes the wild plants operates at the cellular level and is independent of the organization of the cells in the whole plant.

The fact that the response of both calli and dividing protoplasts to salinity was correlated to that of the whole plant raised the question of whether such a correlation characterizes only dividing and relatively undifferentiated cells, or whether it is also, a characteristic of both differentiated and differentiating cells in these plants. This question was studied by comparing the response to salinity of excised fully differentiated tissues (leaves) and differentiating tissues (shoot apices) of the cultivated and wild species of tomato.

MATERIALS AND METHODS

Plant material. The species used in this study included the cultivated tomato, Lycopersicon esculentum cultivar 'VF 234' (VF) and the wild relatives of tomato, L. peruvianum (Lp) and Solanum pennellii accession Atico (Sp). Plants were grown in the greenhouse in aerated half strength Hoagland solution as previously described (Tal and Shannon, 1983).

Leaf discs. The terminal leaflets of the youngest fully-expanded leaves were detached from the plant and surface sterilized by immersion in hypochlorite solution (1% active chloride) for five min. They were then rinsed in sterile water (x 3 passages). Discs, 7 mm in diameter, were punched out from the lamina, avoiding the middle vein, and were transferred in pairs to a sterile agar medium, which contained either 0.0, 1.0, 1.5 or 2.0 % NaCl. The medium consisted of Murashige and Skoog's (MS) mineral salts and inositol (Murashige and Skoog, 1962) and (mg/l): thiamine HCl (10), nicotinic acid (0.5), glycine (2), pyridoxine (0.5), naphthaleneacetic acid (0.5) and kinetin (2).

The cultures were kept at 27°C under 16 h photoperiod with light of 2 W m^{-2} provided by Atlas coolwhite fluorescent tubes. Ten days later, the discs which remained green and turgid were rinsed for 3 min (x 3 passages) in stirring distilled water or in 5 mM CaSO_4 solution and blotted carefully. Placid discs which had changed from green to brown were and classified as dead.

Leaflets. Leaflets were excised from the youngest fully expanded leaves of plants at the onset of flowering. The leaves were surface sterilized as described above. Sterile leaflets were inserted upright in MS medium containing either 0.0, 1.0, 1.5 or 2.0% NaCl, and (mg/l): thiamine HCl (10), glycine (2), nicotinic acid (0.5) pyridoxine (0.5), naphthaleneacetic acid (0.1), and kinetin (6) and incubated as described for the discs.

The response of the leaves to salt was assessed by measuring the retention of chlorophyll in the tissue. Chlorophyll was extracted with dimethylformamide and measured by the absorption at 665 nm.

Shoot apices. Plants at the onset of flowering stage were detopped to induce development of lateral buds. The tips the side branches were detached and sterilized as described above. Pieces, 2-3 cm in length, which included the meristems and the first 1-2 developing leaves were excised and inserted upright in MS medium containing different concentrations of NaCl and incubated as described for the leaflets. The response of the apices to salt was assessed by determining the increment of dry weight during 10 days of incubation.

Ions. Blotted leaf discs and the parts of leaflets and shoot apices which were above the medium were weighed and subsequently dried at 85°C for 48 h. Dry weight was determined, and the dry tissue was transferred into 3 ml of 0.1 N nitric acid for at least 72 h at room temperature. Sodium and potassium were determined with a Corning EEL flame photometer, and Cl^- with a Buchler Cotlove chloridometer.

RESULTS

Leaf discs and leaflets

Survival. The survival of leaf discs of all three species studied was affected by NaCl similarly (Figure 1). Similar results were obtained for leaflets (Table 1).

Ion content. Sodium and chloride level increased in NaCl-treated discs of all three species (Table 2). The increase in Na^+ in Lp and Sp was larger than in VF. Chloride increased in Lp and Sp more than in VF. Potassium level decreased under NaCl salinity in discs of all three species, the decrease being the smallest in Sp and the greatest in Lp.

The accumulation of Na^+ and Cl^- in NaCl-treated leaflets was greater in Sp than in the other species (Table 3). The decrease of K^+ in these leaflets was, like in the leaf discs, the smallest in Sp and the greatest in Lp.

Shoot apices

Growth. Growth of excised apices was negatively affected by salinity in all three species (Table 4). However, the decrease was much greater in VF than in the other two species. The apices of Sp were the least affected by the salinity.

Ion content. Sodium and Chloride were accumulated in apices of all three species under NaCl salinity; the accumulation was high in Sp and low in VF and Lp (Table 5). The decrease of K^+ under salinity was the greatest in Sp.

K^+/Na^+ and Cl^-/Na^+ ratio

The ratios K^+/Na^+ and Cl^-/Na^+ in NaCl-treated excised leaf discs, leaflets and shoot apices are presented in Table 6. K^+/Na^+ ratio was similar in all species in leaflets and apices. In leaf discs, it was higher in VF and Sp than in Lp. The ratio Cl^-/Na^+ was close to 1 in leaf discs of VF and Sp, in leaflets of VF and Lp, and in the apices of all three species.

DISCUSSION

A positive correlation was found between the response of the whole plant and dividing cells to salt in tomato species (Tal et al., 1978; Rosen and Tal, 1981). In the present work it was queried whether such a correlation characterizes only relatively undifferentiated dividing cells or whether it is not necessarily related to the state of differentiation. The results presented here support the former alternative. A positive correlation was found between the response to salinity of excised apices and whole tomato plants as found for dividing calli and similarly to the whole plants. Apices of the two salt-tolerant species Lp and Sp grew better than those of the cultivated tomato on saline medium. In contrast, no correlation was found between the response of isolated fully differentiated tissues and the response of the whole plant. Discs and leaflets of fully differentiated leaves of the cultivated tomato survived to the same extent, or in some cases even to a larger extent, than those of the wild species. These findings demonstrate the central role of cells in the growing zone may have in the tolerance of the whole plant. Wyn Jones et al. (1979) suggested that various steps involved in protein synthesis are very sensitive to changes in the ionic environment. It is possible that these processes are more salt tolerant or more protected from the toxic effect of salts in the growing zone of the wild species Lp and Sp than in the cultivated tomato.

The balance of Na^+ , Cl^- , and K^+ in the shoot apex and the leaflets which were brought in direct contact with salt after their excision appreciably differed from that of the same parts attached to salt-treated plants. The most noticeable differences in ion accumulation under salinity between detached and attached parts are as follows:

1. Na⁺ accumulation. The difference between the wild and the cultivated species with respect to Na⁺ accumulation under salinity was smaller in detached as compared with attached leaves and apices.
2. Cl⁻/Na⁺. The ratio was around 1 in detached parts in both wild and cultivated plants. In attached leaves and apices, it was close to 1 only in the cultivated plants, being much lower in the wild species.
3. K⁺ accumulation. Under salinity, a larger decrease of K⁺ was found in detached leaves and apices than in attached ones in both wild and cultivated species, except for Sp leaflets.
4. K⁺/Na⁺. In all species, the ratio was smaller in detached than in attached parts; the difference was the greatest in VF and the smallest in Sp.

The balance of ions in the leaf and the shoot apex may be determined by the net uptake into the root, the transport from the xylem parenchyma of the root to the vessels, the transport from the shoot xylem to the cells surrounding it, and the rate of retranslocation of ions downward to the root (Lauchli, 1972). The discrepancy in ion balance between detached and attached leaves and apices probably results from their being removed from the major sites controlling the balance of ions in the whole plant.

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Table 1. Chlorophyll content (A_{665} /mg dry weight) in leaflets excised from VF, Lp, and Sp plants cultured on control and saline media. Numbers in brackets represent chlorophyll content as percentage of reading at zero time. Standart error is in the range of ± 0.01 to ± 0.07 .

Species	Discs at zero time*	Cultured discs		
		Control	NaCl (%)	
			1.5	2.0
VF	0.47	0.39 (81)	0.23 (48)	0.25 (53)
Lp	0.41	0.33 (81)	0.12 (30)	0.17 (42)
Sp	0.39	0.33 (86)	0.14 (37)	0.17 (43)

* Chlorophyll content was determined in a sample of discs at the time culturing was initiated.

Table 2. Ion content (mmol g dry weight⁻¹) in leaf discs of VF, Lp, and Sp plants cultured on control (C) and 1% NaCl (S) medium.

Species	Na ⁺		Cl ⁻		K ⁺		% of decrease
	C	S	C	S	C	S	
VF	0.17±0.03	2.20±0.13	0.13±0.05	2.09±0.48	0.94±0.05	0.40±0.04	57.5
Lp	0.37±0.04	3.02±0.96	0.29±0.01	4.18±0.27	0.77±0.07	0.28±0.38	63.6
Sp	0.18±0.02	3.32±0.40	0.17±0.08	3.62±0.19	1.14±0.06	0.68±0.14	40.6

Table 3. Ion content (m mol.g dry weight⁻¹) in leaflets of VF, Lp, and Sp plants cultured on control (C) and 1.55 NaCl (S) medium.

Species	Na ⁺		Cl ⁻		K ⁺		% of decrease
	C	S	C	S	C	S	
VF	0.23±0.02	3.55±0.22	0.01±0.00	3.51±0.23	0.92±0.24	0.63±0.03	31.5
Lp	0.37±0.08	3.88±0.15	0.08±0.04	3.50±0.15	1.38±0.04	0.54±0.06	60.9
Sp	0.29±0.02	4.77±0.28	0.06±0.01	6.11±0.30	0.93±0.25	0.76±0.00	18.3

Table 4. Growth (mg dry weight) of shoot apices of VF, Lp, and Sp plants cultured on control and saline media.

Species	Control	NaCl (%)			
		1.5	% of control	1.8	% of control
VF	36.60±0.17	0.02±0.00	0.0	0.52±0.05	1.4
Lp	24.80±0.16	6.01±1.09	24.2	2.58±0.27	10.4
Sp	26.33±0.09	17.00±1.56	64.5	8.26±1.52	31.4

Table 5. Ion content (mmol.mg dry weight⁻¹) in shoot apices of VF, Lp, and Sp plants cultured on control (C) and 1.5% NaCl (S) media.

Species	Na ⁺		Cl ⁻		K ⁺		% of decrease
	C	S	C	S	C	S	
VF	0.17±0.04	3.55±0.11	0.03±0.00	3.50±0.13	1.38±0.09	0.87±0.32	37.0
Lp	0.45±0.06	3.92±0.22	0.08±0.04	3.50±0.50	1.58±0.62	0.93±0.14	41.1
Sp	0.42±0.11	5.08±0.46	0.00	5.01±0.44	2.10±0.20	1.13±0.06	46.2

Table 6. K^+/Na^+ and Cl^-/Na^+ ratio in NaCl-treated cultured leaf discs, leaflets and shoot apices of VF, Lp, and Sp plants, and in attached leaves and apices of salt-treated VF, Lp and Sp plants. * values of attached parts were taken from Tal and Shannon's (1983) work, which was conducted under conditions similar to those of the present work.

Species	Leaves			Apices	
	Cultured discs	Cultured leaflets	Attached*	Cultured	Attached*
K^+/Na^+					
VF	0.18	0.18	10.00	0.24	4.70
Lp	0.09	0.14	0.80	0.24	1.00
Sp	0.20	0.16	0.40	0.22	0.50
Cl^-/Na^+					
VF	0.95	0.99	1.10	0.99	0.70
Lp	1.38	0.90	0.20	0.89	0.20
Sp	1.09	1.28	0.20	0.99	0.10

Figure legend

Fig. 1. Survival of leaf discs excised from VF, Lp, and Sp plants as function of NaCl concentration in the medium.

VF _____; Lp.....; Sp _____;

Effects of High Temperature, Dehydration and Osmotic Pressure
on the Stability of Leaf Membranes of Lycopersicon esculentum
L. cheesmanii, L. peruvianum, and Solanum pennellii

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ABSTRACT

The stability of leaf membranes under heat, dehydration, and osmotic stress was studied in summer- and winter-grown plants of the cultivated tomato Lycopersicon esculentum and its wild relatives L. cheesmanii, L. peruvianum, and Solanum pennellii. The stability was assessed by determining the integrity of naked leaf protoplasts under heat stress and in mannitol solution of decreasing molarity and the leakage of electrolytes from leaf discs exposed to heat and dehydration stress. In all species, especially in the wild ones, the membrane was found to be less stable under stresses in the winter than in the summer. With some exceptions, the membrane of the wild species appeared to be less stable than that of the cultivated one.

Introduction

The present biochemical and physiological knowledge of the mechanisms controlling stress resistance of plants suggests that the membrane is one of the main cellular targets common to different stresses (Levitt, 1980). It was shown that increased degree of saturation of membranal lipids is correlated with increased heat tolerance of plants, while increased unsaturation is correlated with adaptation to low temperature. The knowledge of the effects of drought on plant membranes is very limited. Leakage studies indicate that some changes occur in the structure of membranes during dessication, some of these changes being explained by lipid peroxidation (Bewley, 1979; Leopold et al., 1981). Some facts propose that ion transport, which is one of the key characteristics related to salt resistance, is controlled by the lipids of the membrane (Kuiper and Kuiper, 1978; Erdei et al., 1980).

Since the membrane may have a central role in the cellular response and tolerance to stresses, as suggested by Levitt (1980), the extent of its damage is commonly used as a measure of tolerance to various stresses in plants, such as freezing (Dexter, 1956), heat, drought (Blum and Ebercon, 1981), and salt (Leopold and Willing, 1983).

The wild salt-tolerant relatives of tomato, Lycopersicon cheesmanii, L. peruvianum, and Solanum pennellii, used in our laboratory, evolved in dry areas in South America. Such areas are characterized by high salinity and long periods of drought, which coincide with high temperatures (Epstein, 1972). The wild relatives of tomato, may have, therefore, mechanisms to resist such stresses. The possibility that these species, which have common characteristics of salt tolerance (Tal, 1971; Dehan and Tal, 1978; Rush and Epstein, 1976), are also tolerant to heat and drought stress was examined in the present work.

The effect of dehydration and heat stress on leaf membranes of the

cultivated tomato L. esculentum, and its wild relatives L. cheesmanii, L. peruvianum and S. pennellii was studied by measuring the leakage of electrolytes from treated leaf discs. The study also included the effect of decreasing external osmotic pressure and of high temperature on the stability of naked protoplasts isolated from leaves of L. esculentum, L. cheesmanii and L. peruvianum. The latter technique is similar to that used by Livne, Kuiper and Meyerstein (1972), who found that the osmotic stability of erythrocytes isolated from heat-acclimated hamsters increased in correlation with the increase in the degree of unsaturation of the membranal lipids. Naked plant protoplasts, in analogy to animal cells, were suggested as a very suitable system for the study of the involvement of the surface membranes to freezing injury due to the absence of cell wall (Wiest and Stiponkus, 1978). Ruesink (1971) proposed that the maintenance of the spherical shape of naked protoplasts can be used as an easily observed bioassay for the changes in the structure of the plasma membrane of living cells.

Materials and Methods

Plant material. The plants used in the present investigation included the cultivated tomato Lycopersicon esculentum cultivar 'VF 234' (VF) and its wild relatives L. peruvianum (Lp), L. cheesmanii ecotypes no. 1400 (Lc 1400) and 1401 (Lc 1401), and Solanum pennellii (Sp). Seedlings were prepared as described by Tal and Shannon (1983). Seedlings bearing 1-2 leaves were transferred to aerated Hoagland solution in the greenhouse with day/night temperatures of 20/10°C during winter and 30/20°C during summer.

Leaf-disc tests

Drought tolerance test. The method used in the present work was adapted with some modifications from Blum and Ebercon (1981). Leaf discs of 10 mm diameter were punched out of the upper fully-expanded leaves of summer- and

winter-grown plants of the cultivated and wild species and washed with de-ionized water to remove electrolytes adhered to the surface. Five disc samples were placed in Erlenmeyer flasks containing 10 ml solution of 35 and 40% polyethylene glycol 6,000 (PEG) or 10 ml of de-ionized water for 24 hr under temperature of 10°C. The discs were then rinsed with de-ionized water and transferred to 10 ml de-ionized water for 24 h under the same conditions. The remaining steps were performed exactly as described by Blum and Ebercon (1981). The extent of injury caused to the leaf discs by dehydration or high temperature was assessed from the change in the leakage from the discs:

$$\% \text{ Injury} = 1 - [1 - (T_1/T_2) / 1 - (C_1/C_2)] \times 100$$

where T_1 and C_1 signify the initial conductivity of the medium of treatment and control groups, respectively, and T_2 and C_2 the respective final conductivity.

Heat tolerance test. The procedure employed was as above except that all samples were placed in 10 ml of de-ionized water and heated for 1 hr at 45 or 50°C in a reciprocal shaker at a frequency of 40 cycles/min.

Naked-protoplast tests

Protoplasts were isolated from leaves of plants bearing 4-5 leaves by a method described by Tal and Watts (1979) with the following modifications: 1) the leaves were not sterilized; 2) the concentrations of pectinase (Macerozyme R10) were 0.125% (w/v) for VF and Lc and 0.25% for Lp; 3) the incubation with 1% cellulase (Onozuka R10) lasted 60 min instead of 90 min. Leaf-disc samples, 250 mg fresh weight, were incubated in 20-ml enzyme solution in a 125-ml Erlenmeyer, two for each species. At the end of the incubation period, the content of the two Erlenmeyers was combined in a conical tube, centrifuged at 100g for 3 min, and the supernatant was discarded. Thirty ml of 0.5 M mannitol solution was then added to each tube, and protoplast concentration was determined using a haemocytometer.

Osmotic tolerance test. Samples of protoplast suspension of known concentration were distributed into conical tubes and centrifuged. After discarding the supernatant, mannitol solutions of different molarity were added to the tubes. After five min, the number of protoplasts remaining intact and spherical was counted.

Heat tolerance test. Thirty-ml samples of protoplast suspension of known concentration were centrifuged, the supernatant was discarded, and 0.5 M mannitol solution was added to each tube. The tubes were then incubated in water bath at 50°C. Protoplasts remaining intact and spherical were counted. Protoplasts of Sp plants were not included in the present experiments since they were destroyed immediately upon their separation from the leaf.

Results

Stability of leaf discs

Effect of external osmotic pressure. Treatment of leaf discs isolated from leaves of summer- and winter-grown plants of the cultivated and the wild species with solutions of 35 and 40% PEG caused an increase of leakage of electrolytes from them (Table 1). Discs derived from winter-grown plants of VF were injured more as compared with discs excised from summer plants. No seasonal difference was found in the leakage of discs of the wild species Lc 1401 and Sp. Leakage increased, however, during winter in discs of VF, Lc 1400 and Lp. Discs of the wild species Lc 1400, Lp, and Sp were more leaky than those of the cultivated species during summer. During winter, the difference between the cultivated and the wild species was less noticeable.

The possibility that electrolytes located outside of the cell contribute to the differences in leakage between the species was examined by comparing the leakage from discs immersed in de-ionized water or in a solution of 10 mM CaSO_4

under room temperature. In all species no difference was found between the two media.

Effect of high temperatures. In all species the leakage at 45°C was higher in discs taken from winter plants than in those taken from summer plants (Table 1). A similar tendency was found in discs of Lp and Sp at 47.5°C and in those of VF at 47.5 and 50°C. Discs of both summer or winter plants of Lc 1400, Lc 1401, and Sp were more leak than those of VF under all three temperatures. No seasonal difference was found between the leakage of VF and Lp discs.

