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Growth and water relations of *Lotus creticus creticus* plants as affected by salinity

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Abstract

Young plants of *Lotus creticus creticus* growing in a hydroponic culture were submitted to 0, 70 and 140 mM NaCl treatments for 28 d and the growth and ecophysiological characteristics of these plants have been studied. The growth of *Lotus* plants was not affected by salinity when applied for a short period (about 15 d); however, 140 mM NaCl induced a decrease in shoot RGR at the end of the treatment. The root growth was not decreased, even it was stimulated by 140 mM NaCl. The osmotic adjustment of *Lotus* plants at 70 and 140 mM NaCl maintained constant pressure potential, avoiding the visual wilting. For a similar leaf water potential, cuticular transpiration of salinized plants was lower than in control plants due to the salinity effect on the cuticle. Moreover, the presence of hairy leaves (60 and 160 trichomes per mm^2 in young and adult leaves, respectively) allows keeping almost 81 % of sprayed water and absorbing the 9 % of the water retained, decreased the epidermal conductance to water vapour diffusion.

Additional key words: cuticular transpiration, leaf hairs, NaCl stress, pressure potential, water potential.

Introduction

Plant salt tolerance is generally related to the ability of the plant to withstand salinity without a significant adverse effect. However, the tolerance of a given crop depends on the growth stage (Lunin *et al.* 1963) and on the degree and duration of saline stress (Neumann 1997). In this sense, Sánchez-Blanco *et al.* (1998) showed that the growth of *Lotus creticus* in a greenhouse decreased only as high salinity was applied for a long period of time (one month) because of the toxic effects of the Cl^- and Na^+ accumulating within the plant (Munns 1993).

On the other hand, it is well-known that several characteristics of the plant could provide a significant increase in the tolerance, minimising the osmotic and toxic effects of salt. In this sense, the leaves of many species vary greatly in morphology, anatomy and physiology having an ecological meaning to resist particular environmental conditions. For example, the leaf trichomes can be considered like an adaptation of the plants to Mediterranean conditions. Besides other functions, the leaf hairs may improve the leaf water status (Grammatikopoulos and Manetas 1994), even increase water potential (Kerr and Beardsell 1975).

Lotus creticus creticus is a Mediterranean species with abundant pubescence in its leaves. This kind of native species are usually considered tolerant and adapted to dry conditions and soil salinity (Caballero and Cid 1993).

For all that, the objective of this paper was to determine whether the morphological and physiological characteristics of *Lotus* plants allow to resist saline conditions. Accordingly, growth, water relations and water absorption by leaves were studied.

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Abbreviations: RWC - relative water content; Tc - cuticular transpiration; ε - bulk modulus of elasticity; Ψ_l - leaf water potential; Ψ_{os} - osmotic potential at saturation; Ψ_p - leaf pressure potential; Ψ_s - leaf osmotic potential.

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Materials and methods

Plants and treatments: *Lotus creticus creticus* plants were used in these experiments. The plants were grown in hydroponic culture with half strength Hoagland solution in a growth chamber under a 16-h photoperiod with the maximum/minimum photosynthetically active radiation (PAR) 245/81 μmol m^{-2} s^{-1}. Day/night average temperatures and relative humidities were 24/20 °C and 60/85 %, respectively. The experiments were performed during the period of May-July 1998.

Salt treatments of 0, 70 and 140 mM NaCl in nutrient solutions were applied gradually until reaching the desired concentrations. An average electrical conductivity (EC) was 1.26 dS m^{-1} for the control treatment and 6.43 dS m^{-1} and 12.34 dS m^{-1} for 70 and 140 mM NaCl, respectively. The salinization period lasted 28 d.

Measurements of growth and water status: Before salt application (28 June), 15 d after the beginning of salt aplication (12 July) and at the end of the salinization period (27 July) plants (10 per treatment) were harvested and stem, root and leaf dry masses were measured. The root and shoot relative growth rate (RGR) was determined according to Beadle (1993).

