BIOLOGIA PLANTARUM 36 (2): 247-254, 1994

Osmotic adjustment in leaves of *Lycopersicon esculentum* and *L. pennellii* in response to saline water irrigation

A. TORRECILLAS, J.J. ALARCON, M.J. SANCHEZ-BLANCO
and M.C. BOLARIN

Centro de Edafología y Biología Aplicada del Segura (CSIC),
P.O. Box 4195, E-30080 Murcia, Spain

Abstract

Two tomato species (*Lycopersicon esculentum* and *L. pennellii*) were grown under unheated plastic greenhouse and irrigated with 0 or 140 mM NaCl. Salinity induces a more important reduction in predawn leaf water potential (ψ_{pd}) in *L. esculentum* than in *L. pennellii*. In both species the osmotic adjustment was achieved by active solute accumulation. The leaf water potential at turgor loss point (ψ_{tlp}) seemed to be controlled by leaf osmotic potential (ψ_{os}). The results revealed the existence of limits to the accumulation of osmotic solutes in leaf tissues and the existence of an ontogenetic effect on the solute accumulation. In both species, but essentially in *L. pennellii* the inorganic solutes contribution especially Na^+ and Cl^- accumulation to ψ_{os} was higher than the organic solutes. Therefore, wild species save energy more markedly.

Introduction

Although breeding for salt tolerance progress without advances in physiological and biochemical knowledge, more rapid improvements depend on a deeper understanding of the physiological processes and genetic controls that limit plant growth in adverse environments (Tal 1985). When plants are grown in saline conditions, they are subjected to three types of stress: water stress, mineral toxicity stress and disturbances in the mineral nutrition of the plant (Blum 1986). In spite of the fact that the water stress is one of the most important effects induced by salinity, it has been studied less than the other effects. High solute concentrations contribute to lower tissue osmotic potential, which leads to the maintenance of turgor potential,

---

Received 9 August 1993, *accepted* 30 September 1993.
Acknowledgements: The authors are grateful to Mrs. M.D. Velasco, Mrs. C. Carreño, Mr. J. Soto and Mr. J. Soto-Montesinos for their technical assistance. Mr. A. Rodriguez prepared the figure and Mrs. M. Aragüés prepared the manuscript. This study was supported under EC (DG VI) PL 900165 and Comisión Interministerial de Ciencia y Tecnología (CICYT) AGF 92-0260 grants to authors.

and improves tolerance to low tissue water potential (Tyree and Jarvis 1982). Consequently, low osmotic potential and the capacity for osmotic adjustment may also serve as useful criteria for selection and breeding of stress-resistant species and cultivars (Sánchez-Blanco *et al.* 1991). High salt tolerance has been reported for various wild relatives of the cultivated tomato (Tal and Shannon 1983; Bolarín *et al.* 1991). These wild species represent a potential source of useful genetic variation (Rick 1988), and they are therefore ideally suited for a comparative physiological-genetic study (Cuartero *et al.* 1992).

The objetive of this study was to determine the salinity effects on predawn leaf water potential, leaf water potential at the turgor loss point, and leaf osmotic potential at full turgor along the growing season and the contribution of inorganic and organic solutes to the osmotic adjustment in domestic tomato leaves (*L. esculentum*) and its wild salt tolerant relative species *L. pennellii.*

Materials and methods

Plant material and culture conditions: Seedlings of two tomato species, *Lycopersicon esculentum* (L.) Mill. cv. P-73 and *L. pennellii* (Correll) D'Arcy ac PE-47 were transplanted in a unheated plastic greenhouse on 25 March.

The experiment was conducted during spring-summer 1990 in the Segura Valley (Murcia, Spain). Plants were drip irrigated daily using two emitters of 2 l h^{-1} per plant. Plant spacing was 1.0 × 2.15 m. The water applied at each irrigation was 28 % of the evaporation for the 3 previous days of a class A pan located outside the greenhouse. The total amount of water applied was 223.1 mm. In the greenhouse maximum/minimum temperature was 38/21 °C, the relative humidity ranged between 29 - 83 % and the maximum irradiance was 1089 µmol(PAR) m^{-2} s^{-1}.