Stability of naked protoplasts

Effect of external osmotic pressure. The osmotic stability of protoplasts isolated from leaves of summer- or winter-grown plants of VF, Lc1400, and Lp was tested by incubating them in mannitol solutions of decreasing molarity (Table 2). The protoplasts of the wild species were more stable than those of the cultivated tomato during summer and less stable during winter. In the wild species, the protoplasts isolated from summer-grown plants were much more stable than those isolated from winter-grown plants. The stability of the protoplasts of the cultivated tomato was about the same in the two seasons.

Effect of high temperature. The thermal stability of protoplasts isolated from leaves of summer- and winter-grown VF, Lc1400 and Lp plants was tested at 50°C (Fig. 1). Protoplasts of wild species were much less stable to heat shock than those of the cultivated tomato during winter but of similar stability during summer. Similarly to their behavior under decreasing external osmotic pressure, the protoplasts isolated from the winter-grown wild species were much less stable at the high temperature than those isolated from the summer-grown wild plants. The stability of the cultivated tomato protoplasts almost did not differ in the two seasons.

Discussion

The membrane is considered to be one of the main cellular targets common to different stresses (Levitt, 1980). In the present work we queried whether salt-tolerance is positively correlated with membrane tolerance to heat and dehydration stress. For that purpose, the stability of leaf membranes to heat, dehydration, and osmotic stress was determined in summer- and winter-grown plants of the cultivated tomato L. esculentum (VF) and its salt-tolerant wild relatives L. cheesmanii (Lc 1400, Lc 1401), L. peruvianum (Lp), and S. pennellii (Sp), which were evolved in arid areas.

The sensitivity to stress of membranes of winter-grown plants was generally higher than that of membranes of summer-grown plants. A noticeable exception to this generalization was the response of VF protoplasts, which behaved similarly in the two seasons.

The wild and cultivated plants differed in the stability of their membranes under the stresses studied. An exception was the similar response of VF and Lp discs to heat stress. In most of the tests, the membrane of the wild plants was less stable than that of the cultivated ones. The only exception was the higher stability of protoplasts isolated from summer-grown plants of Lc 1400 and Lp exposed to decreasing mannitol molarity.

In view of the direct relationship found in various plants between the degree of saturation of membrane fatty acids and temperature (Kulper, 1970 and 1972; Yamada et al, 1980), the seasonal differences in the thermal and osmotic stability of protoplasts and in the permeability of cell membranes in leaf discs may result from seasonal changes in the degree of saturation of fatty acids in the phospholipids of the cell membranes of these plants. This suggestion is also in keeping with Levitt's thesis (1980) that an increase in lipid saturation is related to improved thermostability of plants. The different response to

external stresses of membranes of the wild and cultivated species may also result from differences in the degree of saturation of lipids. This possibility remained to be elucidated.

Since the wild salt-tolerant relatives of tomato originated in arid areas, which are characterized by drought and long periods of high temperatures, they were expected also to be tolerant to heat and dehydration stress. The results of the disc tests, and especially those with Sp, do not support this expectation. In agreement with these results which indicate that the wild plants are less tolerant to drought than the cultivated ones, Tal et al (1979) found that Sp plants were more sensitive to dehydration stress caused by nonpenetrating osmoticum than the cultivated plants. They concluded that the former plants survive under drought conditions due to their ability to accumulate salt ions, which are used as intracellular osmoticum and thus, they avoid dehydration.

The selection of salt-resistant plants in large segregating populations can be enhanced by applying screening tests which are rapid and easy to apply. The application of the disc test, which is rapid and simple, for distinguishing between heat- or drought-tolerant plants was suggested in sorghum and wheat (Blum and Ebercon, 1931). Whether the negative correlation found between the salt tolerance and heat or dehydration tolerance in the tomato species can be used in a disc test for screening salt-tolerant plants is still an open question. This proposition is dependent on the co-inheritance of the traits, i.e. high salt tolerance and high sensitivity to heat or drought stress.

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Table 1. Dehydration and heat injury of membranes of discs excised from summer- and winter-grown cultivated (VF) and wild (Lc 1400, Lc 1401, Lp and Sp) tomato plants

	Dehydration injury (%)				Heat injury (%)					
	% PEG				Temperature (°C)					
	Summer		Winter		Summer		Winter			
	35	40	35	40	45.0	47.5	50.0	45.0	47.5	50.0
VF	66.5±2.7	66.7±1.7	69.0±1.6	76.2±2.4	13.8±0.8	28.1± 4.2	72.2±0.8	21.5±3.2	49.5±4.0	79.6±1.0
Lc 1400	70.9±2.2	74.3±1.3	69.9±4.0	73.3±5.2	24.1±3.2	67.7± 6.7	80.7±2.5	57.0±6.3	67.5±2.5	83.8±1.7
Lc 1401	68.3±2.5	69.2±2.9	73.1±3.2	73.3±5.7	35.2±2.6	74.0± 2.0	81.0±0.8	65.9±4.7	69.4±5.3	81.7±0.4
Lp	70.9±2.1	73.1±2.1	72.3±7.8	88.7±4.6	13.9±1.1	33.6±12.2	74.8±5.1	27.3±4.1	51.1±3.0	70.2±3.5
Sp	74.6±4.1	79.8±1.0	79.0±3.9	77.0±4.8	31.2±2.2	67.4± 3.0	90.8±1.0	72.2±3.7	88.8±1.0	86.8±2.4

Table 2. Percentage of intact spherical protoplasts isolated from summer- and winter-grown VF, Lc 1400; and Lp plants after five minutes in mannitol solutions of different molarity.

Mannitol (M)	% Intact spherical protoplasts					
	VF		Lc		Lp	
	Summer	Winter	Summer	Winter	Summer	Winter
0.50	100.0	100.0	100.0	100.0	100.0	100.0
0.40	- ^a	78.6	-	81.8	-	90.6
0.35	65.0	69.6	78.3	55.0	70.7	71.3
0.30	50.0	53.0	69.7	30.2	71.4	27.0
0.25	40.7	-	59.4	-	55.7	-

^a not determined

Fig. 1. Percentage of protoplasts, isolated from winter(A)- and summer(B)-grown VF, Lc 1400 and Lp plants, remaining intact and spherical under heat of 50°C. VF_____ ; Lc.....; Lp-----.

Salt tolerance in the wild species of the cultivated tomato:
Growth, succulence, and accumulation of Na^+ , K^+ and Cl in NaCl -
treated F_2 hybrids between Lycopersicon esculentum x Solanum pennellii

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Abstract

The elongation rate of the main stem as well as succulence and accumulation of Na^+ , K^+ , and Cl^- in the root, stem, leaf, and shoot tip were studied in NaCl-treated F_2 hybrids derived from selfed F_1 (Lycopersicon esculentum x Solanum pennellii) plants. The latter species is a salt tolerant wild relative of the cultivated tomato.

Similar frequency distributions of ion accumulation as well as succulence were found in the various parts of the plants. In contrast to distribution curves of Na^+ , K^+ , Cl^- and succulence which were more or less typical of quantitative characters, that of K^+ were skewed toward low concentration. The skewness suggests that low accumulation of K^+ , which is characteristic of the wild parent, is dominant over high accumulation.

Contrary to expectation, no correlations were found in the F_2 plants between growth rate and any of the other parameters studied except a low negative correlation between growth and sodium accumulation in the shoot-tip and a positive correlation between growth and succulence in the old leaf. Sodium and potassium levels were found to be negatively correlated in the shoot, leaf, and top. Succulence was positively correlated with Na^+ as well as with Cl^- in all parts.

Introduction

The wild relative of tomato Solanum pennellii was found (Dehan and Tal, 1978) to be more tolerant to salt than the cultivated species Lycopersicon esculentum, and a greater osmotic adjustment has been suggested as an explanation for this higher tolerance. The wild plants accumulated more Na^+ and Cl^- and less K^+ , and their growth decreased relatively (salt-treated as compared with the control) less than that of the cultivated species under salinity.

The dominance relations between the two species for the rate of stem elongation, succulence, and accumulation of Na^+ , K^+ , and Cl^- was studied in leaves of control and NaCl-treated F_1 hybrid plants derived from the cross L. esculentum x S. pennellii (Tal and Shannon, 1983). The results demonstrated the existence of heterosis for stem elongation rate and complete dominance of S. pennellii over the cultivated parent with respect to the relative decrease of growth rate and K^+ content and for the increase of succulence under salinity.

In the present work we describe the response to salinity of the F_2 hybrid plants derived from selfed F_1 plants. Such a study may contribute more information on dominance relationships as well as on possible correlations between growth rate and other attributes which might be essential for salt tolerance. The characteristics studied include elongation rate of the main stem, succulence, and accumulation of Na^+ , K^+ , and Cl^- in the root, stem, old and young leaves, and top of the plant.

Materials and Methods

Plant Material

The F_2 hybrid plants were obtained by selfing plants of F_1 derived from

the cross Lycopersicon esculentum cultivar 'VF 234' (VF) x Solanum pennellii accession Atico (Sp), the cultivated plant being used as the maternal parent.

Plants were grown in the greenhouse during summer with maximum and minimum daily temperatures of 30/20°C. Seeds were germinated in vermiculite, and seedlings bearing 1-2 leaves were transferred to aerated Hoagland solution (20 seedlings per 22-liter container) as described by Tal and Shannon (1983). Thirty two plants of F₂ generation were treated with salt. Salt treatment was initiated at the 3-4-leaf stage by adding NaCl in 25 meq·L⁻¹·day⁻¹ doses up to a final concentration of 150 meq·L⁻¹. Control plants remained in Hoagland solution.

Elongation Rate of the Main Stem

The length of the main stem was measured on the day on which salt was first added (day 0) and then at 3-day intervals to day 15. Stem growth was expressed by the elongation at each time interval as a percentage of the length at day 0. Elongation rate was calculated from the slope of the regression line between stem growth (Y) and time (X).

Succulence and Accumulation of Ions

Succulence and ion content were determined 12 days after the last addition of salt in the top of the plant (including the meristem and very small leaves), the youngest fully-expanded and the oldest leaves, the middle section of the stem and the proximal part (about 20 mm) of the main root. Other details were exactly as described by Tal and Shannon (1983).

Results and Discussion

Frequency Distributions

The distribution of NaCl-treated F₂ plants varied with respect to the

rate of stem elongation (Fig. 1A) and accumulation of ions (Na^+ , Cl^- , and K^+) and succulence. Both ion accumulation and succulence showed a similar pattern of distribution in the different parts studied, i.e. root, stem, old and young leaf, and top and were represented, therefore, by the young leaf (Figs. 1B to 1E). While the distribution of growth rate, succulence, and accumulation of Na^+ , Cl^- was more or less typical of quantitative characters, that of K^+ accumulation is skewed to the left (low accumulation as in the wild parent Sp), especially in the old leaf. This skewness suggests that low accumulation of K^+ is dominant over high accumulation, in agreement with the results obtained in the F_1 (VF x Sp) hybrid (Tal and Shannon, 1983). Further crosses will be performed to test the hypothesis that the difference between the wild and the cultivated tomato with respect to K^+ accumulation is controlled by a small number (may be only one) of genes.

Since each individual in the F_2 hybrid population is genetically unique, it was impossible to determine the effect of salinity on each of the genotypes relatively to its expression under control conditions as done in the highly homogeneous homozygous parental (VF and Sp) and F_1 (VF x Sp) populations (Tal and Shannon, 1983). Such a comparison in the F_2 generation could be made only if the individual plants were propagated vegetatively. The effects of salinity are, therefore, expressed in the present work only in absolute terms. The high succulence characteristic of the Sp parent was found dominant in the F_1 (VF x Sp) hybrid only when described in relative and not in absolute terms (Tal and Shannon, 1983). The use of absolute values instead of relative may explain the lack of skewness of succulence towards high values in the distribution of the F_2 plants.

Correlations

A positive correlation was expected between growth rate and Na^+ accumulation in F_2 plants growing under salinity since high accumulation of Na^+ and high relative growth rate characterize the Sp parent under these conditions (Dehan and Tal, 1978; Tal and Shannon, 1983). The higher accumulation of Na^+ in the wild plants was explained as an expression of the osmotic adjustment in these plants under salinity. A better osmotic adjustment in the wild plants was suggested as an explanation for their superior performance under salinity.

Contrary to our expectation, no correlation was found between growth rate and accumulation of Na^+ in the F_2 population under salinity except for a significant low negative correlation between growth rate and Na^+ content in the top (Table 1). No correlation was also found between growth rate and either accumulation of Cl^- and K^+ or succulence. Positive correlations were, however, found between succulence and either Na^+ and Cl^- level. A relation between Na^+ and succulence was demonstrated in many plants (Rains, 1972). A negative correlation was found between Na^+ and K^+ levels, in agreement with the partial substitution of K^+ by Na^+ in plants of the wild parent Sp (Dehan and Tal, 1978; Tal and Shannon, 1983).

The meaning of the low but significant negative correlation found between growth rate and Na^+ content in the top is that F_2 plants tend to exclude this ion from the growing zone under salinity. The lack of correlation between growth rate and accumulation of Na^+ in the leaves or other parts of the plant in salt-treated F_2 plants means that fast growing plants can be either includers or excluders of Na^+ in these parts. The advantage of Sp, the wild parent of the F_2 plants, over the cultivated species is only relative. As compared with VF plants, those of Sp were

characterized under control conditions by a much lower absolute growth rate and under salinity of $\geq 75 \text{ meq} \cdot \text{l}^{-1}$ by a smaller relative decrease of the growth rate. Consequently, a somewhat lower absolute growth rate was evident (Tal and Shannon, 1983 and unpublished data). This situation probably results from the fact that the genotype of the wild species has been selected for maximal survival, which is not necessarily related to absolute growth, while the cultivated species was selected for maximal yield (Tal, 1983). Pasternak et al. (1979) suggested that yield in tomato is dependent on growth. The ability of the wild species to accumulate high sodium content under salinity seems, therefore, to be an essential component of the survival strategy of this species, which is probably dependent on low absolute growth rate. Plants having an absolute growth rate of 0.30-0.35 as compared with 0.07-0.10 in Sp and 0.12-0.15 in VF (Tal and Shannon, 1983) were found in the NaCl-treated F_2 population. The fact that fast growth rate is independent of Na^+ accumulation suggests that the ability to accumulate high Na content is not a prerequisite for absolute success under salinity in the tomato. These successful F_2 plants are recommended, therefore, as a source for germ plasm for absolute high salt tolerance and as experimental objects for a better understanding of the mechanisms which might be responsible for high absolute growth rate and consequently high absolute yield in tomato under salinity.

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Table 1. Correlations between growth rate (Gr) and the level of Na^+ , K^+ , Cl^- , and succulence (Su) and between succulence and Na^+ and Cl^- in various parts of NaCl-treated ($150 \text{ meq} \cdot \text{g}^{-1}$) F_2 hybrid plants derived from selfed F_1 (VF x Sp).

Correlation					
between	Root	Stem	Leaf		Top
			Old	Young	
(Correlation coefficient, r)					
Gr/Na ⁺	-0.17	0.31	0.24	-0.34	-0.36*
Gr/K ⁺	0.24	0.18	-0.07	0.09	0.16
Gr/Cl ⁻	0.05	-0.02	0.15	-0.20	-0.22
Gr/Su	0.02	0.33	0.44*	-0.25	-0.16
Na ⁺ /K ⁺	0.23	-0.48*	-0.38*	-0.63*	-0.68*
Su/Na ⁺	0.70*	0.87*	0.66*	0.80*	0.65*
Su/Cl ⁻	0.85*	0.74*	0.67*	0.88	0.64*

*Significant at 0.95 level

Figure Legend

Figure 1. Frequency distribution of growth rate (A), content of Na^+ (B), Cl^- (C), and K^+ (D), and succulence (E) in the youngest fully-expanded leaves of NaCl-treated ($150 \text{ meq} \cdot \text{L}^{-1}$) F_2 hybrid plants derived from selfed F_1 (VF x Sp) plants. The numbers represent the means of NaCl-treated parental and F_1 generations as given by Tal and Shannon (1983).

Evaluation of the Research Achievements with Respect to the Original Research Proposal

In accordance with the original proposal, we prepared seeds of F_1 and F_2 generations. These plants included the following:

F_1 - H x Lc 1400; H x Lc 1401; H x Sp; H x Lp; VF x Lc 1400; VF x Lc 1401; VF x Sp; VF x Lp.

F_2 - Selfing of all the above F_1 s together with the crossing of F_1 (H x Lc 1400) x F_1 (H x Sp); F_1 (H x Lc 1401) x F_1 (H x Sp); F_1 (VF x Lc 1400) x F_1 (VF x Sp); F_1 (VF x Lc 1401) x F_1 (VF x Sp).

Back crosses between selected hybrids and the cultivated parent were not performed. Based on the recommendations of the original reviewers we performed only a limited research on naked protoplasts.

The response of hybrids to salinity was studied only in F_1 (VF x Sp) and F_2 (VF x Sp) plants, which were grown in liquid culture salinized by NaCl. The performance of these plants was evaluated by criteria related to vegetative growth. The response of the parental species to salt was studied in plants grown in liquid culture salinized with NaCl or NaCl + CaCl_2 or in sand salinized with NaCl + CaCl_2 . The performance of these plants was evaluated by criteria related to vegetative or reproductive characteristics. The latter were studied only preliminarily and only in plants growing in sand.

The research also included comparison of salt tolerance in seven accessions of S. pennellii. The accession Atico was found to be a good representative of the species in this respect.

As originally proposed, we continued the study of the mechanism of salt tolerance in Lp and Sp and also included both ecotypes of Lc. Various physiological aspects which may contribute to salt tolerance were studied. These included: accumulation of Na^+ , Cl^- , and K^+ , as well as succulence in different parts of the

plant, uptake of Rb^{+} by excised root tips, comparison of the response of excised differentiating shoot tip and fully differentiated leaves to salt, profile of ^{14}C distribution in different fractions of the leaf under control and saline conditions, resistance of leaf membranes to heat and drought stresses, stomatal behavior, water, osmotic and turgor potentials in the leaf, respiration, ion compartmentation, and ATPase activity in the root. Some of these aspects were also studied in the cultivated potato and three of its wild relatives.

The main contributions of this wide-scope research seem to be the following:

Genetic contributions

1. The preparation of seeds of various combinations of F_1 and F_2 generations, the cultivated tomato species and each of its wild relatives - Lp, Sp and Lc (ecotypes 1400 and 1401), as well as hybrid combinations containing genetic contributions of three species - the cultivated one, Sp and Lc.
2. The demonstration that the inheritance of the mechanism controlling K^{+} level in VF and Sp plants is probably controlled by a small number (possibly one) of genes.
3. The demonstration of the existence of a potential source of genes for salt tolerance in the wild relatives of the cultivated potato - S. kurzianum, S. polytrichon, and S. polyadenium.

Physiological contributions

1. The demonstration that high growth rate and accumulation of Na^{+} under salinity in tomato are not necessarily correlated.
2. The demonstration that the growing zone, which contains actively dividing and growing cells, has a central role in the tolerance of whole tomato plants to salt.

