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Leaf pubescence, water relations and chlorophyll fluorescence in two subspecies of *Lotus creticus* L.

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Abstract

The objective of the present study was to compare the plant morphology, water relations and photochemical efficiency of photosystem 2 in two wild Mediterranean species *Lotus creticus creticus* and *Lotus creticus cytisoides*. *L. creticus creticus* showed higher density of trichomes and stomatal density on the adaxial leaf surface than *L. creticus cytisoides*, whereas *L. creticus cytisoides* showed higher stomatal density in abaxial surface than *L. creticus creticus*. These morphological traits promoted clear differences in leaf surface water retention and leaf reflectance. Leaf water potential and photochemical efficiency were lower in *L. creticus cytisoides* than in *L. creticus creticus*.

Additional key words: *Lotus creticus creticus*, *Lotus creticus cytisoides*, relative water content, stomata density, trichomes, xylem water potential.

Introduction

The interest of horticultural researchers in new ornamental species has grown over the last few years parallel to the demand for native plants in land reclamation processes. In Mediterranean environments, a summer stress and winter stress from very cold temperatures (Mitrakos 1980, Terradas and Savé 1992) along with anthropogenic stresses (*e.g.*, fires, grazing) has been considered as major factors influencing Mediterranean landscapes (Margaris and Mooney 1981, Rundel 1981). In order to improve landscape restoration and xerigardening, it s necessary to know mechanisms of the plant responses to environmental factors, their acclimation during nursery production or in the first stages after transplanting to the field (Montenegro *et al.* 1980,1982, Mooney *et al.* 1987, Sachs 1991, Savé *et al.* 1993a, 1997, 1999, Marfà *et al.* 1998).

Leaf pubescence is a very important morphological trait of plants with three functions (Johnson 1975): defence against the physical environment, defence against biotic factors, and a physiological and metabolic function. In this last category, the secondary metabolism compounds secreted by glandular trichomes and water absorption by hairy leaves were included. *Lotus creticus creticus* L. and *L. creticus cytisoides* L. are two Mediterranean subspecies that live in xeric areas close to the coast. Both subspecies have hairy leaves. The leaf trichomes are an adaptation for these plants to the Mediterranean environment (Grammatikopoulos *et al.* 1994, Johnson 1975, Rundel 1982). Leaf pubescence is thought to confer water use efficiency (WUE), since it increases the boundary layer and decreases the transpiration water losses; on the other hand, under high evaporative demand, water losses can be higher because pubescence increases the evaporative surface of leaves (Nobel 1991). The trichomes also play a role in the leaf energy budget, as reflectors of visible and infra-red radiation, resulting in lower leaf heat load (Ehleringer and Mooney 1978). The trichomes have been described as a protective coat against UV-B radiation; when exposed to UV-B radiation, dehaired leaves have a lower

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photosynthetic rate than control ones (Grammatikopoulos *et al.* 1994). Pubescence also protects plants from predators and parasites (Edwards 1992). Another important adaptation to Mediterranean climate is leaf reorientation to intercept a smaller quantity of radiant energy (Morris 1989, Nobel 1991, Pereira and Chaves 1993); this allows the plant to moderate leaf temperature without excessive transpiration.

The water uptake by organs other than roots has been described (Rundel 1982). Some plants in arid habitats possess adaptations that allow them to take advantage of light rain or condensation of water on leaves. A smooth, waxy leaf surface repels water, but presence of trichomes on the leaf surface can increase water retention. This retention improves the leaf water status by reducing transpiration and increasing the probability of water uptake by leaves (Grammatikopoulos and Manetas 1994).

In the present paper we tried to elucidate the role that play leaf pubescence in plant water balance in two indigenous subspecies of *Lotus creticus*, potentially used in landscape restoration under Mediterranean conditions.

Materials and methods

Plants: Fourteen-month-old plants of *Lotus creticus creticus* L. and *Lotus creticus cytisoides* L. were grown from vegetative cuttings in a greenhouse in 14-cm pots with peat:perlite (2:1; by volume) mixture watered daily to full soil moisture capacity with nutrient solution (1:0.6:2)($N:P_2O_5:KO_2$).