15 d after the planting, the plants were subjected to two different treatments, using 0 and 140 mM NaCl irrigation water (control and saline treatments, respectively). Design of the experiment was completely randomized with 4 replications (24 plants per replicate). Both treatments received the same amounts of fertilizers (11.9 g N, 15.1 g P_2O_5, 13.1 g K_2O and 0.1 g MgO per plant). The electrical conductivities of the irrigation solutions were 1.4 and 14.0 dS m^{-1} for control and saline treatments, respectively.

Water relations: Three times during the experimental period (30, 66 and 90 d after beginning the treatments) predawn leaf water potential (ψ_{pd}) was measured on five plants of each species and treatment, using a pressure bomb (Scholander *et al.* 1965). Leaves from the south side of the top third (fourth or fifth leaf) of the plants were taken at dawn, enclosed in a plastic bag and placed in the chamber within 20 s of collection (Turner and Long 1980, Turner 1988). Estimates of leaf osmotic potential at full turgor (ψ_{os}), leaf water potential at the turgor loss point (ψ_{tlp}) and the relative apoplastic water content, were obtained via pressure-volume analyses of leaves (Wilson *et al.* 1980).

Analyses of mineral and organic contents: Leaves similar to those sampled for ψ_{pd} excised at predawn and on the same sampling dates were used. Leaves were washed with distilled water, dried at 70 °C, ground and stored at room temperature for inorganic solute analyses. An aliquot of fresh material was liophylized and stored at -20 °C until organic solutes were determined.

Na^+, Cl^-, NO_3^- and K^+ foliar contents were determined on oven dried leaves. On the aqueous extracts Cl^- and NO_3^- contents were analyzed by potentiometric titration with $AgNO_3$ and by Lambert and Dubois (1971) methods, respectively. In diluted digestion extracts (using the HNO_3 - $HClO_4$ 2:1 mixture), Na^+ and K^+ concentrations were determined by atomic absorption spectroscopy.

Analysis of soluble sugars, free amino acids and organic acids were made on liophylized foliar material. Extraction of soluble sugars and organic acids has been previously described by Bourgeais-Chaillou and Guerrier (1992). Extracts were analyzed for soluble sugars using the method of Morris and Arthur (1948) and for organic acids by a HPLC system using the procedure of Timpa *et al.* (1986). Free amino acids were extracted with phosphate buffer (50 mM) in presence of 1 % PVP and 0.01 % Triton X-100 and determined in a amino acid autoanalyzer using the nynhidrin post-column reaction.

Osmotic contribution of solutes: The contribution of analyzed individual solutes (ψ_{os} calculated) to ψ_{os} measured was determined according the Van't Hoff relation:

$$\psi_{os}\,(\text{calculated}) = -\,0.002479 \times RDW \times C \times 1/M$$

where RDW [kg m^{-3}] is the relative dry mass at saturation [dry mass/(saturated mass-dry mass)], C [g kg^{-1}] is the individual solute concentration on a dry-mass basis, M [g mol^{-1}] is the molecular mass of each solute and 0.002479 m^3 MPa mol^{-1} is the RT value for 25 °C (Nobel 1983). For the Van't Hoff relation it is assumed that solutes behaved as ideal osmotica, with an ionization constant of one (Munns and Weir 1981).

Results

Predawn leaf water potential (ψ_{pd}) showed high values in control plants of both species (Table 1). Values of ψ_{pd} were lower in the saline treatment of the domestic species, whereas in the wild species not significant differences between treatments was noted. In both treatments of *L. pennellii* and in the control treatment of *L. esculentum* a decrease of ψ_{pd} levels was noted between May and June, followed by no changes of these levels in July. Under saline conditions *L. esculentum* showed a progressive decrease in ψ_{pd} values from May to July.