Based on these and other findings, it is possible to define a few central subjects and questions which are recommended for further study:

1. The response of the growing zone of the shoot to salt with respect to the synthesis of macromolecules, accumulation of organic osmotica, and hormone balance.

2. The elucidation of the causes of the low absolute growth rate (and consequently low final size) of the wild plants under both control and saline conditions. The lower absolute growth rate of the wild plants may result from a higher energy price invested in these plants for maintenance and/or operation of the salt tolerance mechanisms, or it may be, relatively, independent of these aspects.

3. The role of the membrane to salt tolerance, including stability of membranes under salinity, lipid composition and ATPase activity.

ABSTRACT

The behavior under salt stress was studied in the cultivated tomato Lycopersicon esculentum cultivars 'VF 234' (VF), 'Heinz 1350' (H), and 'Walter' (W) as compared with its wild relatives L. cheesmanii ecotypes 1400 (Lc 1400) and 1401 (Lc 1401), L. peruvianum (Lp), and Solanum pennellii accession Atico (Sp).

Lp and Sp plants were characterized by a lower relative decrease of : 1. growth rate under NaCl salinity in liquid culture; 2. dry and fresh weight when treated with mixed salt solution (NaCl + CaCl₂), applied for a long time to either liquid or sand cultures. Neither of the Lc ecotypes has proven to be more tolerant than the test cultivar in any of the experiments.

The wild plants accumulated more Na⁺ and less K⁺ and had lower Cl⁻/Na⁺ and K⁺/Na⁺ ratios in most of their parts under salinity. The uptake of Rb⁺ (representing K⁺) by excised root tips was lower in the wild species in both control and saline solutions. Although each of the species seems to possess a different pattern of salt uptake and distribution, all 4 species maintained a low salt level in the growing zone of the shoot. The growing zone seems to have a central role in the salt tolerance of the whole plant, since the response of excised shoot tips to salt was positively correlated with that of the whole plant. The profile of ¹⁴C distribution in different fractions (neutral, cationic and anionic) of the leaf was found to be different in VF as compared with Lp and Sp¹ under both control and saline conditions. Contrary to our expectation, leaf discs of the wild species were found to be more leaky under both heat and drought stress, thus suggesting a greater membranal sensitivity to stress in these species. The results obtained from the study of F₁ (VF x Sp) and F₂ (VF x Sp) generations suggest that a small number (possibly one) of genes control the difference in K⁺ level between VF and Sp plants. No correlation was found between growth rate and Na⁺ level in the F₂ plants. Additional aspects of

the work, not yet completed or are only preliminary, include the study of dry weight in different parts of the plant, stomatal behavior, water, osmotic, and turgor potentials in the leaf, maintenance respiration, compartmentation of ions, and root ATPase activity.

The study also included the cultivated species of potato and three of its wild relatives S. kurzianum, S. polytrichon, and S. polyadenium. Based on this study the wild species seem to be a potential source for genes for salt tolerance and possibly also for drought tolerance.

This wide-ranging work enabled us to define a few central subjects and questions on which to concentrate in a future study.

1. The original proposal (1979) - a summary

Genetic variability. Relatively little is known about the heritable variability of salt tolerance in the important cultivated crop plants or their wild relatives. One of the first demonstrations of variability was in Agropyron (Dewey, 1962). Experiments seeking varietal differences in salt tolerance have been conducted on carrot (Bernstein and Ayers, 1953a) lettuce (Ayers, et al., 1951), onion (Bernstein and Ayers, 1953b), green bean (Bernstein & Ayers, 1951) and tomato (Pasternak et al., 1977). Certain varietal differences were found under moderate salinities but under high salinity all varieties showed similar damage. It seems, therefore, as if the genetic variability for high salinity tolerance in some species is too narrow for effective selection (Bernstein, 1961; Epstein, 1972). Lycopersicon is one of the few genera of crop plants in which genetic variability for salt tolerance has been demonstrated in the wild species. Three wild tomato species, L. peruvianum (Tal, 1971; Tal et al., 1978), Solanum pennellii (Dehan and Tal, 1978) and L. cheesmanii (Rush and Epstein, 1976) were found to be more tolerant to salinity than the cultivated one. A relatively extensive physiological research has been undertaken during recent years on the mechanisms of salt tolerance in these species.

Mechanisms of salt tolerance. Very little is known on the mechanisms of salt tolerance in wild species relatives of important crop plants (Maas and Nieman, 1978). However, in tomato, Tal (1971) and Dehan and Tal (1978) have suggested that better osmotic adjustment is responsible for the superior performance of the wild species L. peruvianum and S. pennellii under NaCl salinity. The wild plants accumulated more Cl^- and Na^+ and less K^+ , they were more succulent, and their relative water content and total dry weight decreased less as compared with the cultivated tomato. Similar differences were found between the callus

tissues derived from leaves, stems and roots of L. peruvianum and S. pennellii and the cultivated tomato species (Tal et al., 1978). Rosen and Tal (1981) also found that protoplasts of L. peruvianum were more tolerant to salinity than those of the cultivated tomato. Similar findings on the level of the whole plant were reported for another wild species of tomato, L. cheesmanii, by Rush and Epstein (1976). The higher Na^+/K^+ ratio which characterizes the wild tomato species is typical of other salt tolerant plants belonging to other families (Walsel, 1972; Greenway and Munns, 1980).

Based on various observations (Greenway and Osmond, 1972; Weimberg, 1975; Flowers et al., 1977), it was suggested that the high Na^+ accumulated in tolerant plants is probably not in contact with the metabolic apparatus within the cytoplasm, but instead, is sequestered in the vacuole. In such plants the osmotic balance between the vacuole and the cytoplasm is maintained by the accumulation of organic solutes in the latter compartment. Proline, which is accumulated during salt stress (Chu et al., 1976), has been implicated as one of the possible cytoplasmic osmotica. Results presented by Tal et al., (1979) suggest that proline does not play an important role in the salt tolerance of L. peruvianum or S. pennellii since under salinity stress less proline was accumulated in their leaves than in those of the cultivated species.

Kylin and Kahn (1973) suggested that $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ which is located in the plasmalemma of salt-sensitive sugarbeet plants is responsible for the uptake of K^+ and the exclusion of Na^+ from the cell to the outer medium, while the ATPase located in the tonoplast in the salt-tolerant variety is responsible for the exclusion of Na^+ from the cytoplasm to the vacuole. These and other possibilities will be tested in the wild tomato species.

Research plan

Conventional Breeding. F_1 and F_2 hybrid generations of crosses between selected cultivated varieties and S. pennellii and L. cheesmanii will be

produced. Plants will be grown in salinized sand cultures and their response to salinity will be determined based upon vegetative and reproductive development. Salt concentrations will be based upon threshold limits determined in preliminary experiments. Back crosses between selected hybrids and cultivated plants will be made.

Seeds of several wild species of potato originating in the dry habitats of South America have been obtained from Inter-regional Potato Introduction Project, Peninsular Experimental Station, Surgeon Bay, Wisconsin, U.S.A. Some of these species hybridized with cultivated potato. The responses of wild and cultivated species to salinity will be tested and tolerant species will be used for further studies. These studies will include breeding between the selected wild and cultivated plants and investigations on the mechanisms of salt tolerance on the level of the whole plant, isolated tissues and cells.

mechanisms of Salt Tolerance. The study of the mechanism of salt tolerance in L. peruvianum and S. pennellii will be continued. The fluxes of ^{22}Na and ^{86}Rb in excised roots will be determined by the methods used by Tal and Benzioni (1977) and Benzioni and Tal (1978). ATPase activity as affected by pH, temperature and Na^+ , K^+ , Mg^{2+} and Ca^{2+} in the roots of the different genotypes will also be studied by the method used by Morton, Tal and Benzioni (in preparation).

Expected Contribution of Research to Israel and the U.S.A.

Soil and water salinity is a world-wide problem which is increasing in severity annually. Both the U.S. and Israel will benefit from any progress in the adaptation of plants to high salinity.

We feel that this research will contribute to the basic understanding of the genetic and physiological factors controlling salt tolerance and accelerate and breeding of salt tolerant cultivars of other important crop plants. Through

these efforts saline waters and soils can be put into productive use and crops of higher cash value may be grown on marginal lands.

Time Schedule of Work Plan.

In the first year sexual hybrids of L. esculentum x L. chessmanii, L. esculentum x S. pennellii will be produced. During this time, parental types will be tested for salinity tolerance thresholds and specific biochemical and physiological characteristics as discussed in previous sections.

By the second year, sexual hybrids will be tested for salt tolerance and appropriate crosses will be made to produce F₂ generation plants. Biochemical and physiological tests will continue. The response of wild species of potato to salinity will be tested and the most tolerance species will be used for further studies. Hybrids of S. tuberosum x salt tolerant wild potato species will be produced.

In the third year, F₂ generation testing will be conducted. Somatic hybrid progeny will be tested to determine if genetic information has been transferred and is stabilized within the genome. Promising F₂ lines will be maintained for breeding research and further screening. If time permits, back crosses will be produced to improve agronomic quality. Experience accumulated from the biochemical and physiological studies and from the breeding work on salt tolerance in tomato model system will be proposed for further improvement in other species of the Solanaceae.

II. Results and achievements

A. Israel

Growth rate (GR)

The present investigation is a stage of a research program planned to improve the salt tolerance of the cultivated tomato, i.e. to minimize the decrease of its yield under salinity. Since yield production is a time and space

consuming process, and since, as suggested by Pasternak et al (1979), the yield of tomato is related to its GR in early stages of development, GR was adopted as the main attribute which represents plant performance. Additional complications related to yield measurements are: 1. the existence of self incompatibility genes in Lp; 2. the need for controlled hand pollination, especially in the wild plants in which the stigma is extruded outside of the pollen column.

Growth rate (its derivation is described in manuscript no. 1) of L. esculentum cultivar 'VF 234' (VF), L. cheesmanii ecotypes no. 1400 and 1401 (Lc 1400 and 1401), L. peruvianum (Lp), and Solanum pennellii accession Atico (Sp) were compared under various concentrations of salt (Fig. 1). The decrease of GR under salinity, relatively to the control, was greater in the cultivated plants which are greater in their absolute size than the wild ones. The final GR under salt concentrations of 75 meq/L or above was similar in wild and cultivated plants.

The major goal of this research is to transfer genes which control the mechanisms of salt tolerance from the wild species to the cultivated one in order to minimize the effect of salinity on GR (~~~~~ or ~~~~~ lines in Fig. 1) and consequently on yield in the latter. A central question in this research is whether the energy-price to be paid for the operation of the mechanisms of salt tolerance to be transferred to the cultivated plants is too high in terms of growth and yield. According to Nieman (1980), the energy required for the operation of the salt-resistance mechanisms is obtained mainly at the expense of growth. Such a knowledge can not be obtained by studying the mechanisms only in the wild species which are small in absolute size and produce small fruits under both control and stress conditions. These species were selected in nature for maximal survival, i.e. maximal production of seeds, which is not necessarily related to absolute size. In order to answer this question it is essential to

expand, in scope and depth, the knowledge and understanding of the components of the mechanisms of salt tolerance and the interrelations between the components and performance under salinity in hybrids between the wild and cultivated species.

Distribution of dry weight (DW) - a preliminary study

As found previously (Tal, 1971; Dehan and Tal, 1978), total plant DW decreased more in the cultivated than in the wild plants Lp and Sp under salinity. The decrease in all three species resulted only or mainly from the inhibition of shoot growth (Table 1).

Table 1. Dry weight of root, shoot, and leaf area in control and salt-treated VF, Lp and Sp plants.

	VF			Lp			Sp		
	C	S	% of control	C	S	% of control	C	S	% of control
	(g)								
Shoot	4.24±0.48	2.40±0.32	56.6	1.00±0.29	0.77±0.00	64.2	0.66±0.06	0.42±0.03	63.6
Root	0.54±0.09	0.67±0.08	124.1	0.18±0.03	0.23±0.03	127.8	0.16±0.02	0.13±0.02	81.2
Total	4.78±0.61	3.07±0.43	64.2	1.38±0.14	1.00±0.04	72.5	0.82±0.07	0.55±0.04	67.1
<u>Leaf</u>									
total	3.27±0.20	1.70±0.09	52.0	0.93±0.09 (mg)	0.57±0.04	61.3	0.54±0.03	0.42±0.02	77.8
per cm ² leaf area (calculated)	3.18	4.47	140.6	3.72	6.90	185.5	4.14	4.91	118.6
	(dm ²)								
area	102.9±153	38.0±2.5	36.9	25.0±8.0	8.3±0.4	33.1	13.0±2.9	8.6±1.5	65.6

The plant parts of the various species showed a differential response to salt; root DW increased in VF and Lp and decreased somewhat in Sp. The decrease of total leaf DW under salinity was smallest in Sp and greatest in VF. In all three species leaf DW/unit leaf area increased and total leaf area decreased under salinity; the increase of DW and decrease of total leaf area were smallest in Sp and greatest in Lp.

The study will be completed during the summer of 1983.

$^{14}\text{CO}_2$ fixation, distribution of ^{14}C and activity of RUBP carboxylase

Photosynthetic rate was measured as $^{14}\text{CO}_2$ incorporation into leaf discs of control and salt treated VF, Lp and Sp plants. The discs were exposed to $^{14}\text{CO}_2$ for 30 min under light of 1500 lux. The rate was not influenced by salinity in VF and Lp plants, but was reduced in Sp.

The distribution of ^{14}C after a pulse of 45 min was examined in detached leaves obtained from control and NaCl-treated plants (Fig. 2). A relatively greater proportion of the label was transferred to charged compounds in the wild species, comprising probably amino acids (cationic fraction), organic acids, sugar phosphates and nucleotides (anionic fraction). The anionic fraction tended to decrease in salt treated Lp and Sp plants. Starch fraction increased and protein fraction decreased under salinity in all three species, these changes being greatest in the cultivated species.

RUBP carboxylase activity was determined in control and treated plants of the three species. Preliminary results indicated that the activity is affected similarly by salinity in these species.

Present investigations include the measurements of: 1. the proportional quantities of the different fractions in the growing zone of the shoot of control and salt-treated plants exposed to labeled CO_2 ; 2. the activity of RUBP carboxylase in control and salt-treated plants of VF, Lp and Sp.

CONTROL , SALT-TREATED

Respiration

Respiration (growth and maintenance components) is being studied at present separately in roots and shoots of control and treated plants in cooperation with Prof. J. Gale from the Department of Biology, The Hebrew University of Jerusalem.

Stomatal distribution and behavior

Density, total area and circumference of stomatal aperture during day and stomatal opening at night were determined in leaves of control VF, Lp and Sp plants during the summer (Table 2).

Table 2. Density and opening (total aperture area and circumference/mm² leaf area) of stomata on the lower (L) and upper (U) leaf surfaces in the cultivated tomato VF and its wild relatives Lc 1400, Lp and Sp.

Species	Number/mm ² area			Aperture		Circum.(mm)		Opening during night (%)
	L	U	Total	Area(mm ²)		L	U	
				L	U			
VF	162.2	12.0	174.2	0.0134	0.0010	5.82	0.43	0
Lc	138.5	42.8	181.3	0.0099	0.0031	4.39	3.05	65.1
Lp	148.2	20.2	168.4	0.0147	0.0020	5.71	0.78	39.8
Sp	119.2	61.0	180.9	0.0085	0.0043	3.91	4.35	0

Stomatal density on the lower side of the leaf was highest in VF and lowest in Sp. The situation was opposite on the upper side. Total area of stomatal aperture per unit leaf area was lowest in Sp and highest in Lp. Total circumference of apertures per unit leaf area was higher in Sp and Lc than in the other two species. Many stomata in Lp and Lc remained open at night, while

those of VF and Sp were closed.

Stomatal movement during the day was determined in control and salt-treated plants using the Automatic Porometer LI-COR Model LI-65 (Figs. 3,4,5). Air temperature, relative humidity, and light intensity were determined, respectively, with an ordinary thermometer, a whorling hygrometer and a light meter LI-COR Model LI-185A (Figs. 6,7,8).

In all three species the stomata were more closed in the salt treated plants; the greatest difference between control and treated plants was found in VF. In all three species stomata were fully opened already at 8:30 AM when light intensity and temperature were maximal. Stomatal opening, as well as the relative humidity, decreased in all three species under salinity between 9:00 and 11:00 AM; the decrease being greatest in VF plants. In all three species stomatal closure lagged behind the decrease of light intensity in the afternoon. Stomata of salt-treated plants closed before those of the control plants.

Salt tolerance in various *S. pennellii* accessions

Seven different accessions of *S. pennellii*, including Atico, were compared for their salt tolerance. It was found that no one of the other six accessions was more tolerant than the Atico accession which represents *S. pennellii* species in our study.

Uptake of ^{86}Rb by root tips

The results of a study performed in the past are summarized in manuscript no.1. The research is being repeated at present because of the contradiction between our results and recent findings of Rush and Epstein (1981). They found that root tips of the wild species *L. cheesmanii* ecotype no.1401 absorbed more ^{86}Rb than those of the cultivated tomato species. We found that, on the contrary, root tips of the wild species Lc1400, Lp and Sp absorbed less ^{86}Rb in comparison with VF.

Accumulation of ions in different parts of the plant

The results are summarized in manuscript no.2.

The response of the plants to salt dilution in the root medium

The results are summarized in manuscript no.3.

Cellular compartmentation

Intracellular ion compartmentation is being studied by the method of Lerner and Reuveni (1982) in co-operation with Dr. Lerner from the Department of Botany, The Hebrew University of Jerusalem. This method requires a constant supply of isolated cells in suspension culture. Such cultures have already been prepared for Lp and Sp, and appropriate conditions for developing a suspension culture of VF are under investigation.

The role of the shoot growing zone in the salt tolerance of the plant

The results are summarized in manuscript no.4.

Screening tests

In order to differentiate between salt sensitive and resistant plants in large segregating breeding populations, it is essential to define a test which is rapid and easy to apply. Accordingly, we studied the response of young seedlings of VF, Lc, Lp, and Sp plants to various shock treatments. These treatments included: a. the transfer of seedlings grown in Hoagland solution without and together with 100 meq/L NaCl to the following solutions: a. Hoagland solution + various concentrations of NaCl (up to 600 meq/L); b. Hoagland solution + various concentrations of mannitol; c. distilled water; d. the spray of young seedlings with NaCl (0, 50, 100, 150 meq/L) solutions.

None of these treatments yielded a result which can be used as a test to differentiate between the cultivated (sensitive) and wild (resistant) plants.

Salt tolerance is a common characteristic shared by the wild species used

in the present study. Since these species evolved in arid areas they might also be tolerant to other stresses, i.e. dehydration and heat, which prevail in such areas. Salt and heat or dehydration tolerance might, therefore, be positively correlated in these species. Such a correlation can justify the use of tests developed in other plants for dehydration or heat tolerance, and which are easy to apply, as screening tests for salt tolerant plants. The results of our study of heat and dehydration tolerance tests in the wild species are summarized in manuscript no.5. The disc test of dehydration tolerance is being repeated using solutions with lower concentrations of polyethylene glycol.

Potato experiments

Eight wild species of potato which evolved in dry areas in South America were compared with the cultivated species Solanum tuberosum for their salt tolerance. Two of these species were found to be more tolerant to NaCl salinity than the cultivated species. The former accumulated more Na^+ and Cl^- ions and their growth was less impaired under salinity. Additional parameters studied included: 1. Rate of transpiration; 2. Stomatal distribution; 3. Accumulation of proline; 4. Response of excised fully differentiated and differentiating leaves to salinity.