Stomata and trichome density: Leaflets of both *L. creticus* subspecies were preserved in 60 % ethanol because they were small (4 to 18 × 2 to 9 mm) and their epidermes were fragile. Leaflets were dehaired by pressing adhesive tape to the leaf surface and removing it gently (Grammatikopoulos *et al.* 1994). To make epidermal prints a small drop of contact glue (*Super-glue*, Loctite) was spread on a slide. A leaflet, with the dehaired side faced down, was put on the contact glue and pressed for a few seconds, then carefully removed from the slide. The trichomes pores and stomata left on the epidermal print were viewed and counted using a light microscope (*Carl Zeiss* 4270177, Jena, Germany).

Chlorophyll fluorescence: *In vivo* chlorophyll fluorescence of five plants each of subspecies was measured with a *Plant Efficiency Analyzer (PEA) (Hansatech Ltd.*, King's Lynn, England), placed on four randomly selected leaves of each plant, adapted in dark for 20 min before measuring. Each plant was irradiated with red light of 600, 1 200, 1 800 and 2 400 μmol m^{-2} s^{-1}, one level per leaf. Base fluorescence (F_0), maximum fluorescence (F_m), variable fluorescence (F_v), and mean time (T_m) were recorded. Among the chlorophyll fluorescence parameters F_v/F_m was selected due to its relation to efficiency of photosystem 2.

Water absorption: The water absorption by the leaves and shoots was evaluated using the method of Grammatikopoulos and Manetas (1994). Detached shoots (≈ 10 cm long) of each subspecies (n = 18) were placed with the cut ends in water for 24 h at 4 °C. The hydrated shoots were weighed and then divided into two main groups (sprayed and unsprayed), and then sealed at 3 different air humidities 100, 75 and 33 %. (VPD of 0, 0.585 and 1.567 kPa). Sprayed shoots were misted with tap water and reweighed before insertion into plastic chambers (2380 cm^3) containing deionized water (RH ≈ 100 %) or saturated solutions of NaCl (RH ≈ 75 %) or $MgCl_2.6\,H_2O$ (RH ≈ 33 %) (Young 1967). Approximately every 30 min during the next 5 h, the shoots were weighted and the relative water content RWC was calculated (Koide *et al.* 1989).

Drying cycle: 5 plants each subspecies were exposed to a drying cycle of 7 d without irrigation followed by 4 d of rehydration. Five control plants of each subspecies were sufficiently daily irrigated. Shoots of both treatments were collected at midday and immediately measurements of the leaf water potential on days 0, 7 and 11 were made with a pressure chamber (*Soilmoisture 3005, Soilmoisture Equipment Corp.*, Santa Barbara, USA) (Koide *et al.* 1989).

Statistical analysis: The statistical analysis of data conducted using *Statgraphics 7.0*. Analysis of variance was used when appropriate. For those data that were not distributed normally, nonparametric tests (Kruskall-Wallis) were used. Mean separations were by Duncan's multiple range test, $P \le 0.05$. The homogeneity of the regression coefficients was tested as in Gomez and Gomez (1984).

Results and discussion

The stomata of studied subspecies were narrow, surrounded by elliptical guard cells, and the pores left by the removed hairs were circular and slightly larger than stomatal pores. Both subspecies are amphistomatous, and show significantly higher number of stomata on the adaxial than abaxial surface. Some specific differences appeared also between them: *L. creticus creticus* had 15 % more stomata on adaxial surface and 12 % less in the abaxial surface than *L. creticus cytisoides* (Table 1). These differences may be related to reversible leaf movements in relation to environmental conditions (Palmer 1985) that show these subspecies. *L. creticus creticus* showed higher paraheliotropism (data not shown) than *L. creticus cytisoides,* which can be associated with greater drought avoidance, by means of protecting the leaf surface that has more stomata on it (Shackel and Hall 1979), because this reduces canopy irradiance interception and consequently leaf water vapour loss is reduced.

The density of thichomes was 42 % greater on adaxial surface of *L. creticus creticus* than in *L. creticus cytisoides.* On the abaxial surface, there were no significative differences between subspecies (Table 1).

Table 1. Densities of stomata and trichomes of *Lotus creticus creticus* and *Lotus creticus cytisoides*. Means ± SE (x - n = 17, y - n = 23, z - n = 14; NS - nonsignificant, * - significant at $P \leq 0.05$).

