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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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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* Mill. and its wild salt-tolerant relatives *L. peruvianum* (L.) Mill. and *Solanum pennellii* Cor. 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 tissues already differentiated. This suggestion is based on the positive correlation found between the response to NaCl of shoot apices and of 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 among 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 different from the balance 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.

Additional key words – Salt tolerance, shoot apex, tomato species.

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Introduction

Lycopersicon peruvianum and *Solanum pennellii* are salt-tolerant relatives of the cultivated tomato and have been suggested as a potential source of germplasm for salt resistance (Tal 1971, Dehan and Tal 1978). Under saline conditions their relative growth rate is superior to that of the cultivated species, and their leaves 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). In saline medium , the plating efficiency 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 whole plants and of calli and dividing protoplasts, which are relatively undifferentiated, led to the suggestion that the better osmotic adjustment, which characterizes the wild tomato plants, operates at the cellular level and is independent of the organization of the cells in the whole

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plant. A similar correlation between the response of the whole plant and the response of the callus to salt was demonstrated in other glycophytic (Orton 1980, Smith and McComb 1981) and halophytic (Von Hedenström and Breckle 1974) species. In some systems, in which no such correlation was found, the organization of the cells in the whole plant was suggested to be essential for the operation of the tolerance mechanisms (Smith and McComb 1981).

The fact that in tomato 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. The question was studied by comparing the response to salinity of excised fully differentiated tissues (leaves) and of 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* Mill. cultivar 'VF 234' and the wild relatives of tomato, *L. peruvianum* (L.) Mill. and *Solanum pennellii* Cor. accession Atico. Plants were grown in the greenhouse during the winter with day/night temperatures of about 20/10°C. Seedlings were prepared as described by Tal and Shannon (1983). Young seedlings bearing one or two leaves were transferred to aerated Hoagland solution, 20 seedlings per container of 22 l. The solution contained 5.0 m*M* $Ca(NO_3)_2 \cdot 4H_2O$, 5.0 m*M* KNO_3, 2.0 m*M* $MgSO_4 \cdot 7H_2O$, 1.0 m*M* KH_2PO_4, 47 μ*M* H_3BO_3, 9 μ*M* $MnCl_2 \cdot 4H_2O$, 0.9 μ*M* $ZnCl_2$, 0.3 μ*M* $CuCl_2 \cdot 2H_2O$, 0.1 μ*M* $H_2MoO_4 \cdot H_2O$ and 0.12 m*M* sodium ferric ethylenediamine di-(o-hydroxyphenylacetate) (sequestrene 138 Fe).

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% w/v active chloride) for 5 min. They were then rinsed three times in sterile water. 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, 0.171, 0.256 or 0.342 *M* NaCl. The medium consisted of Murashige and Skoog's (MS) mineral salts and inositol (Murashige and Skoog 1962) and 29 μ*M* thiamine·HCL, 4 μ*M* nicotinic acid, 27 μ*M* glycine, 2.4 μ*M* pyridoxine, 2.7 μ*M* α-naphthaleneacetic acid, and 9.2 μ*M* kinetin.

The cultures were kept at 27°C under 16 h photoperiod with light of 2 W m^{-2} provided by Atlas cool white fluorescent tubes. Ten days later, the discs which remained green and turgid were rinsed three times for 1 min in stirred distilled water or in 0.5 m*M* $CaSO_4$ solution and blotted carefully. Flaccid discs which had changed from green to brown were 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 as above either 0, 0.171, 0.256 or 0.342 *M* NaCl, and 29 μ*M* thiamine·HCl, 27 μ*M* glycine, 4 μ*M* nicotinic acid, 2.4 μ*M* pyridoxine, 0.52 μ*M* α-naphthaleneacetic acid, and 28 μ*M* kinetin 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 were detopped to induce development of lateral buds. The tips of the side branches were detached and sterilized as described above. Pieces, 1 cm in length, which included the meristem, and the first 1–2 developing leaves were excised and inserted upright in MS medium of the same composition as for leaflets and incubated under identical conditions. 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 *M* 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 and discussion

Survival and growth

A positive correlation between the response of the whole plant and of dividing cells to salt was found 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 occurs also for other states of differentiation. The results presented here support the former possibility. Apices of the two salt-tolerant

Tab. 1. Growth (mg dry weight) of shoot apices of *L. esculentum* (Le), *L. peruvianum* (Lp), and *S. pennellii* (Sp) cultured on control and saline media. Each value is the mean (± SE) of 10 apices. Numbers in brackets give % of control. The table represents the data of one out of 3 similar experiments.

Species	Control	NaCl	
		0.256 *M*	0.308 *M*
Le	36.6±0.2	0.0±0.0(0.0)	0.5±0.0(1.4)
Lp	24.8±0.2	6.0±1.1(24.2)	2.6±0.3(10.4)
Sp	26.3±0.1	17.0±1.6(64.5)	8.3±1.5(31.4)

species *L. peruvianum* and *S. pennellii* grew better than those of the cultivated tomato on saline medium (Tab. 1). In contrast, no correlation was found between the response of isolated fully differentiated tissues and the response of the whole plant. Discs 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 (Fig. 1). Similar results were obtained for excised fully differentiated leaflets. These findings demonstrate the central role that cells in the growing zone may have in the tolerance of the whole plant. The present data agree with Munns et al. (1982) who suggested that the primary cause of reduced growth in barley plants under NaCl salinity is located in the growing tissues and not in the photosynthetic tissues of the leaf. They showed that carbohydrate supply to the former could not be a limiting factor and suggested that water deficit was the main cause for the reduced growth under salinity in the growing tissues. In apices cultured on nutrient medium, reduced supply of metabolites can be ruled out as a cause for the reduced growth under salinity. The inhibition of growth may result from adverse water relations or from adverse effects of Na^+ and Cl^- on metabolism. Wyn Jones et al. (1979) suggested that various steps involved in protein synthesis are very sensitive to changes in the ionic environment. Whether these processes are more protected from the toxic effect of salts in the growing zone of the wild species *L. peruvianum* and *S. pennellii* than in the cultivated tomato is still an open question.