Summary and final conclusions based on Beer Sheva experiments

1. The wild species, especially Sp and Lp, are characterized by a lower relative decrease of growth rate under salinity. This was less certain in the Lc ecotypes which gave different results in different experiments. The absolute growth rate was lower in the wild than in the cultivated species under control conditions. Under salinity of 75 meq/l or above, the growth rate became similar in all species. The proportional decrease of dry weight of different organs under salinity differed in the different species; leaf dry weight, for example, decreased less in proportion to other organs in Sp under salinity. In addition, the central role of

[illegible]

 

the growing zone of the shoot in the tolerance of the whole plant has been demonstrated.

2. Sp plants showed an exceptional increase of succulence under salinity.

3. The wild plants, especially Sp, have a greater ability of Na^+ accumulation and lesser ability of K^+ accumulation in the leaves. The ratio Cl^-/Na^+ under salinity was lower in the wild species, especially in Sp. The ratio K^+/Na^+ in the leaves was lower in the wild species. This tendency was most pronounced in Sp. In excised parts which were exposed to salt in culture, the differences in Cl^-/Na^+ and K^+/Na^+ ratios between the wild and cultivated species disappeared; resulting, probably, from the removal of these parts from ion controlling sites in the whole plant.

4. Uptake and distribution of ions: a. Uptake of Rb^+ (representing K^+) was lower in the root of the wild species than in that of the cultivated one; b. Sp was exceptional in having the highest ratio of stem/root Na^+ accumulation and the lowest ratio for K^+ accumulation; c. The Na^+ ion-accumulation ratios of stem/leaf or stem/apex was lower in the wild than in the cultivated plants. This was true to a lesser extent for K^+ ion-accumulation ratios.

5. The results obtained from the hybrids suggest that a small number (possibly one) of genes control the difference in K^+ accumulation between VF and Sp plants.

6. No correlation was found in F_2 (VF x Sp) plants between salt tolerance as expressed by growth rate under salinity, and accumulation of Na^+ , Cl^- , K^+ and increase of succulence. It seems, therefore, that the success of these hybrid plants under salinity does not depend, necessarily, on osmotic adjustment by inorganic ions.

7. The permeability of leaf discs to electrolytes was affected more in Sp than in VF by heat and dehydration stresses.
8. The distribution of ^{14}C in different leaf fractions differed in the wild and cultivated species.

The inheritance of the different components of the mechanism of salt tolerance will be verified, and the understanding of these components and the interactions between them will be extended and deepened by studying F_2 lines each having different components or different component combinations. Such lines can be used: a. to test the hypothesis of single gene inheritance of the control of K^+ level, by crossing low K^+ with high K^+ lines; b. to verify the role of ATPase in the control of ion balance in these plants; c. to verify the role of shoot apex in the salt tolerance of the whole plant; d. to verify the possibility that the greater sensitivity of the leaf membrane of the wild plants to stresses can be used as a screening test; e. as a source material for genes to be transferred to the cultivated species. Different F_2 lines are being prepared by vegetative propagation of individual F_2 plants.

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Final Report to BARD on Project No. I-111-79

II.

B. United States

Experiments with the following objectives were conducted at the United States Salinity Laboratory:

1. To test salt tolerance differences between different cultivars of the tomato, Lycopersicon esculentum, in an effort to reconcile observations made by other investigators and to determine the range of variability in tolerance and ion uptake characteristics in this species.
2. To determine the effect of different Na/Ca ratios and its interaction with salinity in tomato and its wild relative, L. cheesmanii.
3. To study differences in salt tolerance between tomato, and three relatives that have previously demonstrated high salt tolerance, L. cheesmanii (Lc), L. peruvianum (Lp), and Solanum pennellii (Sp).
4. To test the effects of low salinities (0 to -9 bars osmotic potential) in solution cultures on the growth and ion distribution of tomato and Lp.
5. To test the effects of high salinities (-12 to -24 bars osmotic potential) in solution cultures on growth, ion distribution, and survival of tomato and Lp.

6. To study the salt tolerance of tomato and its wild relatives (Lc, Lp, Sp) in sand cultures and to compare growth and yield under these conditions.
7. To isolate and characterize root membrane ATPase in tomato and to study the effects of salinity on this enzyme.

Methods and results of experiments having the aforementioned objectives follow:

1. Differences Between Cultivars.

METHODS: Seedlings (4 weeks old) of 3 cultivars of L. esculentum ('Heinz 1350', 'VF 36' and 'Walter') were transferred to solution cultures and salinities were increased at a rate of 25 mM per day with NaCl and CaCl₂ (5:1 molar ratio) to final treatment concentrations of 50, 100, and 150 mM. One treatment was not salinized. There were 8 replications for each treatment and cultivar. Two weeks after salinization, 4 replications were harvested. After an additional week a type 1650-A General Electronics impedance bridge was used to measure plant root capacitance and plant root resistance of the three varieties. Two electrodes were used in the measurement: one electrode was submerged in the nutrient solution and one electrode was attached directly to the crown of the plant. Na⁺ and K⁺ determinations were made on leaf discs by flame photometry. Proline content of leaf tissues were determined spectrophotometrically according to the procedures of Bates et al. (1973). Plants were then harvested and fresh and dry weights of roots and shoots were determined.

RESULTS: At both harvests, fresh weights of roots and shoots showed significant differences with salinity and cultivar (Table 1a). Shoot fresh weights of 'VF 36' were higher than the other two cultivars at all salinities; however, relative fresh weight yield reductions were similar and not significantly different (Fig. 1a). Leaf Na increased with treatment salinity and differed with cultivar. Leaf Na^+ was higher in lower, older leaves than in younger leaves located at higher nodes (Table 2a). Generally, 1350 had the highest leaf Na^+ concentrations in nonsaline and 50 mM treatments. VF 36 generally had the lowest leaf Na^+ concentrations and this was especially evident at 100 and 150 mM salt. Leaf proline concentrations averaged between 6.3 and 16.5 $\mu\text{mol gfw}^{-1}$ in the nonsaline treatments. At 150 mM, proline concentrations were as high as 177 $\mu\text{mol gfw}^{-1}$ in the lower leaves. Leaf proline concentration was proportional to Na^+ concentration with respect to cultivar, salinity, and leaf position. The reported root mass, capacitance and resistance reported are the average of 4 plants (Table 3a).

A very good correlation was observed between the measured capacitance or resistance and root mass (Table 4a). The capacitance measurement is preferred over the resistance measurement because it is independent of the path resistance to the outer surface of the roots. A difference in electrical properties among varieties was also observed. This difference could prove useful in genetic selection.

2. Na/Ca Ratios.

METHODS: To determine if Na/Ca ratios interact with salt tolerance in tomato (Lycopersicon esculentum) or its wild relative, L. cheesmanii, seedlings (5-6 weeks old) were transferred to solution cultures and

salinized to -6 bars osmotic potential using NaCl and CaCl₂ in molar ratios of 1:1, 2:1, 5:1, 10:1, and 45:1. One treatment was maintained without salinity as a control. There were 4 plants per replication and 2 replications for each treatment and species. Fresh weight of shoots and roots were measured after 30 days salinization. Cation contents (Na, Ca, K, and Mg) were determined for young and mature leaves by atomic absorption spectrophotometry on nitric-perchloric digests. Cl⁻ was determined by potentiometric titration of nitric-acetic acid extracts (Brown and Jackson, 1955).

RESULTS: A 1:1 molar ratio of Na/Ca at -6 bars resulted in the lowest shoot and root fresh weights in both L. esculentum and L. cheesmanii. Na/Ca ratios did not affect the growth reductions caused by salinity in either species (Figure 2a).

3. Salinity Effects on Species.

METHODS: To observe the effects of salinity on the growth, seedlings (4-6 weeks old) were transferred to solution cultures and salinities were increased by additions of NaCl and CaCl₂. Species used in these experiments included two Lycopersicon esculentum Mill. cultivars, 'Heinz 1350' (Le-1350) and 'VF 234' (Le-234), two ecotypes of L. cheesmanii Riley, ecotype 1400 (Lc 1400) and ecotype 1401 (Lc 1401), L. peruvianum (L.) Mill. (Lp) and Solanum pennellii Cor., accession Atico (Sp).

The experiment was conducted in a greenhouse in October 1981. Day maximum and night minimum temperatures were 34 and 18°C, respectively, and averages were 30.5 and 18.3°C, respectively during the study. Seedlings of the cultivated species (4 weeks-old) and the wild species (6

weeks-old) were transferred to 60-liter solution culture containers. There were a total of 24 containers, each containing 4 plants (replications) of one of the 6 entries (Le-1350, Le-234, Lc 1400, Lc 1401, Lp and Sp). Nutrient solutions contained 6 mM KNO_3 , 6 mM $\text{Ca}(\text{NO}_3)_2$, 3 mM MgSO_4 , 0.18 mM KH_2PO_4 , 0.1 mM Fe as diethylene-triamine pentaacetate, 46 μM H_3BO_3 , 9 μM MnCl_2 , 0.8 μM ZnSO_4 , 0.3 μM CuSO_4 , and 0.1 μM H_2MoO_4 .

Salinity treatments were initiated by adding $\text{NaCl} + \text{CaCl}_2$ (2:1 molar ratio) at a rate of 25 mM day⁻¹ to the culture solutions. Salinity treatments of 0, 50, 100, and 150 mM total $\text{NaCl} + \text{CaCl}_2$ were established. Electrical conductivities of the treatment solutions (κ_s) averaged 1.0, 7.9, 14.2, and 20.7 dS m⁻¹, respectively. Fresh and dry shoot and root weights were measured after 30 days. Roots were given 4 successive 30-second rinses in fresh solutions of 5 mM CaSO_4 , and were centrifuged at 300 x g for 10 minutes in wire baskets before weighing. Samples of young and mature leaf tissues were collected for analyses of ions and organic compounds (Grieve and Gratten, 1983). Osmotic potentials were measured on detached leaf disks using thermocouple psychrometers (Hoffman et al., 1971). Mature, sunlit leaves were sampled between 1130 and 1200 h (PST) one week before harvest. Leaf osmotic potential (ψ_L) was measured after freezing leaf disks in liquid nitrogen.

RESULTS: Total dry and fresh weights of stems and leaves decreased with increasing salinity (Table 5a). Under nonsaline conditions, both ecotypes of L. cheesmanii had significantly higher fresh and dry shoot weights than S. pennellii, this may have been due to initial differences in seedling age at transplanting. L. cheesmanii, ecotype 1400 showed

the most significant yield reductions at 50 and 100 mM salinity; but at 150 mM, only L. peruvianum and Le-1350 had higher fresh and dry weights than the others. Relative salt tolerance values as described by Maas and Hoffman (1977) were calculated based on electrical conductivity of solution cultures (κ_g), (Figure 3a). Thresholds, slope values and their confidence limits were calculated according to van Genuchten (1983), (Table 6a). Generally, Le-1350 was more tolerant than any of the wild species; however, the cultivar, Le-234 was not as tolerant as Sp or Lc-1401 under 50 mM salinity or as tolerant as Sp or Lp under 150 mM salinity (Table 5a).

Leaf water potential (ψ_l), osmotic potential (ψ_π), turgor potential (ψ_p), and relative water content (RWC) decreased with salinity and differed with species (Table 7a). Average ψ_l decreased from -7.4 to -14.6 bars as salinity increased to 150 mM. Correspondingly, ψ_π decreased from -12.1 to -17.6 bars, while ψ_p increased from -4.7 to -3.1 bars across this salinity range. Average leaf RWC decreased from 88.7 to 76.4 percent with increased salinity. The cultivated tomatoes had the greatest RWC decrease whereas Sp changed very little (4.6 percent). Turgor changes were greatest in the Lc ecotypes and decreased by only about one bar in the other species. Lp had the highest ψ_π under both nonsaline and high salt conditions, but total ψ_π changes were intermediate compared to those of other species.

Quaternary ammonium compounds (QAC) did not change significantly with salinity treatment. In young leaf tissues QAC ranged from 46.3 $\mu\text{mol g}^{-1}$ dry weight for Le-1350 to 30.7 $\mu\text{mol g}^{-1}$ for Sp. Mature leaf averages were 28.4 and 16.9 $\mu\text{mol g}^{-1}$, respectively. At salt concentrations as high as 150 mM, QAC concentrations for young and mature leaf

tissues averaged across species were 35.3 and 18.6 $\mu\text{mol g}^{-1}$ dry weight, respectively.

Proline concentrations were lower in mature than in young leaves; salinity increased proline in both tissues (Table 8a). Le-1350 and Lp had higher proline contents, across treatments, than the ecotypes of Lc. Sp was intermediate and Le-234 was similar to Le-234.

Significant differences between lines were found in leaf Na^+ and Cl^- contents at all salinities (Fig. 4a).

4. Effect of Moderate Salinities.

METHODS: To test the salt tolerance of Lycopersicon peruvianum compared to its cultivated relative, the tomato (L. esculentum, 'Heinz 1350'). Seedlings (3-4 weeks old) were transferred to solution cultures and salinities were increased to the equivalent of -3, -6, and -9 bars osmotic potential by additions of NaCl and CaCl_2 (5:1 molar ratio). Additions were made at the rate of -1 bar per day and one treatment was maintained as a nonsalinized control. There were 4 plants per replication and 3 replications per treatment. The experiment was conducted two times during the year. Fresh and dry weights of shoots and roots were measured and ion contents of roots and mature and developing leaves were analyzed by the procedures previously described.

RESULTS: Relative fresh shoot weights were reduced more at -3 bars in L. peruvianum (Lp) than in L. esculentum (Le) (Table 9a). At -6 bars, the average reduction in relative shoot weight of Lp was more than Le in the first experiment but not in the second. Likewise, relative fresh weights of roots were less for Lp at -3 and -6 bars than for Le, in the

first study. But, in the second experiment the differences were only consistent at -3 bars. This indicates that most significant differences may occur between -1 and -6 bars.

Le and Lp differed in ion content of leaf and root tissues (Table 10a). In unsalinized solutions Lp had lower Na^+ and Cl^- , and higher Ca^{2+} concentrations in both young and mature leaves than Le. At salinities between -3 and -6 bars, Na^+ in the developing leaves of Lp was significantly higher than in Le, but Ca^{2+} , K^+ , and Cl^- contents were lower. In mature leaves, the major differences were that Lp had significantly lower Mg^{2+} and Cl^- contents than Le. The roots of Le and Lp had similar Cl^- concentrations in all treatments, but Lp had higher Mg^{2+} concentrations for salinized treatments than Le. Root Na^+ concentrations differed significantly with species in all treatments. Under nonsaline or low saline (-3 bars) conditions, Lp roots were lower in Na^+ than Le, but at higher salinities Na^+ concentrations in the Lp roots were higher than those of the Le roots.

5. Effect of High Salinities.

METHODS: To compare the growth and survival of tomato, Lycopersicon esculentum (Le) to its wild relative, L. peruvianum (Lp) under high salinities, seedlings were transferred to solution cultures and salinities were increased by adding a 5:1 molar ratio of NaCl and CaCl_2 at a rate of -1 bar per day to final osmotic potentials of -12, -18, and -24 bars. One treatment was maintained without salt as a control. For each species, there were 4 plants per replication and 3 replications per treatment. Fresh and dry weights of shoots and roots were determined on

surviving plants and ion (Na^+ , Ca^{2+} , Mg^{2+} , K^+ , and Cl^-) contents were determined for young and mature leaf tissues and root tissues.

RESULTS: L. peruvianum had higher survival rates at all salinities than L. esculentum (Fig. 5a). Dry weights of L. peruvianum shoots averaged 138, 19.5, 11, and 6.7 g for the control, -12, -18, and -24 bar treatments, respectively. Respective L. esculentum dry weights averaged 117, 6.5, 4.2, and 2.6 g. Therefore, Lp plants averaged 3 to 4 times higher dry weights than Le when grown in solutions at salinities equivalent to -12 to -24 bars osmotic potential. Roots of Lp had higher contents of Na^+ , Ca^{2+} , and Cl^- , and less K^+ than Le (Table 11a). In both species, concentrations of Na and Cl were 2 to 5 times higher in mature leaves than in the developing leaves. The Na^+ and Cl^- concentrations of stems were intermediate between mature and developing leaf concentrations and showed no gradient. Under nonsaline conditions, ion contents of roots and leaves of Lp were similar to Lp (Table 11a). Minor variations that were significant were not consistent with results obtained under similar conditions (Table 10a). Root Na^+ concentrations of Lp were higher than those of Le and supported the evidence that Lp Na^+ contents were generally higher than Le in all tissues, leaf, stems, and roots. In mature stems of Lp, Ca^{2+} concentrations were higher than in those of Le; however, K^+ , Mg^{2+} , and Cl^- concentrations were significantly lower.

6. Effect of Salinity and Irrigation Frequency in Sand Cultures.

METHODS: To test for differences in salt tolerance between tomato and its wild relatives in sand cultures having different irrigation frequencies, tomato seedlings (4-5 weeks old) were transplanted into 18,

1.5 x 3 m outdoor, sand culture plots. Three plants each of Lycopersium esculentum, cv. Heinz 1350 (Le); Lycopersicon cheesmanii ecotype 1401 (Lc); Lycopersicon peruvianum ecotype Atico (Lp); and Solanum pennellii (Sp) were transplanted in each plot. After 4 weeks, treatments were established by adding NaCl and CaCl₂ (5:1 molar ratio) at a rate of 25 mM per day to final salinities of 50 or 100 mM. One nonsalinized treatment was kept as a control and each treatment was replicated 6 times. Upon this experimental design, two irrigation treatments were imposed -- one cycle a day and two per day. Approximately 400 liters of solution was applied at each irrigation. After 10 weeks, leaf osmotic potentials and water potentials were determined by psychrometry and leaf samples were obtained for determination of Na⁺ and K⁺. Plants were harvested 12 weeks after salinization. Fruit and shoot fresh weights and shoot dry weights were measured.

RESULTS: In sand cultures, salinity reduced vegetative and fruit yields and at high concentration, showed interaction with irrigation frequency. As in solutions, total fresh weights per plant were higher for Le-1350 and Lc 1401 than for Lp and Sp (Table 12a). Without salinity Le-1350 and Lc 1401 had higher total fresh weights with 2 irrigations per day whereas total fresh weights of Lp and Sp did not differ significantly. At 100 mM salinity two daily irrigations produced the highest total fresh weights regardless of species.

Leaf and stem fresh weights were highest in Lc 1401, median and similar for Le-1350 and Lp, and lowest for Sp when compared in nonsaline media (Table 12a). At 50 salt, mM vegetative fresh weights were lower except that which Lp had been irrigated once daily. Two daily irriga-

tions contributed to higher vegetative fresh weights in all species at 100 mM salinity but had no clear beneficial influence in other treatments. Lc 1401 and Lp both showed some salt tolerance in vegetative growth compared to Le-1350, however much of the apparent sensitivity of Le-1350 can be explained by its capacity to maintain fruit production at 50 mM salinity. Generally, fruit weight was maintained or slightly stimulated at 50 mM salinity on both a fresh weight or percent of total plant weight basis (Table 12a). Fruit weight was reduced at 100 mM salt, especially with only one daily irrigation, but only as a percentage total plant weight.