At the same time, leaf water potential (Ψ_l), leaf osmotic potential (Ψ_s) and leaf osmotic potential at saturation (Ψ_{os}) were measured at the end of the dark period (predawn) in 15 plants of each treatment. Ψ_l was determined using a pressure chamber (*Soil Moisture Equipment Co.*, Santa Barbara, USA) according to Scholander *et al.* (1965). Ψ_s was estimated using a vapour pressure osmometer *Wescor 5000* (Logan, USA) in excised leaves harvested at predawn and immediately frozen and stored at -30 °C. Before the measurements, samples were thawed and leaf sap expressed for immediate osmotic potential determination according to Gucci *et al.* (1991). Leaves used for Ψ_{os} determination were placed in plastic bags and allowed to reach full saturation by dipping the petioles in distilled water overnight. After that, leaves underwent the previous procedure until being measured in vapour pressure osmometer. Leaf pressure potential (Ψ_p) was estimated as the difference between Ψ_l and Ψ_s.

The bulk modulus of elasticity (ε) at 100 % relative water content (RWC) was estimated via pressure-volume analysis of leaves, as outlined by Wilson *et al.* (1979). Leaves were excised in the dark, placed in plastic bags and allowed to reach full turgor by dipping the petioles in distilled water overnight. Pressure-volume curves were obtained from periodic measurements of leaf mass and balance pressure as leaves dried on the bench.

Cuticular transpiration (Tc) was estimated using leaves from pressure-volume analysis and calculated from the rate of water loss with time. Drying leaves period was about 5 h. We assumed that stomata were closed after 30 min of dehydration.

Trichome count: For conventional microscopy, samples were fixed for 2.5 h at 4 °C in a 0.1 M Na-phosphate buffered (pH = 7.2) mixture of 2.5 % glutaraldehyde and 4 % paraformaldehyde (Olmos and Hellín 1996). The samples were then dehydrated in a graded acetone series. The trichomes were observed through a *Jeol JSM-6100* scanning microscope (Tokyo, Japan) using an acceleration voltage of 20 kV and quantified by an image analysis system (*Leica Q 500MC*, Germany).

Water retention, duration, and absortion: Two treatments were done (control and sprayed treatments) to calculate the percentage of water absorption by the leaves, water duration and water retention (Grammatikopoulos and Manetas 1994). A fixed amount of water was sprayed on the upper surface of pre-weighed leaf discs (0.5 cm in diameter) with a sprayer (sprayed treatment). These discs were immediately re-weighed to assess the percentage of water retention. Discs of filter paper with the same size were used as 100 % retention control since they retain all sprayed water as was shown in previous experiments.

To evaluate the water duration on the leaf surface, discs of leaves were left to lose water on the bench at low light until epidermal conductance was close to 0. Half of them were sprayed with a fixed amount of water and then periodic measurements of leaf mass loss in sprayed and non sprayed discs (control discs) was recorded. The mass loss in control discs corresponded to cuticular transpiration, whereas in sprayed discs corresponded to the cuticular transpiration and water evaporation from the leaf surface. When the loss rates in control and sprayed discs became equal, the surface water was considered to be totally evaporated, being the time period since the spray application, the water duration time. At that time, the differences found between sprayed and control discs could indicate the amount of absorbed water.

Results

Salinity effect on plant growth: Salinity did not adversely affect the *Lotus* plants growth (Table 1). The total dry mass of salinized plants was even higher in plants treated with 140 mM NaCl than in control. This effect was more marked on the 15th day after the NaCl application, when in all cases, the values of shoot, leaves and root dry masses of 140 mM NaCl treated plants were the highest. At the end of the salinization period, the differences detected in total dry mass of plants treated with 140 mM NaCl were only due to those found in root dry mass (Table 1). These results are in accordance with the RGR values (Table 2). During the first period of time (28 June - 12 July), the plants submitted to 140 mM NaCl presented the highest shoot-RGR values, and after this period, a decrease of these values with respect to the control and 70 mM NaCl treatments were observed. The root RGR values were higher through all the experimental time in salinized plants.

Table 1. Total dry mass, leaf dry mass, stem dry mass and root dry mass for *Lotus creticus creticus* plants in the control (0 mM NaCl), 70 and 140 mM NaCl recorded at three different times during the experimental period. Means within a column and time that do not have the same common letter are significantly different by $LSD_{0.05}$ test.