Values of leaf osmotic potential at full saturation (ψ_{os}) in the saline treatment of the domestic species and in the two treatments of the wild species decreased between the first and the second sampling date (Table 1). In this period no changes in ψ_{os} levels in the control treatment of the cultivated species was observed. Subsequently, in both treatments of *L. pennellii* and in the saline treatment of

L. esculentum a stabilization of ψ_{os} levels was observed, whereas in the control treatment of the domestic species a decrease of ψ_{os} was noted.

Table 1. Predawn leaf water potential (ψ_{pd}), leaf osmotic potential at full turgor (ψ_{os}), leaf water potential at turgor loss point (ψ_{tlp}) and contribution of measured inorganic solutes (ψ^{i}_{os}) and organic solutes (ψ^{o}_{os}) to leaf osmotic potential at full turgor (ψ_{os}) for *L. esculentum* and *L. pennellii* in control and saline treatments. Values within parentheses indicate percentage of the ψ_{os} measured. Means within a column for each time and species that do not have a common letter are significantly different by $LSD_{0.05}$ test.

Species	Time	NaCl [mM]	ψ_{pd} [MPa]	ψ_{os} [MPa]	ψ_{tlp} [MPa]	ψ^{i}_{os} [MPa]	ψ^{o}_{os} [MPa]
L. esculentum	May	0	-0.26a	-0.83a	-1.01a	-0.43a (51.8)	-0.36a (43.4)
		140	-0.49b	-0.97b	-1.17b	-0.55a (56.7)	-0.30a (30.9)
	June	0	-0.36a	-0.82a	-1.12a	-0.46a (56.1)	-0.30a (36.6)
		140	-0.60b	-1.10b	-1.35b	-0.63b (57.3)	-0.32a (29.1)
	July	0	-0.35a	-1.10a	-1.23a	-0.59a (53.6)	-0.33a (30.0)
		140	-0.75b	-1.12a	-1.42b	-0.64a (57.1)	-0.35a (31.3)
L. pennellii	May	0	-0.28a	-0.73a	-1.06a	-0.35a (47.9)	-0.14a (19.2)
		140	-0.30a	-0.87b	-1.20b	-0.42b (48.3)	-0.09a (10.3)
	June	0	-0.40a	-0.96a	-1.25a	-0.39a (40.6)	-0.13a (13.5)
		140	-0.42a	-1.20b	-1.46b	-0.52b (43.3)	-0.08b (6.7)
	July	0	-0.39a	-0.95a	-1.43a	-0.40a (42.1)	-0.17a (17.9)
		140	-0.43a	-1.21b	-1.54b	-0.55b (45.5)	-0.15a (12.4)

Leaf water potential at the turgor loss point (ψ_{tlp}) was higher for control plants than for salt-treated plants of both species (Table 1). In all cases, ψ_{tlp} showed a similar behaviour as ψ_{os} throughout the experimental period.

In general, the calculated ψ_{os} values in control and salt-treated domestic plants were approximated to the measured ψ_{os} values (Table 1). In both treatments of the wild species, the inorganic solute contribution to ψ_{os} was several times higher than that of organic solutes, whereas in *L. esculentum* this difference was less important. In the salt treated plants of *L. pennellii* the contribution of inorganic solutes to ψ_{os} increased significantly in relation to the control at all sampling dates. In *L. esculentum* this contribution was only increased in June. However, the organic solute contribution to ψ_{os} was similar in both treatments of *L. esculentum*, whereas in the wild species a certain tendency to decrease in June was noted.

In the control plants of both species, the major solutes which contribute to ψ_{os} were organic acids, Cl^-, K^+ NO_3^- (Fig. 1). In salt treated plants, mainly in *L. pennellii*, an important increase in the contribution to ψ_{os} by the elements provided by the saline water and a reduction of the contribution of K^+ and organic acids was observed. Although soluble did not belong among the major components of ψ_{os} in control plants, their contribution under salt stress increased significantly in the cultivated species in June.