Relative salt tolerances in sand cultures based on relative total fresh weights, differed from those found in solution cultures. Since the differences may be attributed to differences in growth media, sand versus solutions, and some differences may be due to the effects of prolonged growth and fruit production. Under the two-irrigation regime, Le-1350 and Lp showed the highest tolerance in total fresh weights at 50 mM salt, but Lp was most tolerant at 100 mM (Table 12a). With only one daily irrigation, Le-1350 and Lc 1401 maintained relatively higher total fresh weights than Lp or Sp at 50 mM salinity. Again, Lp was the most tolerant at 100 mM.

7. Characterization of Root Membrane ATPase.

METHODS: To isolate and characterize root membrane ATPase in tomato and to study the effects of salinity on this enzyme, roots from nonsaline and saline-treated tomato (Lycopersicon esculentum) plants were ground and filtered. Plasma membrane-enriched fractions were isolated from each sucrose gradient using ultra centrifugation. The activity and

sensitivity of these fractions were determined in vitro with various substrates and inhibitors.

RESULTS: The ATPase of a plasma-membrane enriched fraction isolated from tomato roots was identified by its high specificity for ATP or substrate, and its sensitivity to known inhibitors of plasma membrane ATPase. The enzyme was activated by Mg^{2+} and stimulated by monovalent cations, but there was no evidence of K^+/Na^+ synergism in its stimulus. The pH optimum for the ATPase was 6.5 Km and Vmax for KMg-ATP were 0.59 mM Mg-ATP and 95 μ mol/mg protein/hr, respectively. The enzyme's activity was found to be sensitive to sodium vanadate, diethylstilbestrol, and N,N'-dicyclohexylcarbodiimide. The properties of the ATPase from isolated tomato roots grown in a solution containing salt (5:1 molar ratio of NaCl and $CaCl_2$) equivalent to -4 bars osmotic potential were also examined. This ATPase was found to have a lower Km and Vmax for KMg-ATP than for that isolated from nonsalinized roots (Table 13a). Experiments where the plasma membrane fractions from control and salinized roots were mixed indicate that the reduced Vmax of the salinized-root enzyme is not due to the presence of an inhibitor of enzyme activity. Experiments with ionophores (carbonylcyanide, p-trifluorophenoxyphenolhydrazine and valinomycin) have shown that the lower activity of the enzyme isolated from roots grown in saline solutions is not due to a less permeable vesicle which might be dissipating the electrochemical gradient generated by the ATPase. The detergent, Triton X-100 (0.005%), caused a greater stimulation of enzyme activity of the ATPase isolated from salinized roots. This may indicate that differences exist in the lipid matrices surrounding the enzymes.

DISCUSSION

Salt tolerance of wild species to mixed salt solutions was less than expected from results obtained from studies using NaCl or seawater as salinizing media (Dehan and Tal, 1978; Tal, 1971; Phillips et al., 1979; Rush and Epstein, 1976). Tal (1971) reported dry weight reductions of 62% for Le cv. 'Marmande' and only 48% for Lp when 3-week-old plants were salinized for 1 week with 196 mM NaCl. Longer salinization intensified damage; dry weights were reduced by 84% for the cultivar and 78% for Lp after 5 weeks of NaCl treatment. Relative dry weights of Le-1350 and Lp in these studies were reduced by about 60% after 4 weeks of salinization to 150 mM (Table 5a); however, Le-234 suffered an 80% dry weight reduction. Furthermore, the salt tolerance of the Le-1350 cultivar was higher than any of the other species at other salinity concentrations.

Dehan and Tal (1978) noted that dry shoot weights of 9-week-old Sp plants had been reduced by only 21% after being grown for 5 weeks in 100 mM NaCl. Mixed salt solutions of 100 mM were found to reduce Sp dry weight growth by 53% in solution culture studies (Table 5a). In sand cultures, 100 mM mixed salt reduced Sp fresh weight by 77% (Table 12a); nevertheless, Sp plants were more tolerant than Le at this salinity. At 150 mM salt, Sp was also more tolerant than Lc or Le-234 in solution culture but not as tolerant as Lp or Le-1350 (Table 5a).

Lc ecotypes have been reported to differ markedly in their survival in seawater and less so in growth in seawater dilutions (Rush and Epstein, 1976). Fresh weight reductions in 0.2 seawater of about 70% were reported for Lc ecotypes grown in 0.2 seawater for almost 6 weeks.

(Rush and Epstein, 1976). Respective fresh weight reductions for the cultivar 'VF 36' were as high as 85%. The κ of the 0.2 seawater was given as 16 dS m^{-1} and is close to that of a mixed salt solution of 150 mM. Growth reductions of the cultivars used in this study (Le-234 and Le 1350) were not as great as those previously reported for 'VF 36', and reductions in growth of the Le ecotype were greater than those reported by Rush and Epstein (Table 5a). Furthermore our findings also indicate that there are no differences in salt tolerance between the cultivated species, Le-1350 (Heinz), 'VF 36', or 'Walter' (Table 1a). On the other hand, rather large differences existed in the abilities of these species to exclude ions from the shoots (Table 2a). Although all cultivars excluded Na^+ equally from the youngest leaves, VF 36 was more effective than Walter and Le 1350 at maintaining low Na^+ in the rest of the shoot. Le 1350 had the highest leaf Na^+ contents overall. Rush and Epstein (1976) attributed some of the salt tolerance of Lc 1401 to its ability to maintain high Na^+ concentrations in the shoots. Cultivated species also show significant differences in this character.

The ability of a plant to accumulate salt may be advantageous because it may allow rapid osmotic adjustment. Leaf water and osmotic potential measurements indicated that turgor decreased with increasing salinity in all species (Table 7a). Relative water content also decreased as salinity increased. Le, Le 1401, and Lp had the highest leaf osmotic potentials, suggesting high solute concentrations. Sp had the lowest change in relative water content, demonstrating its high drought potential.

Clearly, rather large differences exist in the reported salt tolerances of tomato cultivars and their relatives and it is difficult to

determine the reason. Salt tolerance is known to differ with development and consequently the age at which treatment begins is important. In our greenhouse studies, seedlings were 4 weeks old, in the case of cultivars, and 6 weeks old in the case of the wild species. The cultivars were more vigorous in germination and seedling growth and by 4 weeks were approximately similar in size and development to 6-week-old wild species. Wild species used in these studies were generally 2-3 weeks older, when salinized, than plants used in other tests (Dehan and Tal, 1976; Tal, 1971; Rush and Epstein, 1976). However, this would be expected to make them more tolerant, not less. Final shoot dry weights for the nonsalinized plants in this study were more than twice those reported for Lp (Dehan and Tal, 1976) and 100 times those reported for Sp (Tal, 1971). Fresh weights of Lc ecotypes in this study were larger by a third than those reported previously (Rush and Epstein, 1976).

Salt tolerance differences, though not as large as expected, were apparent as differences in the relative yield decreases per unit salinity increases (slope values) between the genotypes examined (Table 6a). Based on these values Lp and Sp were the most salt tolerant (lowest slope) and Lc-1400 was the most sensitive. Threshold values ranged from 4.1 to 6.9 dS m⁻¹ and had relatively high standard errors due to the fact that most of the treatments used in this study were at high salinities. In cases in which the threshold value could not be calculated (Le-234, Lp, and Lc-1400), the value of 4.1 dS m⁻¹ was used as a conservative estimate (see Maas and Hoffman, 1977; van Genuchten, 1983).

It is interesting that Rush and Epstein (1976) showed no differences between growth of Lc-1400 and Lc-1401 in 0.2 seawater, in spite of the evidence of rather large differences in survivability in seawater.

Present studies show that significant differences in salt tolerance as measured by growth, exist between these ecotypes across a wide range of salinities (Tables 5a and 6a).

Salt tolerance as measured by relative total fresh weights of plants grown in sand under two irrigation schemes indicate that wild species are not significantly more tolerant than cultivated species at 50 mM salinity; however, both Lp and Sp showed greater tolerance than the Lc-1350 at 150 mM salt (Table 12a). Such evidence supports the results obtained from solution cultures; and likewise, supports the premise that these species may differ in salt tolerance under some conditions. An analysis of slope and threshold values, however is not possible because of the limited number of salinity treatments that were used in this study.

Irrigation practices are known to interact with salt tolerance and osmotic stress is an important part of salt stress. Sand cultures, because of the lower water potential can accentuate salinity stresses in much the same way as soils. Plants must adjust to lower external water potentials by increasing their osmotic potentials either through ion uptake or the production of organic osmotica. Rush and Epstein reported higher leaf Na^+ accumulation in Lc 1401 compared to 'VF 36'. Tal (1971) found slightly higher leaf Na^+ concentrations in salt-treated Lp than in a cultivar similarly stressed, also Sp was shown to accumulate more Na^+ under saline conditions than its cultivate counterpart. Differences in leaf Na^+ and Cl^- contents among the tomato species increases with salt concentration and varies dramatically within the plant. High salt concentrations are found in the older leaves. Hayward and Long (1941) suggested that plants might regulate excessive ion concentrations by

partitioning such ions in tissues, like old leaves, that are metabolically less active. Bernstein and Hayward (1958) pointed out that $^{36}\text{Cl}^-$ accumulated mainly in old leaves, stems, and fruit petioles. Young leaves maintain lower ionic concentrations and thus may be protected from some of the affects of salt stress. Developing tissues are less vacuolated than mature tissues and consequently have higher osmotic potentials -- even without high salt ions. Furthermore, the xylem elements may be less developed so that ions in the xylem stream cannot readily enter younger tissues. It is unknown whether the low Na^+ and Cl^- concentrations of young tissues is actually beneficial or merely a consequence of plant design and development, however, it is certain that a concentration gradient exists throughout the plant (Table 2a and 11a). While some investigators have concluded that leaf Na^+ is positively correlated with salt tolerance in much the same way that it is in halophytes, Sacher et al (1982) found no close correlation between salt tolerance and either Na^+ or Cl^- in tomato lines derived from *Le x Sp* crosses.

Salinity composition may play an important role in the determination of salt tolerance. Our studies indicated that salt tolerance in *Le*-1350 and *Lc* 1401 is relatively insensitive to differences in $\text{Na}:\text{Ca}$ ratios, except at the extremes as low as 1:1. Rush and Epstein have found that *Lc* 1401 is more sensitive to high K^+ concentrations than is the cultivated species. Sea water mixes, as used in their study, are generally low in this cation but often have high concentrations of Mg^{2+} . Recently, the salt tolerance of a clone of *Agrostis stolonifera* was attributed, in part, to cotolerance to MgCl_2 and CaCl_2 (Hodson et al., 1981).

Additional studies, with more replications, were conducted to determine differences in salt tolerance between Le-1350 and Lp. In two solution culture experiments, Lp was more sensitive to low salinity (equivalent to -3 bars osmotic potential in the root media) than Le-1350 (Table 9a). However, at moderate salinities (up to -9 bars) differences were negligible and both species exhibited similar tolerances. Ion uptake differences between the species were evident (Table 10a) and indicate that although tolerances are similar, the mechanism by which each species achieves tolerance is different. This provides optimism that crosses between the species could provide new recombinations of salt tolerant genes and more highly tolerant offspring. Rush and Epstein (1981) have reported success using this technique with crosses of Lc-1401, a genotype that these studies show is less tolerant than Lp (Table 6a).

At high salinities, Lp exhibited more tolerance than Le-1350. Tolerance was chiefly expressed as increased survival at osmotic potentials from -12 to -24 bars (Fig. 5a). However, differences were again noted in ion concentrations in leaves, stems, and roots (Table 11a).

The rather large variability in ion uptake patterns between the wild relatives of tomato needs to be studied and genetically exploited. The relationship between salt tolerance, high ion uptake rates, and high growth rates is not well understood. The variability among the relatives of tomato can be used to study these relationships and, potentially, to develop more tolerant cultivars. Rush and Epstein (1981) have reported some success in increasing salt tolerance by back crossing Lc 1401 to a cultivated tomato and Robinson, Sacher, and Staples (personal communication) have been conducting a similar program using a

cross between Sp and a cultivated parent. In lieu of a complete understanding of the mechanisms, progeny of these crosses show increased salt tolerance. Thus, tomato is a good system to further potentiate the salt tolerance character and to develop a model system for the basic understanding of this character in all species.

Osmotic adjustment and ion regulation are obviously two key factors involved in salt adaptation and tolerance. The wild species show wide variation in these characters. Such differences may ultimately be linked to unique characteristics of membranes or membrane-bound proteins. We have shown the salinity induces physical or chemical changes in the ATPase enzyme of tomato plasma membrane (Table 13a). These changes lower the activity of the enzyme and may be the effect of changes in the lipid matrix surrounding the membrane.

Finally, the analytical description of water transport in saline soils that have actively transpiring plants is usually given by adding a sink term to the Richards equation and use of the appropriate initial and boundary conditions. The sink term describes water extraction by roots and usually assumes a spatial distribution with regards to density. Clearly, root mass and distribution are very important and yet difficult parameters to obtain. Nevertheless, they may have as important role in contributing to difference found in salt tolerance in solution cultures and tolerance in soil. The relation between root mass and some electrical property of roots (capacitance, resistance) was sought, so that the root mass and eventually surface area could be determined in situ. The objective here was to study these relationships in a well defined environment. Our studies report differences between cultivars (Tables 3a and 4a), but results not finished in time to be

included in this report indicate that greater differences exist among the wild species. An in situ method for measuring root mass may be an effective tool for genetic selection.

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Table 1a. Partial 2-way Analysis of Variance table showing the significance and interaction of salinity on 3 tomato cultivars.

	DF	Probability of Significance, PR(F)			
		1st Harvest		2nd Harvest	
		Shoot	Root	Shoot	Root
Salinity (A)	3	0.001	0.003	0.001	0.001
Cultivar (B)	2	0.003	0.001	0.022	0.014
A * B	6	NS	NS	NS	0.096
Error	36	--	--	--	--
Total	47	--	--	--	--

Table 2a. Na contents of leaves at different positions on 3 tomato cultivars.

Leaf Position	Salinity (mM)	Na contents of fresh leaf discs			
		Cultivar			S.E.
		1350	VF 36	Walter	
Highest, fully expanded	0	9	5	5	2
	50	21	15	13	8
	100	25	16	21	7
	150	87	45	73	26
Third from base	0	10	5	5	1
	50	36	15	18	5
	100	48	20	63	8
	150	170	39	163	45
Second from base	0	11	7	9	2
	50	83	31	20	20
	100	188	32	97	41
	150	270	73	221	68
Lowest, true leaf	0	14	7	15	3
	50	127	65	27	15
	100	204	108	54	27
	150	318	114	134	32

S.E. Standard error of the means.

Table 3a. Electrical properties of roots of three varieties of tomatoes grown at 4 levels of salinity.

Type	Salinity* mM	Root-Mass (gdw)	C _p (nf)	R _p (x 10kΩ)
<u>Walter</u>	0	1.90 ± .47	3.00 ± .37	1.28 ± .45
	50	1.93 ± .50	3.10 ± .12	1.11 ± .43
	100	1.35 ± .51	2.80 ± .74	1.58 ± .62
	150	0.78 ± .15	1.50 ± .17	3.40 ± .53
<u>VF-36</u>	0	2.23 ± .21	3.80 ± .54	1.31 ± .45
	50	2.25 ± .19	4.00 ± .84	1.28 ± .71
	100	2.13 ± .50	3.76 ± .59	1.59 ± .36
	150	1.10 ± .42	2.70 ± .67	2.63 ± .90
<u>Heinz</u>	0	2.78 ± .45	4.21 ± .21	0.93 ± .19
	50	2.20 ± .59	4.03 ± 1.03	1.26 ± .55
	100	1.28 ± .50	3.37 ± .52	0.76 ± .24
	150	1.18 ± .38	3.00 ± 1.02	0.93 ± .29

Table 4a. Linear regression analyses between capacitance (C) or resistance (R) and root mass (RM) for the three varieties.

<u>Variety</u>	<u>Linear Regression Equation</u>	
Walter	$RM = .685 C - .291$	$r = .938$
	$RM = -.485 R + 2.383$	$r = .924$
V-36	$RM = .938 C - 1.416$	$r = .992$
	$RM = -.867 R + 3.404$	$r = .992$
Heinz	$RM = 1.302 C - 2.897$	$r = .961$

Table 5a. Average shoot fresh and dry weights, and relative dry weights of cultivated and wild tomato species grown in salinized solution cultures.

Line	Salinity			
	0	50	100	150
	Average Shoot Fresh Weights, g plant ⁻¹			
Le-1350	633	697	298	201
Le-234	621	442	207	113
Lc-1400	593	263	185	59
Lc-1401	641	571	230	106
Lp	405	258	147	132
Sp	329	210	131	92
LSD _{0.05}				
	Average Shoot Dry Weights, g plant ⁻¹			
Le-1350	42.0	44.3	21.9	16.8
Le-234	45.9	32.6	16.1	9.1
Lc-1400	51.3	16.5	10.8	4.2
Lc-1401	56.5	50.9	17.5	9.8
Lp	41.2	26.9	14.5	15.5
Sp	31.5	26.6	14.8	8.2
LSD _{0.05}	14.8	18.3	9.4	5.4
	Relative Dry Weights			
Le-1350	---	1.05	0.52	0.40
Le-234	---	0.71	0.35	0.20
Lc-1400	---	0.32	0.21	0.08
Lc-1401	---	0.90	0.31	0.17
Lp	---	0.65	0.35	0.38
Sp	---	0.84	0.47	0.26

Table 6a. Salt tolerance slope, threshold and optimal yield values for two tomato cultivars (L-1350 and L-234) and four wild relatives.

Genotype ^y	Value	S-E ^z	95% Confidence Limits	
			Lower	Upper
	SLOPE			
Relative yield decrease (%) per unit salinity increase				
Le-1350	7.0	1.6	3.5	10.4
Le-234	7.0	0.7	5.4	8.5
Lp	5.7	0.9	3.8	7.5
Sp	6.2	1.9	2.6	10.7
Lc-1400	10.7	1.5	7.5	13.9
Lc-1401	7.5	2.2	2.7	12.3
THRESHOLD				
Salinity (dS m ⁻¹ at first significant yield reduction				
Le-1350	6.9	1.9	2.8	11.0
Le-234	4.1	-	-	-
Lp	4.1	-	-	-
Sp	4.4	2.8	-1.7	10.4
Lc-1400	4.1	-	-	-
Lc-1401	5.4	2.8	-1.6	10.7
OPTIMUM YIELD				
Dry Wt per plant (g)				
Le-1350	41.9	4.0	33.3	50.6
Le-234	42.7	3.3	35.5	49.9
Lp	36.4	3.2	29.5	43.2
Sp	31.5	3.4	24.2	38.8
Lc-1400	41.2	4.5	31.4	51.0
Lc-1401	56.5	7.0	41.4	71.7

Table 7a. Leaf water potential (ψ_l), osmotic potential (ψ_π), turgor potential (ψ_p), and relative water content (RWC) of 6 tomato genotypes grown in nonsaline and saline (150 mM) solution cultures.