	Density of stomata [mm^{-2}]			Density of trichomes [mm^{-2}]		
	adaxial	abaxial		adaxial	abaxial	
L. creticus creticus	309.42 ± 15.31^x	220.78 ± 11.11^y	*	189.05 ± 13.76^x	193.64 ± 18.11^y	NS
L. creticus cytisoides	269.64 ± 8.74^x	251.03 ± 4.23^z	*	133.63 ± 6.33^x	150.70 ± 7.75^z	NS
	*	*		*	NS	

Table 2. Relative water content versus time (RWC = b·t + a) in unsprayed (control) and sprayed shoots of both *L. creticus* subspecies at three relative humidity treatments (n = 8, being each point the mean of five values, * - significant at $P \leq 0.05$, r^2 - correlation coefficient).

Subspecies	Treatment	RH	r^2		b ± SE	a
L. creticus creticus	control	33	0.8885	*	-0.0255 ± 0.004	98.529
L. creticus creticus	control	75	0.9520	*	-0.0259 ± 0.002	99.168
L. creticus creticus	control	100	0.7382	*	-0.0034 ± 0.001	100.460
L. creticus creticus	sprayed	33	0.7499	*	-0.1479 ± 0.038	122.490
L. creticus creticus	sprayed	75	0.7519	*	-0.0944 ± 0.024	111.450
L. creticus creticus	sprayed	100	0.8000	*	-0.0580 ± 0.013	117.110
L. creticus cytisoides	control	33	0.7289	*	-0.0421 ± 0.011	95.162
L. creticus cytisoides	control	75	0.8104	*	-0.0422 ± 0.008	97.076
L. creticus cytisoides	control	100	0.9415	*	-0.0131 ± 0.001	100.090
L. creticus cytisoides	sprayed	33	0.7812	*	-0.1301 ± 0.031	112.980
L. creticus cytisoides	sprayed	75	0.8182	*	-0.1139 ± 0.024	113.680
L. creticus cytisoides	sprayed	100	0.8985	*	-0.0557 ± 0.008	119.710

Leaf trichomes have a particularly strong influence on the formation, repulsion and surface location of water. The high frequency of dew condensation on leaves in Mediterranean habitat (Pitacco *et al.* 1992, Savé *et al.* 1999), joint with broad range of leaf's wettability among species, and potentially strong effects on photosynthesis and growth seems indicate an evolutionary effect related to leaf surface water (Brewer and Smith 1997).

In sprayed treatments, the slopes of RWC versus time did not show significant differences between subspecies nor among relative humidity treatments, except the RH 100 %, which have a smaller slope than the others (Table 2). In contrary, when we compare the control with the sprayed treatments there are clear and significant differences between them and among different relative humidity treatments (Table 2). From these results (Table 2) it was not possible to distinguish if the RWC in the sprayed treatment increase was due to simple surface water deposition and/or water absorption. In spite, it seems more reliable to relate the phenomenon to a simple water deposition. This is in contrary to that described by Grammatikopoulos and Manetas (1994) in a very similar assay, who described leaf water absorption from hairy leaves of *Phlomis fruticosa*. The relationship between the slopes of sprayed versus control groups in all studied treatments (Table 2) showed as these were ≈ 260 % lower in *L. creticus cytisoides* than in *L. creticus creticus*. This seems to indicate greater water retention or suface water maintainance in *L. creticus cytisoides*. This had to be due, according to Brewer and Smith (1997), to pubescence differences. So leaves of *L. creticus cytisoides* with lower leaf hair density, but higher than 1 trichome·mm^{-2}, showed a relative "segregating strategy". Hydrophilic trichomes encircle patches of water where water remained in pools until removed by evaporation. Leaves that segregated water into patches had higher droplet retention. In the same way leaves of *L. creticus creticus* showed a "lifting strategy", in which dense trichomes prevented surface moisture from reaching the leaf epidermis. Leaf surfaces exhibiting this feature had low droplet retention.