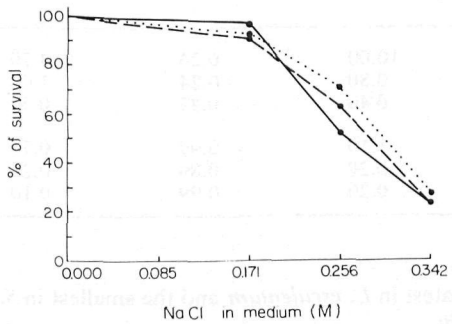

Fig. 1. Survival of leaf discs excised from *L. esculentum* (Le), *L. peruvianum* (Lp), and *S. pennellii* (Sp) plants as a function of NaCl concentration in the medium. Each point is the mean of 6 experiments, each one with 10 replications. Le, continuous line; Lp, dotted line; Sp, broken line.

Ion content

Sodium and chloride levels increased in NaCl-treated discs of all three species (Tab. 2). The increase of both ions was larger in *L. peruvianum* and *S. pennellii* than in the cultivated species. The accumulation of Na^+ and Cl^- in NaCl-treated leaflets was greater in *S. pennellii* than in the other two species (Tab. 3). Potassium level decreased under NaCl salinity in discs and leaflets of all three species, in both parts the decrease was the smallest in *S. pennellii* and the greatest in *L. peruvianum*.

Sodium and chloride were accumulated in apices of all three species under NaCl salinity; the accumulation was high in *S. pennellii* and low in *L. esculentum* and *L. peruvianum* (Tab. 4). The decrease of K^+ under salinity was the greatest in *S. pennellii*.

The ratios K^+/Na^+ and Cl^-/Na^+ in NaCl-treated excised leaf discs, leaflets and shoot apices are presented in Tab. 5. K^+/Na^+ ratio was similar in all species in leaflets and apices. In leaf discs, it was higher in *L. esculentum* and *S. pennellii* than in *L. peruvianum*. The ratio Cl^-/Na^+ was close to 1 in leaf discs of *L. esculentum* and *S. pennellii,* in leaflets of *L. esculentum* and *L. peruvianum* and in the apices of all three species.

The balance of Na^+, Cl^-, and K^+ in the shoot apices and the leaflets which were brought in direct contact with salt after their excision appreciably differed from

Tab. 2. Ion content [mmol (g dry weight)$^{-1}$] in leaf discs of *L. esculentum* (Le), *L. peruvianum* (Lp), and *S. pennellii* (Sp) cultured on control (C) and 0.171 *M* NaCl (S) medium. Each value is the mean (± SE) of 5 discs. The table presents the data of one out of 5 similar experiments.

Species	Na^+		Cl^-		K^+		
	C	S	C	S	C	S	% decrease
Le	0.17±0.03	2.20±0.13	0.13±0.05	2.09±0.48	0.94±0.05	0.40±0.04	58
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	64
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	41

Tab. 3. Ion content [mmol (g dry weight)$^{-1}$] in leaflets of *L. esculentum* (Le), *L. peruvianum* (Lp) and *S. pennellii* (Sp) plants cultured on control (C) and 0.265 *M* NaCl (S) medium. Each value is the mean (± SE) of 5 leaflets. The table presents data of one out of 4 similar experiments.

Species Species	Na^+		Cl^-		K^+		
	C	S	C	S	C	S	% decrease
Le	0.23±0.02	3.55±0.22	0.01±0.00	3.51±0.23	0.92±0.24	0.63±0.03	32
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	61
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

Tab. 4. Ion content [mmol (mg dry weight)$^{-1}$] in shoot apices of *L. esculentum* (Le), *L. peruvianum* (Lp), and *S. pennellii* (Sp) plants cultured on control (C) and 0.265 *M* NaCl (S) media. Each value is the mean (± SE) of 5 apices. The table presents data of one out of 4 similar experiments.

Species	Na^+		Cl^-		K^+		
	C	S	C	S	C	S	% decrease
Le	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
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
Sp	0.42±0.11	5.08±0.46	0.00	5.01±0.44	2.10±0.20	1.13±0.06	46

Tab. 5. K^+/Na^+ and Cl^-/Na^+ ratio in NaCl-treated cultured leaf discs, leaflets and shoot apices of *L. esculentum* (Le), *L. peruvianum* (Lp) and *S. pennellii* (Sp) plants, and in attached leaves and apices of salt-treated 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.

Ratio	Species	Leaves			Apices	
		Cultured discs	Cultured leaflets	Attached*	Cultured	Attached*
K^+/Na^+	Le	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^+	Le	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

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 than in 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 *S. pennellii* leaflets.
4. *K^+/Na^+.* In all species, the ratio was smaller in detached than in attached parts; the difference was the greatest in *L. esculentum* and the smallest in *S. pennellii*.

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 (Läuchli 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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