Discussion

The high ψ_{pd} values suggest that the applied irrigation amounts were not a limiting factor for both cultivars (Table 1). The ψ_{pd} behaviour confirms that salinity induces a more apparent reduction in the leaf water potential in *L. esculentum* than in the wild species (Alarcón *et al.* 1993). The decrease in ψ_{pd} by salinity could be due to the reduction of ψ_{os}, and the decrease in the overall ψ_{pd} values, during the season, may be related to leaf senescence. Rudich *et al.* (1981) indicated that leaf senescence induces an increase in resistance to water flow in the xylem.

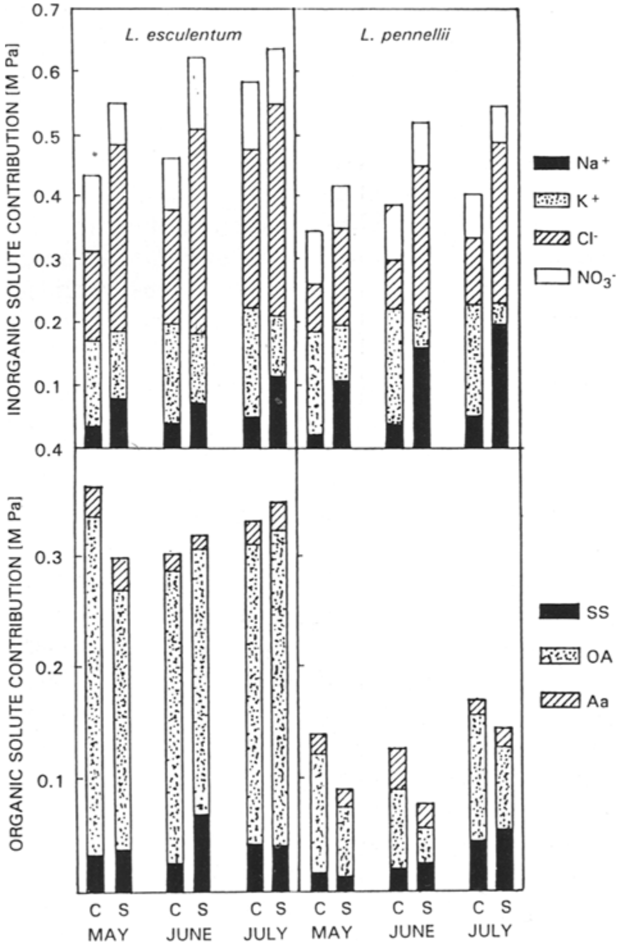

Fig.1. Contribution of inorganic and organic solutes to leaf osmotic potential at full turgor (ψ_{os}) in *L. esculentum* and *L. pennellii* under control (*C*) and saline (*S*) treatments at three different times during the experimental period.

Wilson *et al.* (1980) indicated that osmotic adjustment is accounted by decreases in the saturated mass/dry mass ratio, increases in the apoplastic water content, and direct solute accumulation. No significant changes in the two first parameters (data not shown) were observed; therefore, in our experimental conditions the ψ_{os} reductions in both species by saline stress effect may be due to a active accumulation of solutes (Table 1). The accumulation of solutes during leaf ontogeny could explain the observed ψ_{os} reduction in control plants during the season (Parker *et al.* 1982). The stabilization of ψ_{os} levels between June and July in both treatments of the wild species and in the saline treatment of the domestic species confirm the existence of limits to accumulation of osmotically active solutes in the leaves (Tyree and Jarvis 1982, Sánchez-Blanco *et al.* 1991). The overall parallel pattern observed in both species between ψ_{os} and ψ_{tlp} (Table 1) confirms that ψ_{tlp} is controlled by ψ_{os} (Alarcón *et al.* 1993).

The fact that the organic solutes contribution to ψ_{os} in the domestic species was greater that in the wild species could be related to the glycophytic characteristic of *L. esculentum* (Table 1). Marschner (1986) indicated that uptake of inorganic solutes prevails over the synthesis of organic solutes in halophytes, since in the latter case the process results in a higher expenditure of energy.