Time [d]	NaCl [mM]	Total d.m. [g plant^{-1}]	Leaf d.m. [g plant^{-1}]	Stem d.m. [g plant^{-1}]	Root d.m. [g plant^{-1}]
0	0	0.73	0.15	0.39	0.19
15	0	1.19 a	0.20 a	0.63 a	0.36 a
	70	1.14 a	0.26 a	0.54 a	0.34 a
	140	2.31 b	0.66 b	1.04 b	0.61 b
28	0	3.39 a	0.99 a	1.83 a	0.57 a
	70	3.99 a	1.25 a	1.85 a	0.89 ab
	140	4.83 b	1.32 a	1.96 a	1.55 b

Table 2. Relative growth rate (RGR)[g g^{-1}d^{-1}] of shoot and root of *Lotus creticus creticus* plants in the control (0), 70 and 140 mM NaCl recorded throughout the experimental period. Means within a column and time that do not have the same common letter are significantly different by $LSD_{0.05}$ test.

Time period	NaCl [mM]	Shoot RGR [d^{-1}]	Root RGR [d^{-1}]
0 - 15	0	0.031 a	0.046 a
	70	0.029 a	0.042 a
	140	0.081 b	0.084 b
15-28	0	0.087 a	0.033 a
	70	0.096 a	0.069 b
	140	0.047 b	0.066 b

Salinity effect on plants water status: At the end of salinization, NaCl induced a progressive decrease in predawn leaf water potential (Ψ_l) (Table 3), reaching minimum values of -0.6, -1.0, and -1.14 MPa for control, 70 and 140 mM NaCl treatments, respectively. Predawn leaf osmotic potential (Ψ_s) presented a similar behaviour to that observed for Ψ_l in all treatments (Table 3). Predawn leaf pressure potentials were similar throughout the experimental period (Table 3).

The osmotic adjustment (decrease of Ψ_{os}) was not observed in control plants through the experiment (Table 3). However, in treated plants, a rapid decrease of this parameter was detected and significant differences between both saline treatments, with values of -1.08 MPa for 70 mM NaCl and -1.27 MPa for 140 mM NaCl, were found within 15 d. At the end of the experimental period no differences between salinized *Lotus* plants were noted. The values of bulk modulus of elasticity (ε) remained unchanged (Table 3).

Table 3. Leaf water potential (Ψ_l), leaf osmotic potential (Ψ_s), leaf osmotic potential at full saturation (Ψ_{os}), leaf pressure potential (Ψ_p), bulk modulus of elasticity (ε) and cuticular transpiration (T_C) for *Lotus creticus creticus* plants in 0, 70 and 140 mM NaCl at three different times of the experimental period. Means within a column and time that do not have the same common letter are significantly different by $LSD_{0.05}$ test.

Time [d]	NaCl [mM]	Ψ_l [MPa]	Ψ_s [MPa]	Ψ_{os} [MPa]	Ψ_p [MPa]	ε [MPa]	T_C [mg g^{-1}(d.m.) s^{-1}]
0	0	-0.42	-1.12	-0.89	0.69	0.73	0.218
15	0	-0.44 a	-1.12 a	-0.96 a	0.68 a	0.78 a	0.380 a
	70	-0.46 a	-1.28 a	-1.08 b	0.82 a	1.10 a	0.279 b
	140	-0.74 b	-1.58 b	-1.27 c	0.84 a	0.77 a	0.287 b
28	0	-0.63 a	-1.20 a	-1.02 a	0.57 a	0.65 a	0.424 a
	70	-1.00 b	-1.61 b	-1.46 b	0.61 a	0.75 a	0.289 b
	140	-1.14 b	-1.71 b	-1.50 b	0.58 a	0.87 a	0.250 b

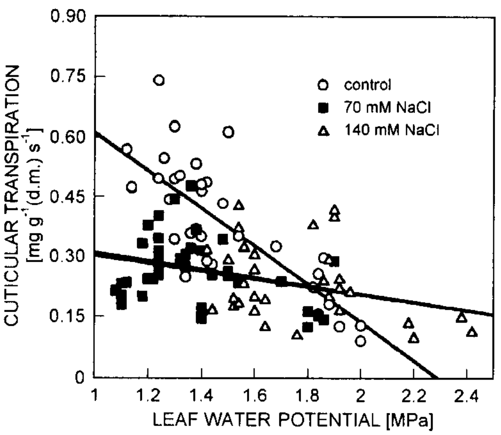

Fig. 1. Relationship between leaf water potential and cuticular transpiration for *Lotus creticus creticus* plants in control (*circles*), 70 mM NaCl (*squares*) and 140 mM NaCl (*triangles*). This relationship has been established considering the values recorded from pressure-volume isoterm leaves during the dehydration period.