Species	Salt	ψ_l	ψ_π	ψ_p	RWC
	mM		-bars		%
Le-1350	0	8.2	12.4	11.2	90.9
	150	15.0	18.3	13.2	72.0
	Change	6.8	5.9	2.0	18.9
Le-234	0	7.8	11.6	11.2	95.8
	150	15.0	17.9	14.8	82.2
	Change	7.2	6.3	3.6	13.6
Lc-1400	0	6.4	12.4	10.9	88.4
	150	13.7	16.6	12.6	75.9
	Change	7.3	4.2	2.7	12.5
Lc-1401	0	7.4	12.1	10.2	83.9
	150	15.6	17.9	13.2	73.6
	Change	6.3	5.4	2.4	13.4
Lp	0	7.9	13.5	11.8	87.9
	150	14.2	18.9	14.2	74.5
	Change	6.3	5.4	2.4	13.4
Sp	0	6.9	10.6	9.0	85.0
	150	13.1	15.7	12.6	80.4
	Change	6.2	5.1	3.6	4.6

Table 8a. Leaf free proline concentrations of 6 tomato genotypes grown in nonsaline and salinized solution cultures.

	$\mu\text{mol Proline g}^{-1}$ fresh weight							
	Young leaves				Mature leaves			
	mM salt				mM salt			
	<u>0</u>	<u>50</u>	<u>100</u>	<u>150</u>	<u>0</u>	<u>50</u>	<u>100</u>	<u>150</u>
Le-1350	19	82	309	931	24	42	142	337
Le-234	18	188	494	719	19	45	130	225
Lc-1400	53	112	297	518	24	42	19	51
Lc-1401	36	58	171	667	21	45	23	59
Lp	149	258	695	1002	23	125	90	339
Sp	133	162	395	328	16	15	59	147
LSD _{0.05}	81	76	222	323	n.s.	40	65	85

Table 9a. Effect of salinity on shoot and root fresh weights of two tomato species, Lycopersicon esculentum (Le) and L. peruvianum (Lp).

<u>Tissue</u>	<u>Species</u>	<u>Salinity, ψ</u>			
		<u>0</u>	<u>-3</u>	<u>-6</u>	<u>-9</u>
		<u>Fresh wt.</u>	<u>Weights relative to the control</u>		
		<u>g</u>			
<u>First Experiment</u>					
Shoot	Le	543	0.85	0.41	0.14
	Lp	305	0.61	0.23	0.10
Root	Le	77	1.05	0.79	0.45
	Lp	52	0.86	0.39	0.39
<u>Second Experiment</u>					
Shoot	Le	454	0.91	0.32	0.13
	Lp	340	0.65	0.34	0.16
Root	Le	66	1.02	0.56	0.33
	Lp	64	0.80	0.49	0.31

Table 10a. Ion contents of leaf and root tissues from two tomato species, *Lycopersicon esculentum*, 'Heinz 1350' (Le) and *L. peruvianum* (Lp), grown in moderately saline solutions.

Species	ψ^z (-bars)	Developing Leaves ^y				
		Na	Ca	K	Mg	Cl
Le	0	31	169	872	239	105
	3	28	297	778	134	493
	6	68	437	902	123	814
	9	174	498	808	115	1165
Lp	0	17 ^x	264*	853	213	57*
	3	168*	274*	672	143	231*
	6	266*	327*	608*	117	548*
	9	356*	332*	602*	106	707*
Mature Leaves						
Le	0	137	752	1068	304	101
	3	285	1208	586	166	1566
	6	866	1682*	640	164	2996
	9	1698	1769*	462	169	4228
Lp	0	23*	1236*	1008	301	54*
	3	700*	1273	715	135*	891*
	6	712	1027*	643	131*	1191*
	9	1385	1246*	922*	140*	2777*
Roots						
Le	0	449	198	403	166	76
	3	894	229	576	121	1108
	6	825	207	1092	84	1352
	9	1153	216	1030	75	1601
Lp	0	31*	307*	1110*	170	78
	3	723*	289	1070*	181*	1235
	6	1139*	214	940	182*	1640
	9	1565*	272	705*	106*	1628

^z ψ Osmotic potential of nutrient solutions.

^y All ion values given as mmol Kg⁻¹ dry weight.

^x Asterisks indicate a significant difference from corresponding values in Le.

Table 11a. Ion contents of leaf and root tissues of two tomato species, *Lycopersicon esculentum*, 'Heinz 1350' (Le) and *L. peruvianum* (Lp), grown in highly saline solutions.

Species	ψ^z (-bars)	Developing Leaves ^y				
		Na	Ca	K	Mg	Cl
Le	0	15	254	705	180	50
	12	493	731	895	132	1763
	18	--	--	--	--	--
	24	--	--	--	--	--
Lp	0	19	258	858*	204*	49
	12	622 ^x	501*	649	111	887*
	18	1700	641	596	80	1903
	24	1470	570	574	77	2219
Mature Stems						
Le	0	8	390	532	283	13
	12	1650	951	786	120	3064
	18	2130	1078	526	141	3519
	24	3010	1292	609	109	5261
Lp	0	9	218*	481*	119*	24
	12	1160	432*	521*	79*	1677*
	18	2330	686*	470*	73*	3067*
	24	2440	900*	390*	76*	3298*
Roots						
Le	0	276	358	549	190	54
	12	877	498	649	68	1114
	18	1237	362	600	80	1509
	24	1270	316	520	96	1487
Lp	0	265	391	595	254*	110*
	12	1483*	1093*	602	90	1538*
	18	2057*	900*	318*	85	1982*
	24	2027*	754*	318*	78	2080*

^z ψ Osmotic potential of nutrient solutions.

^y All ion values given as mmol Kg⁻¹ dry weight.

^x Asterisks indicate a significant difference from corresponding values in Le.

Table 12a. Effects of salinity and irrigation frequency on vegetative growth and fruit production of 4 tomato species: Lycopersicon esculentum (Le), L. cheesmanii (Lc), L. peruvianum (Lp), and Solanum pennellii (Sp).

Salinity	2 X Irrigations				1 X Irrigations			
	Average Total Fresh Weight, g/plant							
mM	Le	Lc	Lp	Sp	Le	Lc	Lp	Sp
0	8520	9181	4440	2688	7729	7623	4354	2943
50	6766	6468	3561	1368	7424	8317	3381	2034
100	1125	1287	1356	620	737	400	749	195
	Leaf & Stem Fresh Weight, g/plant							
0	3766	9152	4250	2688	4157	7567	4206	2943
50	1952	6383	3380	1368	2505	8253	3106	2034
100	474	1209	1150	620	311	400	720	195
	Fruit Weight, g/plant							
0	4754	29	190	0	3572	56	148	0
50	4813	85	181	0	4919	65	276	0
100	651	78	205	0	426	0	29	0
	Fruit Weight, % of Total Plant							
0	55.8	0.32	5.52	0	46.2	0.73	3.40	0
50	71.1	1.31	5.08	0	66.3	0.78	8.16	0
100	57.9	6.06	15.12	0	57.8	0	3.87	0
	Relative Total Fresh Weight, Tolerance							
50	0.79	0.70	0.80	0.51	0.96	1.09	0.78	0.68
100	0.13	0.14	0.31	0.23	0.10	0.05	0.17	0.07
	Relative Vegetative Weight, Tolerances							
50	0.52	0.70	0.76	0.51	0.60	1.09	0.74	0.69
100	0.13	0.13	0.35	0.23	0.07	0.05	0.17	0.07

Table 13a. Kinetics of ATPases isolated from plasma-membrane enriched fractions of tomato roots.

<u>Treatment</u>	<u>Km</u>	<u>Vmax</u>
	<u>mM Mg-ATP</u>	<u>μmol/mg protein/hr</u>
Control	0.59	95.4
+ Salt	0.33	51.2

Figure 1a. Leaf fresh weights of 3 tomato cultivars after 2 weeks (closed symbols) and 3 weeks (open symbols) growth in salinized solution cultures. (Δ - 'Walter', $\bullet$ - 'VF-36', $\square$ - 'Heinz 1350').

Figure 2a. Effect of different molar ratios of NaCl and CaCl₂ on the shoot fresh weight of *Lycopersicon esculentum* (1350) and *L. cheesmanii* (1401).

Figure 3a. Relative yield reduction of 6 tomato genotype due to salinity in solution cultures.

Figure 4a. Salinity effects on Na and Cl contents of mature (m) and developing (d) leaves of Lycopersicon esculentum (1350), L. cheesmanii (1400), and Solanum pennellii (Sp).

Figure 5a. The influence of salinity as measured by solution osmotic potential on the survival of Lycopersicon esculentum (1350) and L. peruvianum (Lp).

SUMMARY AND FINAL CONCLUSIONS BASED ON U.S. SALINITY
LABORATORY STUDIES

1. Experiments using mixed salt treatments applied for longer periods of time yield slightly different results than those conducted over short periods using single salt solutions. Nevertheless, Lp shows greater salt tolerance than the cultivated variety of tomato under high salinities in solution cultures (greater than -9 bars osmotic potential) and in sand culture. The relative salt tolerance of Sp increases as moisture stress increases, thus this species may have valuable gene resources in the development of salt tolerant plants adapted to arid environments. The Lc ecotypes differ from one another in tolerance, but neither has proven to be more tolerant than the test cultivar in any of our experiments.

2. Basic physiological factors which contribute to salt tolerance can not be specified. Each species possesses a different pattern of salt exclusion, accumulation and sequestering capacities that cannot be specifically linked to salt tolerance - with one exception. Each species maintains low salt concentrations in the region of the growing meristems. Salt tolerance may be a function of how efficiently the species does this and therefore may be dependent upon a number of processes acting in harmony. Eventually, the study of segregating progeny from crosses between the different species may contribute more information concerning the roles and values of mechanisms controlling ion uptake and distribution. Studies of ion distribution in hybrid populations indicates that gene control for certain ion specificities (Na^+ and K^+) may not be complex. Preliminary studies with ATPases suggest a possible site for the action of such genes and indicate that this enzyme may be influenced by salinity.

General summary

Many different aspects of plant responses to different salt treatments were studied in various cultivars of tomato, in related wild species and in their hybrids. The treatments included addition of NaCl to nutrient solution, addition of NaCl + CaCl₂ to nutrient solution and to sand culture. The study included: growth of the whole plant and of isolated organs, photosynthesis, stomatal behavior, respiration, distribution of ¹⁴C, membrane response to stresses, ATPase activity, distribution of ions in the various parts of the plant. Some of the studies are completed, some are partial and some are only preliminary. The work can be regarded, therefore, as a broad base for the establishment of the final and integrated structure whose completion awaits further experimentation.

Manuscript No. 1

Salt tolerance in the wild relatives of the cultivated tomato :

$^{86}\text{Rb}^+$ uptake by excised root tips of Lycopersicon esculentum,
L. peruvianum, L. cheesmanii, and Solanum pennellii.

The three wild relatives of tomato, Lycopersicon peruvianum, L. cheesmanii and Solanum pennellii were found to be more salt-tolerant than the cultivated species L. esculentum (Tal, 1971; Rush and Epstein, 1976; and 1981, Dehan and Tal, 1978). As compared with the cultivated tomato, the growth of the wild species was less impaired under NaCl salinity. The latter species were also found to accumulate more Cl^- and Na^+ and less K^+ . A better osmotic adjustment was suggested as the mechanism responsible for the superior performance of the wild species under salinity. The osmotic adjustment in these plants seems to be regulated mainly by their ability to absorb and to transport large amounts of inorganic ions.

In order to survive in saline environments several plants possess mechanisms regulating the internal content of essential ions (such as K^+) absorbed from a solution which contains high levels of chemically similar ions (e.g. Na^+). An efficient selective mechanism, designated as the dual mechanism of ion transport, was suggested as an integral component of plants response to salinity (Rains and Epstein 1967a and 1967b; Rains, 1972 and 1979).

In contrast to the cultivated tomato, the three wild species do not absorb K^+ selectively to Na^+ under high NaCl salinity (Tal, 1971; Rush and Epstein, 1976 and 1981; Dehan and Tal, 1978). The level of K^+ in the leaf may be controlled by mechanisms located at different sites (Läuchli, 1972). Nissen (1974) proposed that the uptake through the plasmalemma of the

cortical roof cells is the rate-limiting step in K^+ transport to the shoot. Hiatt (1963) suggested that the differences between field-grown plants of certain tobacco varieties with respect to K^+ -uptake were due to differences in the ability of their roots to absorb K^+ .

The present paper was aimed at studying the role of the root in controlling the level of K^+ in the cultivated tomato and its three wild relatives under control and saline conditions. For that, the uptake of $^{86}\text{Rb}^+$ (used as a tracer for K^+) was studied in excised roots of L. esculentum, L. cheesmanii, L. peruvianum, and S. pennellii as a function of time, KCl concentration, and as affected by NaCl concentration.

Materials and Methods

Plant material

The species used in the present study were the cultivated tomato Lycopersicon esculentum cv. VF (VF) and the wild species L. peruvianum (Lp), L. cheesmanii ecotype No. 1400 (Lc), and Solanum pennellii accession Atico (Sp).

Plants were grown in the greenhouse during the summer with day/night temperatures of about 30/20° C. Seedlings were prepared as described in manuscript No. 1.

The plants were transferred to 0.5 mM CaSO_4 solution 48 h before the beginning of the experiments.

$^{86}\text{Rb}^+$ uptake as a function of time

Root tips, 3.5 cm long, were excised from plants bearing 6-8 leaves, weighed, 10 per replicate, and transferred to 250-ml Erlenmeyer flasks

containing 150 ml aerated solution of 0.1 mM KCl, 0.5 mM CaSO_4 , and 10,000 cpm/ml $^{86}\text{Rb}^+$ (The Radiochemical Centre, Amersham, England). The flasks were incubated in a water bath at 27°C. Root samples were taken at various time intervals and washed for 10 min in cold 0.5 mM CaSO_4 . The roots were then blotted carefully and transferred to vials containing 10 ml scintillation solution toluene:triton 2:1 (by vol.), POPOP 0.1 g/l, PPO 5 g/l). The vials were counted for β -emission.

$^{86}\text{Rb}^+$ uptake as a function of KCl concentration

Root tips were prepared as described above. Groups of 10 roots were transferred to 50-ml Erlenmeyer flasks containing 10 ml aerated 0.5 mM CaSO_4 solution containing various concentrations of KCl and 10,000 cpm/ml $^{86}\text{Rb}^+$. The flasks were incubated at 29°C for 80 min. At the end of the experiment, the roots were blotted, and the radioactivity was determined as above.

Effect of NaCl on $^{86}\text{Rb}^+$ uptake

The effect of NaCl on Rb^+ uptake was studied in root tips prepared as described above. Groups of 10 roots were transferred to 50 ml Erlenmeyer flasks containing 10 ml solution of 5 mM CaSO_4 , 0.5 mM KCl, various concentrations of NaCl, and 10,000 cpm/ml $^{86}\text{Rb}^+$.

Results

$^{86}\text{Rb}^+$ uptake as a function of time

In all four species, the rate of $^{86}\text{Rb}^+$ uptake was found to be linear with time up to at least 120 min (Fig. 1), the rate being much higher in the cultivated than in the wild species. Among the wild species, Sp roots had

the lowest rate of uptake.

$^{86}\text{Rb}^+$ uptake as a function of KCl concentration

The rate of $^{86}\text{Rb}^+$ uptake in roots excised from the cultivated plants was higher than in those of the wild plants throughout the range of concentrations used (0.001 to 0.1 mM) (Fig. 2).

Effect of NaCl on $^{86}\text{Rb}^+$ uptake

$^{86}\text{Rb}^+$ uptake was stimulated in all four species by the increase of NaCl concentration from 0.01 up to 50 mM (Fig. 3). At a higher concentration of NaCl (100 mM), the uptake was lowered in VF, Lp, and Lc roots to a level which was still higher than that of the control.

Discussion

Saline environments are characterized by the predominance of Na^+ , a nonessential element for several plants, over essential elements such as K^+ (Rains, 1979). The ability of the root to regulate the internal content of essential ions, i.e. to absorb K^+ selectively to Na^+ in saline media, might be, therefore, an important factor in their adaptation to saline environments. In contrast to the cultivated tomato, its wild salt-tolerant relatives do not absorb K^+ preferentially over Na^+ under high NaCl salinity. These plants accumulate large amount of Na^+ , and their K^+ content decreases drastically under NaCl salinity (Tal, 1971; Rush and Epstein, 1976 and 1981; Dehan and Tal, 1978). As suggested by Rush and Epstein (1981) for L. cheesmanii, the response to salinity of the three wild species studied here, L. cheesmanii, L. peruvianum, and S. pennellii, resembles that of typical halophytes in which

Na^+ replaces K^+ in, at least, part of its physiological role.

The level of K^+ in the leaf is determined by the balance between the influx into the cortical cells of the root, the efflux to the surrounding medium, the transport from the xylem parenchyma to the vessels, the transpiration rate, and the transport from the xylem vessels into the leaf cells (Läuchli, 1972). The influx into the cells of the root was suggested as the rate-limiting step controlling the internal balance of K^+ in the plant (Nissen, 1974; Hiatt, 1963). The major finding of the present study is that excised root tips of the three salt-tolerant wild species have a lower ability to absorb K^+ than the cultivated tomato. This lower ability of the roots of the wild plants to absorb Rb^+ and K^+ may be the consequence of a difference in Na^+/K^+ carrier system and/or in membrane permeability between the wild and cultivated species. Our finding is in a sharp contradiction to the results of Rush and Epstein (1981), who found that the excised root tips of L. cheesmanii ecotype No. 1401 absorbed more Rb^+ than those of the cultivated species. This discrepancy is difficult to explain at the present stage. Whether it results from the difference in K^+ level in the plants growth media (our plants were transferred to 0.5 mM CaSO_4 solution two days before the excision of the roots, while those used by Rush and Epstein were maintained in the Hoagland solution) remains to be elucidated.

The finding of Tal, Heikin and Dehan (1978) suggests that the difference between the cultivated species and its wild relatives in the regulation of the K^+/Na^+ ratio is a cellular characteristic, which is not confined to a specific organ or tissue. They found that calli derived from either the leaf, stem, or root of the wild species Lp and Sp behave under salinity in a similar fashion to the whole plant. These calli accumulated more Na^+ and less K^+ than those

of the cultivated species under salinity. In order to confirm this suggestion we plan to study the uptake of $^{86}\text{Rb}^+$ into cells or naked protoplasts isolated from the leaves of the cultivated and wild species of tomato.

Another finding of the present study which requires a further elaboration is the enhancement of Rb^+ uptake in both wild and cultivated species by Na^+ .

Summary

Root tips of the wild relatives of the cultivated tomato, Lycopersicon cheesmanii, L. peruvianum, and Solanum pennellii accumulated less $^{86}\text{Rb}^+$ than those of the cultivated tomato. This phenomenon was evident in root tips excised from plants maintained for two days in CaSO_4 solution (low salt plants). The ability of the roots of the wild plants to absorb Rb^+ , which represents K^+ , may be the consequence of a difference in a Na^+/K^+ carrier system and/or in membrane permeability between the wild and the cultivated species.

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Figure Legends

Fig. 1. $^{86}\text{Rb}^+$ uptake by excised root tips of L. esculentum (———), L. peruvianum (-----), L. cheesmanii (-.-.-.-.-), and Solanum pennellii (.....) plants as a function of time.