The fact that the calculated ψ_{os} values in *L. pennellii* explained only the 58% of measured ψ_{os} and in *L. esculentum* the percentage of explanation was 89%, can be justified by two main possibilities. One possibility is that solutes inside cells do not always behave as ideal osmotica, so that a change in solute concentration might contribute more to ψ_{os} than explained by ideal osmotica. Another possibility is the contribution to ψ_{os} of solutes which have been not considered (Storey and Wynn Jones 1977, Gimmler and Moller 1981, Newton *et al.* 1989). In this sense, Alarcón *et al.* (1993) indicated that the calculated ψ_{os} values for the studied solutes were similar to the measured ψ_{os} values, when both tomato species were submitted to a sudden saline shock.

The higher Na^+ and lower K^+ contribution to ψ_{os} in plants under saline stress (Fig. 1), could be related with the K^+ partial substitution by Na^+ on its osmotic and metabolic functions (Marschner 1986). The reduction in the organic acids contribution to ψ_{os} by salinity effects could be due to the uptake and foliar accumulation of Cl^- (Hamza 1980). The increase in the contribution of soluble sugars to ψ_{os} in salt-treated plants of *L. esculentum* is an adaptative response of the plants to the stress (Hanson and Hitz 1982).

According to the solutes involved in the osmotic adjustment (Fig. 1), it is clear that the wild species confront a long-term saline stress with a osmotic adjustment based exclusively on the Cl^- and Na^+ accumulation, with marked energy savings. The domestic species shows a more glycophytic response due to the contribution of soluble sugars.

The above results and those obtained in a previous work (Sánchez-Blanco *et al.* 1991) indicate that *L. pennellii* had a more efficient mechanism for tolerating the saline stress than *L. esculentum*. Nevertherless, further research should focus on the identification of other stress metabolites and/or inorganic solutes not studied that could explain the measured ψ_{os} values.

References

Alarcón, J.J., Sánchez-Blanco, M.J., Bolarín, M.C., Torrecillas, A.: Water relations and osmotic adjustment in *Lycopersicon esculentun* and *L. pennelli* during a short-term salt exposure and recovery. - Physiol. Plant. (in press), 1993.

Amzallag, G.N., Lerner, H.R., Poljakoff-Mayber, A.: Induction of increased salt tolerance in *Sorghum bicolor* by NaCl pretreatments. - J. exp. Bot. **41**: 29-34, 1990.

Blum, A.: Salinity resistance. - In: Blum A. (ed.): Plant Breeding for Stress Environments. Pp. 163-169. CRC Press, Boca Raton 1986.

Bolarín, M.C., Fernández, F.G., Cruz, V., Cuartero J.: Salinity tolerance in four wild tomato species using vegetative yield-salinity response curves. - J. amer. Soc. hort. Sci. **116**: 286-290, 1991.

Bourgeais-Chaillou, P., Guerrier, G.: Salt-responses in *Lycopersicon esculentum* calli and whole plants. - J. Plant Physiol. **140**: 494-501, 1992.

Cuartero, J., Yeo, A.R., Flowers, T.J.: Selection of donors for salt-tolerance in tomato using physiological traits. - New Phytol. **121**: 63-69, 1992.

Gimmler, H., Moller, J.C.: Salinity-dependent regulation of starch and glycerol metabolism in *Dunaliella parva*. - Plant Cell Environ. **4**: 367-375, 1981.

Hamza, M.: Résponses des végetatux à la salinité. - Physiol. vég. **18**: 69-81, 1980.

Hanson, A.D., Hitz, W.D.: Metabolic response of mesophytes to plant water deficits. - Annu. Rev. Plant Physiol. **33**: 163-203, 1982.

Lambert, R.S., Du Bois, R.J.: Spectrophotometric determination of nitrate in presence of chloride. - Anal. Chem. **43**: 955-957, 1971.

Marschner, H.: Mineral Nutrition in Higher Plants. - Academic Press, London - Orlando 1986.