These had important ecological and productive implications, because CO_2 diffuses about 10^4 times more slowly in water than in air, and consequently could reduce the assimilation rate when the leaf surface is covered by water (Grammatikopoulos and Manetas 1994, Brewer and Smith 1997). This phenomenon must be very important in Mediterranean habitat, where the night dews and fogs promoted that the leaves remained wet 2 h or more after dawn (Pitacco *et al.* 1992). This is a very important period of time for stressed plants and/or plants with erectophyllous leaves. After dawn the radiation level is sufficiently high and the evaporative demand sufficiently low in order to improve the plant water use efficiency and, consequently, maintain the growth rate. This has been described (Evans *et al.* 1997, personal communication) in plants of *Arctostaphyllos uva-ursi.*

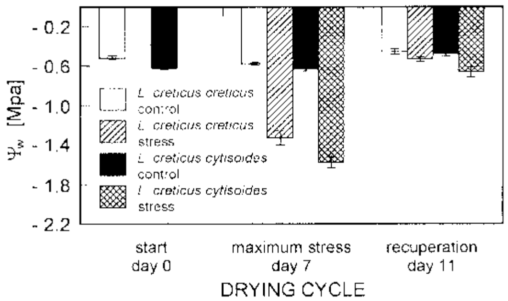

Fig. 1. Leaf water potential of *Lotus creticus creticus* and *L. creticus cytisoides* on start day (0), maximum stress day (7) and four days after rewatering (11), in control and stress groups. Means ± SE, *n* = 5.

Pubescence promoted also a reduction in the rate of leaf water losses, so *L. creticus creticus* sprayed plants showed a 6 % lower transpiration rate than *L. creticus cytisoides*. This avoidance mechanism to drought stress (Savé *et al.* 1993b) promoted low level of tissue dehydration and better recuperation after stress (Fig. 1). So leaf water potential values were always significantly lower in *L. creticus cytisoides* than in *L. creticus creticus* along water stress cycle.

Development of highly reflective leaf pubescence decreases both leaf heat-load and the reliance on transpirational cooling for maintenance of favorable leaf temperatures (Sandquist and Ehleringer, 1997). However, leaf hairs are costly to produce and they reflect photosynthetically active radiation, which reduces carbon assimilation (Ehleringer and Werk, 1986). However, leaf pubescence provide a protection against UV-B radiation which inhibit photosynthetic rate due to stomatal closure and reduction in photosystem 2 photochemical efficiency (Grammatikopoulos *et al.* 1994, Bisba *et al.* 1997). *In vivo* chlorophyll fluorescence is used as a sensitive probe for studying photosynthesis. So the F_v/F_m ratio is a measure of PS 2 photochemical efficiency, and its a sensitive indicator of photoinhibition and other stresses (Araus and Hogan 1994). The obtained results agree with this idea, and the more pubescent subspecies, *L. creticus creticus*, showed higher values of F_v/F_m ratio in all the range of irradiance studied (Fig. 2).

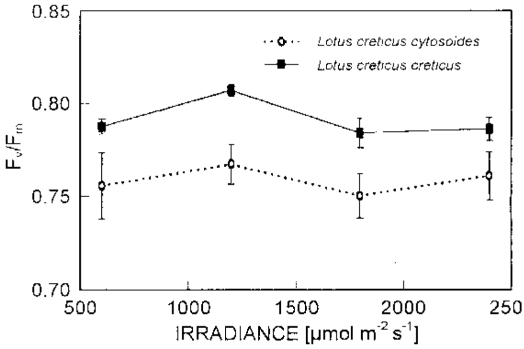

Fig. 2. Chlorophyll fluorescence ratio F_v/F_m of *L. creticus creticus* and *L. creticus cytisoides* at four irradiances (µmol·m^{-2}·s^{-1}). Means ± SE, *n* = 5.

The combination of the avoidance mechanisms (*e.g.*, paraheliotropism of leaflets, leaves and shoots, leaf pubescence, low transpiration rate) and the tolerance mechanisms (*e.g.*, osmotic and elastic adjustment; Savé *et al.* 1996a), create very important adaptation of species to Mediterranean climate (Terradas and Savé 1992).

In previous works about these species (Savé *et al.* 1996a, b) *L. creticus creticus* showed a great resprouting capability and important growth rates under a rainfall of 300 mm. From the results obtained in the present and previous works, it can be deduced that this is a very useful species for revegetation in restored areas. Some effort must be conduced in improve its growth patterns under minimum irrigation under arid and semi-arid Mediterranean conditions.

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