Fig. 2. $^{86}\text{Rb}^+$ uptake by excised root tips of L. esculentum (———), L. peruvianum (-----), L. cheesmanii (-.-.-.-.-) and S. pennellii (.....) as a function of KCl concentration.

Fig. 3. Effect of different NaCl concentrations on $^{86}\text{Rb}^+$ uptake by excised root tips of L. esculentum (———), L. peruvianum (-----), L. cheesmanii (-.-.-.-.-), and S. pennellii (.....).

Salt tolerance in the wild relatives of the cultivated tomato: Responses of Lycopersicon esculentum, L. cheesmanii, L. peruvianum, Solanum pennellii and F_1 hybrids to high salinity.

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Abstract

The performance of three wild relatives of the cultivated tomato, Lycopersicon cheesmanii, L. peruvianum, and Solanum pennellii, and two tomato cultivars in control and saline media was studied comparatively. The parameters studied were elongation rate of the main stem, succulence, and accumulation of Na^+ , K^+ , and Cl^- in the roots, stem, leaf, and shoot tip. The same parameters but in the leaf only were also studied comparatively in two of the wild species L. cheesmanii and S. pennellii, the same two cultivars and F_1 hybrids.

Under control conditions, the elongation rate of the stem of the two cultivars was higher than that of the wild plants, but under salinity it was relatively lower. Among all species, S. pennellii was the most succulent in all its major parts under both control and saline conditions. The wild species, especially S. pennellii, showed high accumulation of Na^+ in the leaf and top and a greater decrease in K^+ content under salinity as compared with the cultivated plants. In all three species, Na^+ probably substitutes for potassium in, at least, some of its physiological functions.

Complete dominance of S. pennellii over the cultivated plants is indicated for the relative decrease of elongation rate and K^+ level for the increase of succulence under salinity. In contrast, L. cheesmanii seems to be completely dominant only for the relative decrease of K^+ under salinity.

Introduction

One approach to the exploitation of saline soils is the improvement of the salt tolerance of cultivated species. This may be achieved by exploiting intra-specific variability (Dewy, 1962; Greenway, 1962; Epstein et al., 1979), or in species lacking such variability, genes may be transferred from closely related wild species adapted to high salinity.

Wild relatives of the cultivated tomato can be used as a source of genes for high salt tolerance. Tal (1971) and Dehan and Tal (1978) found that plants of Lycopersicon peruvianum and Solanum pennellii suffered under salinity less than the cultivated tomato. Compared with the cultivated tomato, these wild species showed a lower decrease in dry weight and relative water content under salinity, and they were more succulent and accumulated more Na^+ and Cl^- and less K^+ . Rush and Epstein (1976, 1981) reported similar results for L. cheesmanii grown in sea water.

In the present work, intact plants of the three wild species L. cheesmanii, L. peruvianum, and Solanum pennellii, two tomato cultivars and F_1 hybrids were compared with respect to their response to high salinity. Such comparative studies of different species will facilitate the evaluation of their relative performance and the characterization of the mechanisms which contribute to their salt tolerance. Such a study may also reveal the dominance relationships between the wild species, on the one hand, and the cultivated species, on the other hand, for these characteristics, a knowledge which is essential for an effective breeding for salt tolerance.

Materials and Methods

Plant Material

The species used in the present research included the cultivated tomato Lycopersicon esculentum Mill. cv. 'Heinz 1350' (H) and 'VF 234' (VF), the wild species L. cheesmanii Riley ecotype No. 1400 (Lc), L. peruvianum (L.) Mill. (Lp), and Solanum pennellii Cor. accession Atico (Sp), and the F_1 hybrids between each of the cultivars

and each of the two wild species Lc and Sp, the cultivars being used as the maternal parents. Seeds of the wild species were collected by Dr. C.M. Rick of the University of California, Davis, from plants growing in dry habitats: Lc in the Galapagos Islands, Lp in Chile and Sp in Peru.

Plants were grown in the greenhouse during the winter with day/night temperatures of about 20/10°C. Seeds of H. VF and Lp and the F₁ hybrids were sown in vermiculite. Seeds of Lc and Sp were sown on wet filter paper in petri dishes after treatment with a solution of hypochlorite (3.5% active chlorine), those of Lc for 90 min and those of Sp for 45 min. The hypochlorite dissolves the seed coat, which prevents germination (Rick and Bowman 1961). Before sowing, the seeds were rinsed with tap water. Upon emergence of roots the seeds were transferred to vermiculite. Young seedlings bearing one or two leaves were transferred to aerated Hoagland solution, 20 seedlings per container of 22 litres. The solution contained (mg l⁻¹): Ca(NO₃)₂·H₂O, 1235; KNO₃, 535; MgSO₄·7H₂O, 525; KH₂PO₄, 145; Sequestrene 138 Fe, 50; H₃BO₃, 2.9; MnCl₂·4H₂O, 1.8; ZnCl₂, 0.12; CuCl₂·2H₂O, 0.048; H₂MoO₄·H₂O, 0.04. Salt treatment was initiated at the 3-4 leaf stage by adding NaCl (25-meq l⁻¹ day⁻¹ doses) to the containers of the salt-treated plants up to a final concentration of 100 or 150 meq l⁻¹. Control plants remained in Hoagland solution.

Stem Elongation Rate

The length of the main stem was measured on the day on which salt was first added (day 0) and then at 3-day intervals to day 15. Stem growth was measured by expressing the length at each time interval as a percentage of the length at day 0. Elongation rate was calculated from the slope of the regression line between stem growth (Y) and time (X). The measurements included 8 plants in each species-treatment combination.

Succulence and Accumulation of Ions

Succulence and ion content were determined 12 days after the last addition of salt in the top of the shoot (including the meristem and the very small leaves), the youngest fully-expanded leaf, the middle section of the stem, and the proximal part (about 20 mm) of the main root in VF, H, Lc, Sp, and Lp, and only in the youngest fully-expanded leaf in VF, H, Lc, Sp, and F_1 hybrids. Fresh material was weighed immediately after excision and again after oven-drying at 85°C for 24 h, and succulence was calculated as the ratio fresh weight/dry weight. The roots were rinsed for 5 min in a solution of 10 mM CaSO_4 and 0.5 M mannitol and blotted carefully before weighed.

Na^+ , K^+ , and Cl^- were determined in dried samples. The weighed dried samples were transferred to 3 ml of 0.1 M nitric acid for 72 h at room temperature. Chloride was determined in samples of this solution with a Buchler-Cotlove chloridometer, and sodium and potassium were determined with a Corning-EEL flame photometer. The measurements included three samples taken from three different plants in each species-treatment combination.

Results

Stem Elongation Rate

Stem elongation rate of both cultivars was higher than that of the wild plants under control conditions. Under salinity of 100 meq l^{-1} , the rate decreased in all plants, the decrease being smaller in the wild species (Table 1).

The decrease of elongation rate under salinity in three of the F_1 hybrids, VF $\times$ Sp, H $\times$ Sp, and H $\times$ Lc, was smaller than in the cultivated parents. The rate decreased similarly in the two cultivars and the hybrid VF $\times$ Lc (Table 2).

Succulence

In all four species, the ratio of fresh weight to dry weight was the highest in the stem, lower in the root, and the lowest in the leaf (Table 3). Among the wild species, Sp was the most succulent in all its major parts under both control and saline conditions. Lp was the least succulent except in its leaf. Salinity

increased succulence most noticeably in leaves of Sp and decreased it in the top of the cultivars.

Succulence was higher in the VF $\times$ Lc and VF $\times$ Sp hybrids than in the VF parent, and similar in H $\times$ Lc and H $\times$ Sp to that of the H parent (Table 4). Under salinity, the succulence increased in the wild parents and the four F₁ hybrids but remained unchanged in the two cultivars. The increase in VF $\times$ Sp and H $\times$ Sp was similar to that in the Sp parent. The increase in VF $\times$ Lc and H $\times$ Lc was intermediary between the parental values.

Accumulation of Ions

Sodium

In all four species, sodium concentration per unit dry weight increased under salinity, its content was the highest in the stem, lower in the root, and the lowest in the leaf (Table 3). The wild species, and especially Sp, accumulated under salinity much more Na⁺ than the cultivars; the differences between the species was most noticeable in the leaf and top.

The accumulation of Na⁺ in the leaves of the wild parents and the F₁ hybrids was higher than in the cultivated varieties (Table 6). The accumulation was lower than the midparental values in VF $\times$ Lc, VF $\times$ Sp, and H $\times$ Sp, and equal to it in H $\times$ Lc.

Potassium

Potassium concentration in control plants was the highest in the stem, lower in the root and top, and the lowest in the leaf in all four species, similarly to the distribution of Na⁺ in salt-treated plants (Table 5). Under control conditions, Sp stem and roots accumulated the largest amount of K⁺ among the species studied, but at the same time Sp leaves contained the lowest concentration of K⁺. Under salinity, the concentration of K⁺ decreased in all major parts studied in all four species, the decrease being the greatest in the stem, smaller in the leaf and root,

and the smallest in the top. In all parts, decreases in K^+ under salinity are greater in the wild than in the cultivated species.

K^+ concentration in the leaves of the VF $\times$ Sp and H $\times$ Sp hybrids was similar to that of Sp, while K^+ in VF $\times$ Lc and H $\times$ Lc hybrids was similar to that of the cultivated parents (Table 6). Under salinity, potassium content decreased in leaves of all plants; the decrease was, however, much greater in the wild species and in all F_1 hybrids.

Chloride

Like Na^+ , Cl^- concentration increased in all four plant types under salinity. Unlike Na^+ , chloride content in NaCl-treated plants of all four species was the highest in the root, lower in the stem, and the lowest in the leaf and the top (Table 3). The greatest difference in Cl^- accumulation was found between the stem and the leaves. The accumulation of chloride in the root, stem, leaf and top was higher in Lc and Sp plants as compared with the two cultivars. Chloride accumulation was most noticeable in the root and stem in Sp and in the top in Lc.

The accumulation of Cl^- in the wild parents and the F_1 hybrids, except in H $\times$ Sp, was higher than in the cultivars (Table 6). The value of Cl^- accumulation in VF $\times$ Sp and H $\times$ Lc was higher than the midparental values, equal to it in VF $\times$ Lc and lower in H $\times$ Sp.

K^+/Na^+ and Cl^-/Na^+ Ratios

The ratios K^+/Na^+ and Cl^-/Na^+ in the root, stem, leaf and top of H, VF, Lc, Lp and Sp plants treated with 100 meq l^{-1} NaCl are presented in Table 7. In all parts studied, K^+/Na^+ ratio was larger in the cultivated than in the wild species; the greatest difference was found in the root and shoot in Lc and in the shoot, leaf and top in Sp.

The K^+/Na^+ ratio in the leaves was similar to that of the wild parent in the VF $\times$ Sp, H $\times$ Lc, and H $\times$ Sp hybrids and almost equal to the midparental value in VF $\times$ Lc (table 8).

Cl^-/Na^+ ratio in the root and stem was similar in all four species, being greater than one (except in Lc) in the root and smaller than one in the stem (Table 7). The situation was different in the leaf and top. While in the cultivated plants the ratio was close to one, it was much lower than one in the wild plants.

The Cl^-/Na^+ ratio in the leaves was equal to that of the cultivated parent in VF $\times$ Lc, equal to the midparental value in VF $\times$ Sp, and lower in H $\times$ Lc and H $\times$ Sp (Table 8).

Discussion

The wild relatives of the cultivated tomato L. cheesmanii, L. peruvianum, and S. pennellii are distinguished from the cultivated species by: 1) a lower absolute elongation rate of the stem under control conditions and a smaller relative decrease of this rate under high salinity; 2) a higher accumulation of Na^+ , which was most pronounced in the leaf and top of the shoot; 3) a greater decrease of K^+ content under salinity, especially in the stem and to a lesser extent in the leaf and the top.

A positive correlation between Na^+ accumulation and salt tolerance was reported previously in studies in which each of the wild species included here was studied separately (Tal, 1971; Dehan and Tal 1978; Rush and Epstein 1976, 1981). This correlation was confirmed by the present results.

In contrast to the cultivated plants, all three wild species do not absorb K^+ selectively to Na^+ under high NaCl salinity. Consequently, their Na^+ content increases and K^+ content decreases more under this condition. The substitution of K^+ by Na^+ is clearly indicated by their distribution in the control and salt-treated plants, respectively. In all plant types studies, the relative distri-

bution of K^+ in the major parts under control conditions is identical to the distribution of Na^+ under NaCl salinity. Rush and Epstein (1981), working with Lc accession 1401, suggested that the accumulation of Na^+ can be used as a key characteristic in the evaluation of potential germplasms in selection and breeding programs aimed at the improvement of the salt tolerance of the tomato.

The extent and nature of the physiological functioning of Na^+ in the wild relatives of tomato under salinity is still an open question. Another open question is how the electrical neutrality is maintained in the leaves and top of the wild species, in which the Cl^-/Na^+ ratio is much lower than one.

While the two tomato cultivars resembled each other in most of their responses to salinity, differences were found between the responses of the wild species. Among the wild plants, those of Sp are distinguished by their high succulence of all their major vegetative parts. Resulting from the dilution effect of succulence, Sp plants have the lowest concentration of Cl^- per unit fresh weight under salinity as calculated from Table 3. Similarly, this species accumulates the largest amount of Na^+ in the shoot and the largest amount of K^+ in the root, as expressed per unit of dry or fresh weight. Consequently, the ratio of K^+/Na^+ in Sp relatively to Lc and Lp plants is lowest in the shoot and highest in the root. A high succulence can be also used as a key characteristic to evaluate the potential of hybrids containing Sp germplasm in selection and breeding programs for improved salt tolerance. Plants of the wild species Lp are the least succulent among the plants studied except its leaf. Plants of Lc are distinguished by higher concentration of chloride in their shoot under salinity.

In all five plant types, the relative degree of succulence in the various parts of the plant is positively correlated with the distribution of sodium, both being the highest in the stem, lower in the root and the lowest in the leaf and top. The relation between Na^+ and succulence, which was demonstrated previously in other investigations, was discussed by Rains (1972).

The dominance relations for the various characteristics between the parental species as expressed in the F_1 hybrids are presented in Table 9. In all of the F_1 combinations except for $\text{VF} \times \text{Lc}$, the stem elongation rate under both control conditions and salinity was higher than in both parents, indicating heterosis (Allard 1960). The present results suggest a complete dominance of Sp over the cultivated parents with respect to the relative decrease of the growth rate and K^+ level and for the increase of the succulence under salinity. In contrast to Sp, Lc was dominant only for the relative decrease of K^+ under salinity.

Rick (1960) ascertained that 17 out of 30 morphological differences between L. esculentum and Sp showed dominance in the F_1 hybrid between them, 6 for the characteristics of the former and 11 for those of the latter parent. According to Dobzhansky (1951), clear dominance expression, which is common in intraspecific crosses, is an exception in interspecific crosses mainly because of the high interaction of different modifier gene complexes of the two parental species. The high proportion of characters which show clear dominance in the cross between L. esculentum and Sp has been explained by their cross-compatibility, which indicates that a high proportion of modifier complexes is common to the two species, and the finding that several of these morphological characters are controlled by a small number of genes (Tal 1967). Such an explanation might also be correct for the dominant expression of some of the characteristics studied here in the $\text{VF} \times \text{Sp}$ and $\text{H} \times \text{Sp}$ crosses.

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Table 1. Elongation rate of the main stem of the cultivated tomato (VF, H) and its wild relatives (Lc, Sp, Lp) as affected by 100 meq l⁻¹ NaCl. The values represent the slope of the regression line between stem growth and time $\pm t_{.05} \cdot S_b^1$ (for more details see Materials and Methods).

Plant type	Control	NaCl	% of control
VF	0.24 $\pm$ 0.03	0.12 $\pm$ 0.01	50.0
H	0.21 $\pm$ 0.03	0.09 $\pm$ 0.00	42.8
Lc	0.10 $\pm$ 0.01	0.09 $\pm$ 0.00	90.0
Lp	0.15 $\pm$ 0.00	0.11 $\pm$ 0.00	68.7
Sp	0.17 $\pm$ 0.02	0.10 $\pm$ 0.02	58.8

1 Following Snedecor and Cochran (1956).

Table 2. Elongation rate of the main stem of the cultivated tomato (VF, H), its wild relatives (Lc, Sp), and F_1 hybrids as affected by 150 meq^{-1} NaCl. Other details are as in Table 1.

	VF	H	Lc	Sp	VF×Lc	VF×Sp	H×Lc	H×Sp
Control	0.39 ± 0.11	0.28 ± 0.07	0.16 ± 0.04	0.15 ± 0.03	0.27 ± 0.07	0.41 ± 0.12	0.35 ± 0.12	0.48 ± 0.15
Salt-treated	0.15 ± 0.03	0.11 ± 0.01	0.10 ± 0.02	0.07 ± 0.01	0.11 ± 0.02	0.20 ± 0.03	0.17 ± 0.03	0.25 ± 0.07
% of control	38.5	39.3	62.5	46.7	40.7	48.8	48.6	52.1

Table 3. Responses of the cultivated tomato (VF, H) and its wild relatives (Lc, Lp and Sp) to 100 meq l^{-1} NaCl (values are given $\pm$ S.E.).