The cuticular transpiration (T_C) decreased significantly under salinity (Table 3). Salinized plants presented similar values through the experimental period for both treatments. In all treatments the lowest Ψ_l values coincided with the lowest T_C levels. However, similar values of Ψ_l corresponded to higher T_C in control plants than in saline treatments (Fig. 1).

Surface water on hairy leaves: *Lotus* plants showed hairy leaves on both sides of the leaflets. The number of trichomes was approximately about 60 per mm^2 in young leaves and 160 per mm^2 for adult leaves

Hairy leaves of *Lotus* were effective in falling droplets, keeping almost 81 % of sprayed water. The time course of mass loss was recorded and corresponded to 26 min. At that time, the transpiration rate on sprayed and non-sprayed discs was similar indicating the complete evaporation of the surface water. In that moment, the amount of absorbed surface water by sprayed discs was about 9 % with respect to the retained water.

Discussion

In general, the plant growth under salinity conditions is reduced. Nevertheless, it is known that the degree to which the growth is reduced by salinity differs greatly with species and even with cultivars within a species (Shannon and Grieve 1999). In our conditions, the fact that the growth of *Lotus* plants increased with respect to the control plants could be related to the influence of duration of the saline stress (Neumann 1997). Similar results were obtained in field conditions, where *Lotus* plants submitted to salinity during one month showed a similar development or even better than the control ones (Sánchez-Blanco *et al.* 1998). Similar effect had been observed by many halophytic and in some glycophytic succulent plants in which the growth was stimulated by low to moderate salinity. On the contrary, after a longer period of salt stress, NaCl began to inhibit growth (shoot RGR; Table 2).

The fact that the roots growth was not affected by NaCl is in accordance with the results obtained in many studies (*e.g.* Kumar and Bharadwaj 1981, Khavari-Nejad and Najafi 1990, Munns and Termaat 1986, Niemann *et al.* 1988). This behaviour has been explained by a relatively greater proline accumulation in roots than in shoots during the salt stress. Proline plays an important role in the cellular osmoregulation and acts as a reserve of nitrogen to sustain root growth (*e.g.* Kalaji and Pietkiewicz 1993, Misra *et al.* 1996).

In this assay, osmotic adjustment of *Lotus* plants in both saline treatments (Table 3) maintained constant the leaf Ψ_p, avoiding the visual wilting and it was sufficient to maintain the growth during the first 15 d after the salt application. Changes in tissue elasticity can also affect the relationship between Ψ_p and cell volume (Davies and Lakso 1979, Ruiz Sánchez *et al.* 1993); however, changes in leaf tissue elasticity in *Lotus* plants were not observed as response to saline stress (Table 3). This has been also described in the same plants under salinity in the field (Sánchez-Blanco *et al.* 1998).

Besides the osmotic adjustment, the dehydration induced by salinity was avoided by reductions on the leaf water losses, thus, for similar Ψ_l salinized plants presented lower T_C. Possibly, the accumulation of some ions in plant tissues could alter the properties of cuticle (thickened leaf cuticle) causing lower water loss (Cuartero and Fernandez-Muñoz 1999). Moreover, the presence of hairy leaves in *Lotus* plants could contribute to decrease T_C (Grammatikopoulus *et al.* 1994).

The hairy leaf may increase the water retention and its duration increasing the probability of water absorption. Our observations showed that *Lotus* plants had percentage values of absorbed water (9 %) similar to those found in other native mediterranean species such as *Phlomis fructicosa* (6.5 %) and *Verbascum mallophorum* (7.5 %) and higher from those observed for leaves of species without trichomes such as *Nerium oleander* (Grammatikopoulus and Manetas 1994). In general, although hairs do not constitute the gate for water entrance, their presence may assist in retaining and prolonging the duration of water droplets on the leaf surface and their final absorption.

References

Beadle, C.L.: Growth analysis. - In: Hall, D.O., Scurlock, J.M.O., Bolhàr-Nordenkampf, H.R., Leegood, R.C., Long, S.P. (ed.). Photosynthesis and Production in a Changing Environment. Pp. 36-46. Chapman & Hall, London 1993.