Martin, B., Thorstenson, Y.R.: Stable carbon isotope composition ($\delta^{13}C$), water use efficiency, and biomass productivity of *Lycopersicon esculentum, Lycopersicon pennellii* and the F1 hybrid. - Plant Physiol. **88**: 213-217, 1988.

Martin, B., Nienhuis, J., King, G., Schaefer, A.: Restriction fragment length polymorphisms associated with water use efficiency in tomato. - Science **243**: 1725-1728, 1989.

Morris, D.A., Arthur, D.: Invertase activity in sinks undergoing cell expansion. - Plant Growth Regul. **2**: 327-337, 1948.

Munns, R., Weir, R.: Contribution of sugars to osmotic adjustment in elongating and expanded zones of wheat leaves during moderate water deficits at two light levels. - Aust. J. Plant Physiol. **8**: 93-105, 1981.

Newton, R.J., Sean, S., Puryear, J.D.: Solute contributions to osmotic potential in loblolly pine (*Pinus taeda* L.) callus. - J. Plant Physiol. **134**: 746-750, 1989.

Nobel, P.S.: Biophysical Plant Physiology and Ecology. - W.H. Freeman and Company, New York 1983.

Parker, W.C., Pallardy, S.G., Hinckley, T.M., Teskey, R.O.: Seasonal changes in tissue water relations of three woody species of the *Quercus-Carya* forest type. - Ecology **63**: 1259-1267, 1982.

Rick, C.M.: Molecular markers as aids for germplasm management and use in *Lycopersicon*. - HortScience **23**: 55-57, 1988.

Rudich, J., Rendon-Poblete, E., Stevens, M.A., Ambri, A.I.: Use of leaf water potential to determine water stress in field-grown tomato plants. - J. amer. Soc. hort. Sci. **106**: 732-736, 1981.

Sánchez-Blanco, M.J., Bolarín, M.C., Alarcón, J.J., Torrecillas, A.: Salinity effects on water relations in *Lycopersicon esculentum* and its wild salt-tolerant relative species *L. pennellii*. - Physiol. Plant. **83**: 269-274, 1991.

Scholander, P.F., Hammel, H.T., Bradstreet, E.D., Hemingeen, E.A.: Sap pressure in vascular plants. - Science **148**: 339-346, 1965.

Storey, R., Wyn Jones, R.G.: Quaternary ammonium compounds in plants in relation to salt resistance. - Phytochemistry **16**: 447-453, 1977.

Tal, M.: Genetics of salt tolerance in higher plants: Theoretical and practical considerations. - Plant Soil **89**: 199-226, 1985.

Tal, M., Shannon, M.C.: Salt tolerance in the wild relatives of the cultivated tomato: Response of *Lycopersicon esculentum, L. cheesmanii, L. peruvianum, Solanum pennelli* and F1 hybrids to high salinity. - Aust. J. Plant Physiol. **10**: 109-117, 1983.

Turner, N.C.: Measurement of plant water status by the pressure chamber technique. - Irrig. Sci. **9**: 289-308, 1988.

Turner, N.C., Long, M.J.: Errors from arising from rapid water loss in the measurement of leaf water potential by the pressure chamber technique. - Aust. J. Plant Physiol. **7**: 527-537, 1980.

Tyree, M.T.: Physical parameters of the soil-plant-atmosphere system: breeding for drought resistance characteristics that might improve wood yield.- In: Caunell, H.G.R, Last, F.T (ed.): Tree Physiology and Yield Improvement. Pp 329-348. Academic Press, New York 1976.

Tyree, M.T., Jarvis, P.G.: Water in tissues and cells. - In: Lange, O.L, Nobel, P.S, Osmond, C.B, Ziegler, H. (ed.): Encyclopedia of Plant Physiology. II Physiological Plant Ecology. Pp 35-73. Springer-Verlag, Heidelberg - New York 1982.

Wilson, J.R., Ludlow, M.M., Fisher, M.J., Schulze, E.-D.: Adaptation to water stress of the leaf water relations of four tropical forage species. - Aust. J. Plant Physiol. **7**: 207-220, 1989.

Communicated by J. POSPÍŠILOVÁ