Plant type	Data for plant parts:							
	Root		Stem		Leaf		Top	
	Control	NaCl	Control	NaCl	Control	NaCl	Control	NaCl
(a) Succulence (fresh wt/dry wt)								
VF	15.6 $\pm$ 0.7	12.5 $\pm$ 0.1	23.3 $\pm$ 1.0	20.4 $\pm$ 1.6	7.8 $\pm$ 0.1	6.4 $\pm$ 0.2	9.5 $\pm$ 0.2	7.9 $\pm$ 0.7
H	14.1 $\pm$ 0.1	13.6 $\pm$ 0.9	24.7 $\pm$ 0.3	22.0 $\pm$ 2.7	7.8 $\pm$ 0.2	6.8 $\pm$ 0.1	8.6 $\pm$ 0.3	6.9 $\pm$ 0.5
Lc	17.7 $\pm$ 1.3	14.2 $\pm$ 0.8	19.4 $\pm$ 1.0	19.3 $\pm$ 0.5	7.9 $\pm$ 0.2	7.5 $\pm$ 0.2	9.3 $\pm$ 0.6	9.0 $\pm$ 0.6
Lp	13.4 $\pm$ 0.8	11.7 $\pm$ 0.9	12.7 $\pm$ 0.8	13.5 $\pm$ 1.9	8.5 $\pm$ 0.2	8.6 $\pm$ 0.8	7.5 $\pm$ 0.2	6.8 $\pm$ 0.4
Sp	17.1 $\pm$ 1.1	17.3 $\pm$ 1.0	26.7 $\pm$ 2.4	31.8 $\pm$ 0.5	10.3 $\pm$ 0.4	12.4 $\pm$ 0.8	9.2 $\pm$ 0.3	9.1 $\pm$ 0.3
(b) Sodium (meq g^{-1} dry wt)								
VF	0.07 $\pm$ 0.03	1.00 $\pm$ 0.01	0.11 $\pm$ 0.04	2.65 $\pm$ 0.21	0.01 $\pm$ 0.00	0.15 $\pm$ 0.02	0.02 $\pm$ 0.00	0.26 $\pm$ 0.09
H	0.06 $\pm$ 0.03	1.05 $\pm$ 0.16	0.20 $\pm$ 0.09	2.37 $\pm$ 0.09	0.01 $\pm$ 0.00	0.21 $\pm$ 0.03	0.06 $\pm$ 0.06	0.15 $\pm$ 0.06
Lc	0.04 $\pm$ 0.04	2.40 $\pm$ 0.59	0.59 $\pm$ 0.24	4.68 $\pm$ 0.51	0.07 $\pm$ 0.00	0.81 $\pm$ 0.06	0.32 $\pm$ 0.08	1.10 $\pm$ 0.15
Lp	0.00 $\pm$ 0.00	1.15 $\pm$ 0.26	0.06 $\pm$ 0.06	2.05 $\pm$ 0.05	0.16 $\pm$ 0.04	1.30 $\pm$ 0.25	0.08 $\pm$ 0.08	1.03 $\pm$ 0.26
Sp	0.21 $\pm$ 0.12	1.69 $\pm$ 0.14	0.38 $\pm$ 0.27	7.16 $\pm$ 0.07	0.01 $\pm$ 0.00	1.60 $\pm$ 0.12	0.03 $\pm$ 0.01	2.16 $\pm$ 0.33
(c) Chloride (meq g^{-1} dry wt)								
VF	0.14 $\pm$ 0.05	1.48 $\pm$ 0.05	0.28 $\pm$ 0.05	1.16 $\pm$ 0.04	0.02 $\pm$ 0.01	0.17 $\pm$ 0.00	0.07 $\pm$ 0.01	0.19 $\pm$ 0.03
H	0.20 $\pm$ 0.02	1.58 $\pm$ 0.12	0.32 $\pm$ 0.05	1.32 $\pm$ 0.12	0.04 $\pm$ 0.01	0.27 $\pm$ 0.01	0.06 $\pm$ 0.00	0.14 $\pm$ 0.02
Lc	0.03 $\pm$ 0.03	1.80 $\pm$ 0.14	0.12 $\pm$ 0.00	1.41 $\pm$ 0.05	0.01 $\pm$ 0.01	0.35 $\pm$ 0.03	0.03 $\pm$ 0.02	0.40 $\pm$ 0.05
Lp	0.09 $\pm$ 0.00	1.47 $\pm$ 0.25	0.13 $\pm$ 0.03	0.91 $\pm$ 0.12	0.02 $\pm$ 0.00	0.32 $\pm$ 0.07	0.04 $\pm$ 0.01	0.17 $\pm$ 0.04
Sp	0.17 $\pm$ 0.06	2.26 $\pm$ 0.13	0.23 $\pm$ 0.03	1.78 $\pm$ 0.05	0.02 $\pm$ 0.02	0.28 $\pm$ 0.02	0.04 $\pm$ 0.00	0.25 $\pm$ 0.03

Table 4. Succulence (fresh wt/dry wt $\pm$ S.E.) in leaves of the cultivated tomato (VF, H), wild species (Lc, Sp), and F_1 hybrids as affected by 150 meq l^{-1} NaCl.

	VF	H	Lc	Sp	VF $\times$ Lc	VF $\times$ Sp	H $\times$ Lc	H $\times$ Sp
Control	7.4 $\pm$ 0.1	8.1 $\pm$ 0.2	9.0 $\pm$ 0.1	10.6 $\pm$ 0.3	8.5 $\pm$ 0.2	8.8 $\pm$ 0.1	8.8 $\pm$ 0.2	8.8 $\pm$ 0.3
Salt-treated	7.5 $\pm$ 0.2	7.8 $\pm$ 0.2	10.9 $\pm$ 0.4	13.0 $\pm$ 0.5	9.1 $\pm$ 0.2	10.7 $\pm$ 0.4	9.5 $\pm$ 0.3	11.0 $\pm$ 0.3
% of control	102.6	96.0	120.6	122.1	107.9	122.0	107.4	125.0

Table 5. Potassium content (meq g^{-1} dry wt $\pm$ S.E.) in root, stem, leaf and top of plants of the cultivated tomato (VF, H) and its wild relatives (Lc, Lp and Sp) as affected by 100 meq l^{-1} NaCl.

Plant type	Root		% of Necr-ease	Stem		% of Decr-ease	Leaf		% of Decr-ease	Top		% of Decr-ease
	Control	NaCl		Control	NaCl		Control	NaCl		Control	NaCl	
VF	2.04 $\pm$ 0.14	1.36 $\pm$ 0.06	33.3	3.74 $\pm$ 0.16	2.32 $\pm$ 0.18	38.0	1.28 $\pm$ 0.03	1.01 $\pm$ 0.04	21.1	1.56 $\pm$ 0.02	1.36 $\pm$ 0.10	12.6
H	2.14 $\pm$ 0.01	1.61 $\pm$ 0.08	24.8	3.72 $\pm$ 0.02	1.97 $\pm$ 0.28	47.1	1.19 $\pm$ 0.03	0.93 $\pm$ 0.01	21.9	1.31 $\pm$ 0.06	1.26 $\pm$ 0.08	3.8
Lc	1.72 $\pm$ 0.20	0.84 $\pm$ 0.10	50.2	3.11 $\pm$ 0.23	0.73 $\pm$ 0.12	76.5	1.22 $\pm$ 0.06	0.81 $\pm$ 0.05	33.6	1.48 $\pm$ 0.16	0.97 $\pm$ 0.03	34.5
Lp	1.93 $\pm$ 0.01	1.08 $\pm$ 0.05	44.1	2.69 $\pm$ 0.25	1.09 $\pm$ 0.18	59.5	1.11 $\pm$ 0.04	0.72 $\pm$ 0.04	35.1	1.28 $\pm$ 0.05	0.95 $\pm$ 0.03	25.8
Sp	2.91 $\pm$ 0.17	2.28 $\pm$ 0.07	21.7	4.29 $\pm$ 0.34	0.99 $\pm$ 0.06	76.9	0.57 $\pm$ 0.05	0.43 $\pm$ 0.02	24.6	1.43 $\pm$ 0.02	1.23 $\pm$ 0.06	14.0

Table 6. Content (meq g⁻¹ dry wt ± S.E.) of Cl⁻, Na⁺, and K⁺ in leaves of the cultivated tomato (VF, H) and wild species (Lc, Sp) and F₁ hybrids as affected by 150 meq l⁻¹ NaCl.

Plant type	Cl ⁻ accumulation in salt-treated plants*	Na ⁺ accumulation in salt-treated plants*	K ⁺ accumulation	
			Control	Salt-treated
				% of control
VF	0.80 ± 0.06	0.81 ± 0.18	1.34 ± 0.06	0.94 ± 0.04
H	1.07 ± 0.04	0.46 ± 0.08	1.31 ± 0.04	0.95 ± 0.05
Lc	1.58 ± 0.08	2.23 ± 0.22	1.46 ± 0.05	0.65 ± 0.05
Sp	1.32 ± 0.12	2.95 ± 0.25	1.00 ± 0.06	0.35 ± 0.05
VF × Lc	1.18 ± 0.08	1.21 ± 0.15	1.30 ± 0.04	0.70 ± 0.06
VF × Sp	1.20 ± 0.08	1.63 ± 0.19	1.05 ± 0.03	0.33 ± 0.05
H × Lc	1.43 ± 0.04	1.32 ± 0.28	1.33 ± 0.03	0.54 ± 0.05
H × Sp	1.04 ± 0.06	1.29 ± 0.09	1.01 ± 0.03	0.41 ± 0.05

* Control plants contained only trace amounts of Na⁺ and Cl⁻ ions.

Table 7. K^+/Na^+ and Cl^-/Na^+ ratios in different parts of NaCl-treated (100 meq l^{-1}) cultivated (VF, H) and wild (Lc, Lp and Sp) plants of tomato.

Plant type	K^+/Na^+				Cl^-/Na^+			
	Root	Stem	Leaf	Top	Root	Stem	Leaf	Top
VF	1.4	0.9	10.0	4.7	1.5	0.4	1.1	0.7
H	1.6	0.7	9.0	13.0	1.5	0.5	1.3	0.9
Lc	0.3	0.1	1.6	0.9	0.7	0.3	0.4	0.4
Lp	0.9	0.5	0.8	1.0	1.3	0.4	0.2	0.2
Sp	1.3	0.1	0.4	0.5	1.3	0.2	0.2	0.1

Table 8. K^+/Na^+ and Cl^-/Na^+ ratios in leaves of NaCl-treated (150 meq l^{-1}) cultivated (VF, H), wild (Lc, Sp) and F_1 hybrids of tomato

	VF	H	Lc	Sp	VF × Lc	VF × Sp	H × Lc	H × Sp
K^+/Na^+	1.2	2.1	0.3	0.1	0.6	0.2	0.4	0.3
Cl^-/Na^+	1.0	2.3	0.7	0.4	1.0	0.7	1.1	0.8

Table 9. Dominance relations in the F_1 hybrids between the cultivated (VF, H) and the wild species (Lc, Sp).

Het - heterosis; D () - Dominance of the parental species in parentheses; I - an intermediate expression; PD () - partial dominance of the parent in parentheses

	VF × Lc	VF × Sp	H × Lc	H × Sp
<u>Elongation rate</u>				
Control level	I	D(VF)	Het	Het
NaCl-treated	PD(Lc)	Het	Het	Het
NaCl-induced decrease	D(VF)	D(Sp)	I	Het
<u>Succulence</u>				
Control level	I	I	I	I
NaCl-treated	I	I	I	I
NaCl-induced decrease	PD(VF)	D(Sp)	I	D(Sp)
<u>Cl⁻ content</u>				
Salt-treated	I	PD(Sp)	PD(Lc)	D(H)
<u>Na⁺ content</u>				
Salt-treated	PD(VF)	I	I	PD(H)
<u>K⁺ content</u>				
Control level	D(VF)	D(Sp)	D(H)	D(Sp)
NaCl-treated	PD(Lc)	D(Sp)	D(Lc)	D(Sp)
NaCl-induced decrease	D(Lc)	D(Sp)	D(Lc)	D(Sp)

Salt tolerance in the wild relatives of the cultivated tomato:

Responses of NaCl-treated Lycopersicon esculentum,
L. cheesmanii, L. peruvianum, and Solanum pennelli plants
to decreasing the salt concentration in the root medium.

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Abstract

The response of NaCl-treated plants of the cultivated tomato, Lycopersicon esculentum (VF) and its wild salt tolerant relatives Lycopersicon cheesmanii (Lc), L. peruvianum (Lp), and Solanum pennellii (Sp) to decrease in salt concentration in the root medium was studied. The study included the determination of Na^+ , K^+ and Cl^- content as well as succulence in the root, stem, old and young leaf, and shoot tip.

Special points worth mentioning are: 1. sodium and Cl^- content decreased during salt dilution in all parts of all plants studied except in the old leaf; 2. the top of Lp and Sp plants excludes more Cl^- and accumulates more Na^+ as compared with the other species; 3. the changes of the ions, i.e. an increase of Na^+ and Cl^- and a decrease of K^+ , in the old leaf were exceptionally large in Lp; 4. potassium level was exceptionally low in old and young leaves of Sp plants; 5. contrary to the other parts of the plant, the top of the plant maintained an almost constant K^+ content; 6. the succulence in the top increased exceptionally more in VF plants during and after salt dilution.

Introduction

The wild species Lycopersicon peruvianum, L. cheesmanii, and Solanum pennellii suffer under salinity less than the cultivated relative tomato (L. esculentum). L. peruvianum and S. pennellii show smaller decrease in dry weight and in relative water content under NaCl salinity, and they are more succulent and accumulate more Na^+ and Cl^- and less K^+ than the cultivated species (Tal, 1971; Dehan and Tal, 1978). Similar results were reported for L. cheesmanii growing in sea water (Rush and Epstein, 1976, 1981).

In our previous investigations, plants of the cultivated and wild tomato species were exposed to constant high salinity levels. In the present work the responses of these plants to decrease in salt concentrations in the external medium were studied. Such a study is important since the salt level in saline media may change in both directions, i.e. up and down.

Materials and Methods

Plant Material

The species used in the present research included the cultivated tomato Lycopersicon esculentum cultivar 'VF 234' (VF) and the wild species L. cheesmanii ecotypes No. 1400 and 1401 (Lc 1400, Lc 1401), L. peruvianum (Lp), and Solanum pennellii accession Atico (Sp).

Plants were grown during winter in the greenhouse with daily maximum and minimum temperatures of 20/10°C. The germination procedure and the composition of the growth solution were as described by Tal and Shannon (1983). Salt treatment was initiated at the 3-4-leaf stage by adding NaCl to the containers in 25-meq·L⁻¹ doses up to a final concentration of 150 meq·L⁻¹. Twelve days after the last addition of salt, the level of NaCl in the root medium was

lowered stepwise in three days to 75, 37.5, and 0 meq·ℓ⁻¹.

Succulence and Ion Accumulation

Succulence and accumulation of ions were determined as described by Tal and Shannon (1983), starting twelve days after the last addition of salt and then every three days, in the top of the plant, the youngest fully-expanded and the oldest leaves, in the middle section of the stem, and the upper section of the root.

Results and Discussion

The changes of Na⁺, Cl⁻, K⁺, and succulence were determined in the root, stem, old and young leaves, and top of NaCl-treated VF, Lc, Lp, and Sp plants during dilution of NaCl in the root medium. The level of these variables at the beginning and the end of the experiment will be designated, respectively, as the 'original' and 'final' levels, and the difference between them as the 'relative' changes.

Sodium

Sodium content decreased during salt dilution in all parts studied except in the old leaf (Fig. 1A). The rate of decrease was greater in the wild species, especially in Sp and Lc 1400, in which it was higher originally. The final levels were similar, more or less, in all species, especially in the young leaf and the top. In the old leaf, sodium content increased in all species during the first days of dilution; the increase continued for a longer time in Lp. The final level of Na⁺ in the old leaf was higher in Sp, Lp, and Lc 1400 than in the cultivated species. The greatest relative change (an increase) in this leaf was found in Lp.

Chloride

Similarly to sodium, chloride content decreased during salt dilution in all parts but the old leaf (Fig. 1B). The species differed less in their Cl^- content in the root, stem, and the young leaf than they did for sodium. The only exceptions were the roots of Lp and Lc 1401 and the stem of Lp, which had lower original levels of Cl^- . The original level of Cl^- in the top was much lower in Sp and Lp, remaining almost unchanged with time. The other two species had a higher original level, which decreased sharply. Like Na^+ , Cl^- content increased in the old leaves during the first days of dilution in all species, the increase continuing longer in Lp. The increase was followed by a decrease in VF, Lc 1400, and Sp. The final level in old leaves was much higher in Lp and Lc 1400 than in the other species. Like for Na^+ , Lp had the greatest relative change (an increase) of Cl^- in the old leaf.

The only explanation for the increase of both Na^+ and Cl^- in the older leaves during NaCl dilution in the medium is their transport from other parts of the plant. This transport downward to the older leaves also explains in part the decrease of Na^+ and Cl^- content in the young leaf. An additional factor, affecting this decrease is a dilution effect of the growth burst, which followed the lowering of NaCl concentration in the root medium.

Potassium

Potassium content decreased in the roots of VF, Lp, and Sp and the old leaves in all species during the first days of the experiment (Fig. 1C). Later, the content remained unchanged or increased somewhat. An exception was the progressive decrease of K^+ in the old leaf of Lp. Other exceptions were the low level of K^+ in the root and stem of Lp and in the old and young leaves of Sp. Relative changes (a decrease) were found only in the root of

VF and to a lesser extent in Lp and in the old leaves of Lp and Sp. In the stem, potassium increased during salt dilution, the increase being the largest in Lc 1400 and the smallest in Lp. In the young leaf, it increased only in VF and Sp, in which the absolute level of K^+ was exceptionally low. When the concentration of K^+ was calculated per unit fresh weight it was found to decrease in the leaves of Sp and Lc 1401. Contrary to other parts of the plant, the top of the various species exhibited a great similarity with respect to both the original level of K^+ and its relative change when expressed per unit dry weight. The concentration of K^+ per unit fresh weight was lower in absolute terms in the tops of Lc 1400 and Sp than in Lc 1400 and Lp, while the relative decrease was the largest in VF.

Generally, the changes of K^+ in the stem, old and young leaves, and the top were in a direction opposite to those of Na^+ and Cl^- . This opposite tendency was most noticeable in the old leaf of Lp.

The older leaves, which serve as accumulators of excessive Na^+ and Cl^- , are probably the source of potassium for the young leaves and the top. It was previously suggested that old leaves of tomato act as a source for the export of large amounts of potassium to the growing organs of the plant (Humphries and Devonald, 1977).

Succulence

A relative change (a decrease) in succulence was evident in all species in the root and young leaf (Fig. 1D). The greatest changes in the root were in VF and Sp, and in the leaf in Sp. In the stem, it increased sharply in VF, Lc 1400, Lc 1401, and Sp during the first days and then decreased. In Lp, the succulence remained almost unchanged. In all species the final value of succulence in the stem was almost identical to the original one. In the

old leaf, succulence increased in all species; the increase was for a short time for VF and Lc 1401 and lasted longer in Lp, Sp, and Lc 1400. The values then decreased or remained unchanged. The greatest relative change (an increase) was found in Lc 1400 and to a lesser extent in VF, Lp, and Sp. In the top of the plants of all species, there was a relative change (an increase) in succulence. The increase was exceptionally large in VF.

Special points worth mentioning are:

1. The higher accumulation of Na^+ and lower accumulation of Cl^- in the top of Lp and Sp plants as compared with that of the cultivated species. The top of these wild plants seems, therefore, to be a Cl^- excluder and a Na^+ includer.

2. The exceptionally large changes of Na^+ and Cl^- (an increase) and K^+ (a decrease) in the old leaf of Lp when NaCl in the external medium is lowered.

3. The similarity of K^+ content per unit dry weight in the top of all plants. However, the content of K^+ per unit fresh weight in the top is higher in Lc 1401 and Lp and lower in Lc 1400 and Sp. In VF its level is similar to that in the first two species under high salinity and to the last two species after salt dilution.

4. The lowest level of K^+ in the old and young leaves of Sp per unit fresh weight as well as dry weight. A similar result was reported by Tal and Shannon (1983).

5. The lowest content (per dry weight) of K^+ in the root and stem of Lp. However, since succulence value in these parts of Lp is also exceptionally low, the concentration of K^+ when calculated per fresh weight unit is not lower in the root and stem of this plant than in the other plants. Similar results for succulence in Lp were reported by Tal and Shannon (1983).

6. The exceptional change of succulence in the top of the cultivated species during salt dilution. The succulence in the top of the wild species (low in Lp and Lc 1401 and high in Sp and Lc 1400) remained relatively unchanged.

The significance of the differences between the wild and the cultivated species with respect to accumulation of ions in the growing region (top) of the plant is being investigated.

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Figure Legend

Figure 1. Sodium (A), chloride (B), and potassium (C) content, and succulence (D) in the root, stem, old and young leaves, and top of NaCl-treated cultivated and wild species of tomato during dilution of NaCl in the external medium.

Cultivated species (VF) ———; Lc 1400 -----; Lc 1401 -----;

Lp; Sp — —.