Caballero, M., Cid, M.C.: Development of new ornamentals from Canary Islands native plants as alternative crops for Mediterranean areas. - In: Martmnez, P.F. (ed): Environmental Constraints in protected Cultivation: Possibilities for New Growing Techniques and Crops. Pp. 163-167. Commision of the EC, Brussels 1993.

Cuartero, J., Fernández-Muñoz, R.: Tomato and salinity. - Scientia Hort. **78**: 83-125, 1999.

Davies, F.S., Lakso, A.N.: Diurnal and seasonal changes in leaf water potential components and elastic properties in response to water stress in apple trees. - Physiol. Plant. **46**: 109-114, 1979.

Grammatikopoulos, G., Karabourniotis, G., Kyparissis, A., Petropoulou, Y., Manetas, Y: Leaf hairs of olive (*Olea europaea*) prevent stomatal closure by ultraviolet-B radiation. - Aust. J. Plant Physiol. **21**: 293-301, 1994.

Grammatikopoulos, G., Manetas, Y.: Direct absortion of water by hairy leaves of *Phlomis fruticosa* and its contribution to drought avoidance. - Can. J. Bot. **72**: 1805-1811, 1994.

Gucci, R., Xiloyannis, C., Flore, J.A.: Gas exchange parameters water relations and carbohydrate partitioning in leaves of field-grown *Prunus domestica* following fruit removal. - Physiol. Plant. **83**: 497-505, 1991.

Kalaji, M., Pietkiewicz, S.: Salinity effects on plants growth and other physiological processes. - Acta Physiol. Plant. **15**: 89-124, 1993.

Kerr, J.R., Beardsell, M.F.: Effect of dew on leaf water potentials and crop resistences in a *Paspalum* pasture. - Agron. J. **67**: 596-599, 1975.

Khavari-Nejad, R.A., Najafi, F.: Growth parameters in sunflower plants as affected by Ca/Na interactions under NaCl salinity. - Photosynthetica **24**: 155-162, 1990.

Kumar, S., Bharadwaj, P.: Studies on genotype difference in the early seedling growth of various crop plants under saline conditions. I. Mung bean (*Vigna radiata* (L.) Wilczek). - Indian J. Plant Physiol. **24**: 123-127, 1981.

Lunin, J., Gallatin, M.H., Batchelder, A.R.: Saline irrigation of several vegetable crops at various growth stages I. Effect on yields. - Agron. J. **55**: 107-114, 1963.

Munns, R.: Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. - Plant Cell Environ. **16**: 15-24, 1993.

Munns, R., Termaat, A.: Whole plant responses to salinity. - Aust. J. Plant Physiol. **13**: 143-160, 1986.

Neumann, P.: Salinity resistance and plant growth revisited. - Plant Cell Environ. **20**: 1193-1198, 1997.

Niemann, R.H., Clark, R.A., Pap, D., Ogata, G., Mass, E.V.: Effects of salt stress on adenine and uridine nucleotide pools, sugars and acid soluble phosphate in shoots of pepper and sunflower. - J. exp. Bot. **39**: 301-309, 1988.

Misra, A.N., Murmu, B., Singh, P., Misra, M.: Growth and proline accumulation in mungbean seedlings as affected by sodium chloride. - Biol. Plant. **38**: 531-536, 1996.

Olmos, E., Hellín, E.: Cellular adaptation from a salt-tolerant cell line of *Pisum sativum*. - J. Plant Physiol. **148**: 727-734, 1996.

Ruiz-Sánchez, M.J., Sánchez-Blanco, M.J., Planes, J., Alarcón, J.J., Torrecillas, A.: Seasonal changes in leaf water potential components in two almond cultivars. - J. agr. Sci. **120**: 347-351, 1993.

Sánchez-Blanco, M.J., Morales, M.A., Torrecillas, A., Alarcón, J.J.: Diurnal and seasonal osmotic potential changes in *Lotus creticus creticus* plants growth under saline stress. - Plant Sci. **136**: 1-10, 1998.

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

Shannon, M.C., Grieve, C.M.: Tolerance of vegetable crops to salinity. - Scientia Hort. **78**: 5-38, 1999.

Wilson, J.R., Fischer, M.J., Schulze, E.-D., Dalby, G.R., Ludlow, M.M.: Comparison between pressure-volume and dewpoint-hygrometry techniques for determining the water relations characteristics of grass and legume leaves. - Oecologia **41**: 77-88, 1979.