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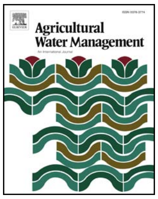

Long term responses and adaptive strategies of *Pistacia lentiscus* under moderate and severe deficit irrigation and salinity: Osmotic and elastic adjustment, growth, ion uptake and photosynthetic activity

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ABSTRACT

Pistacia lentiscus is a wild species that grows widely in the Mediterranean area. However, despite it appearing to be particularly resistant to some stressful conditions, drought and salinity may alter its physiological and morphological behavior. While the responses of *P. lentiscus* to both stresses have been partially studied, its avoidance and tolerant mechanisms are poorly understood. In particular, changes in leaf tissue cell wall and the photosynthetic activity during a prolonged water deficit and salinity are unknown. Nursery grown plants were subjected to four irrigation treatments lasting eleven months: control ($1\ \mathrm{dS\ m^{-1}}$, 100% water holding capacity), two deficit treatments (moderate and severe water deficit, corresponding to 60 and 40% of control) and saline treatment ($4\ \mathrm{dS\ m^{-1}}$, same amount of water supplied as control). Biomass accumulation was affected more by deficit irrigation than by salinity. Salt tolerance in *P. lentiscus* was associated with the restricted uptake of Cl^- and its storage in roots. However, the cumulative effect of irrigating with saline water involved an over-accumulation of Na^+ and Cl^- in leaves, which probably contributed to the pronounced decrease in photosynthesis, confirming the importance of the length of exposure of the plants to salt stress. Plants under saline or severe deficit irrigation exhibited slight dehydration throughout the experiment, as indicated by the lower leaf water potential and relative water content, due to the low availability of substrate water (osmotic effect). The response of plants to severe water stress, which resulted in stomatal closure and a decrease in net photosynthesis rate, involved a marked decrease in plant height and growth, especially in the first months of the experiment, after which a slight acclimation may have occurred in these plants. Under moderate water stress, most of these responses were mitigated. Salinity induced active osmotic adjustment and decreased leaf tissue elasticity. Due to its tolerance of water stress and salinity, *P. lentiscus* is a suitable ornamental species for gardening in arid and saline area.

1. Introduction

Drought tolerance in plants may be explained by functional and structural adaptations at cellular and whole plant, such as growth regulation, osmotic adjustment, changes in cell wall elasticity and in leaf water potential, stomatal closure, all of which may help alleviate the harmful effects of stress (Zheng et al., 2010; Suárez, 2011). Exposure to salt may affect plant metabolism through an osmotic effect, causing water deficit, or through a specific ion effect, causing excessive ion accumulation (Azza Mazher et al., 2007). Under saline conditions, plants have to activate different physiological and biochemical mechanisms to cope with the salt stress, which include changes in morphology, anatomy, water relations, photosynthesis, the hormonal profile, toxic ion distribution and antioxidative metabolism response (Acosta-Motos et al., 2017). However, although salinity and drought stress are physiologically related and the tolerance mechanisms

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Abbreviations: C, control; DW, dry weight; EC, electrical conductivity; g_s, stomatal conductance; J, absorption rate of ions by the root system; LMA, leaf mass per area; P, significance; P_n, net photosynthesis rate; P-V, pressure-volume; RWC_{tpl}, relative water content at turgor loss point; S, saline treatment; SW, severe water deficit treatment; MW, moderate water deficit treatment; Ψ_l, leaf water potential; Ψ_{100s}, leaf osmotic potential at full turgor; Ψ_{tpl}, leaf water potential at turgor loss point; ε, bulk modulus of elasticity; F_v/F_m, maximal PSII photochemical efficiency

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overlap, some aspect of plant physiology and metabolism may differ if the plant experiences saline or water stress. In particular, the changes in leaf tissue cell walls and the factors limiting photosynthesis under these conditions and their possible interactions with leaf tissue damage are not well understood. However, while some Mediterranean species appear to be particularly resistant to some stressful conditions and have developed mechanisms/strategies, such as efficient stomatal control linked to a conservative use of water resources and increases in the water uptake with deep root systems that may allow plants to survive during intense drought conditions, this does not necessarily mean that the plant will be of high visual quality. Even plants that have some degree of drought and/or salinity tolerance may show reductions in quality when exposed to these stresses, which is an important factor in ornamental plants destined for use in gardens and landscaping (Cameron et al., 1999).

The use of Mediterranean shrubs for revegetation in semiarid areas has increased in recent years due to its ability to adapt to severe conditions of drought, which is considered one of the most important factors affecting plant survival and species distribution (Filella et al., 1998; Vallejo et al., 2000; Vilagrosa et al., 2014). Among these, *Pistacia lentiscus* L. (mastic tree or lentisc), is considered a good alternative for landscaping and restoration projects because it responds well to the conditions of Mediterranean summers. The effect of severe drought on the physiological and morphological parameters of *P. lentiscus* and its strategies of drought-avoidance have been well established by Vilagrosa et al. (2003a, 2010) and by Gratani et al. (2013). Indeed, many studies in plants in water-limited ecosystems have regarded the role of plant hydraulic conductivity and their relationship with other traits as a key step in plant growth and survival (Vilagrosa et al., 2010; Lens et al., 2013). Although the general effects of withholding irrigation during the summer period on plant growth and survival of *P. lentiscus* have been studied (Vilagrosa et al., 2003a), further work is required to quantify its physiological responses to different levels of deficit irrigation, as in urban gardening projects, unlike in most revegetation projects, plants are usually watered and fertilized as needed, or at least partially watered to maintain an acceptable appearance. Such deficit irrigation strategies involve the application of water at a volume lower than that needed to compensate the evapotranspiration rate, and may be used in potted ornamental plants to improve plant quality by reducing excessive vigour and promoting a more compact habit (Cameron et al., 2006). However, the degree and duration of the water stress imposed on each species is also critical (Álvarez et al., 2009). In this sense, numerous works in ornamental plants have demonstrated that plant quality decreases as the severity of deficit irrigation increases (Hansen and Petersen, 2004; Henson et al., 2006; Katsoulas et al., 2006; Chyliński et al., 2007; Silber et al., 2007; De Lucia, 2009; Álvarez et al., 2009; Sánchez-Blanco et al., 2009; Bolla et al., 2010; Andersson, 2011; Bernal et al., 2011).

Furthermore, as the competition for high quality water increases, the use of saline waters and reclaimed water has become an option for irrigating ornamentals plants in urban gardening (Cassaniti et al., 2009; Acosta-Motos et al., 2014; Acosta-Motos et al., 2016). Tattini et al. (2006) and Tattini and Traversi (2008) tested the influence of salinity on *P. lentiscus* during a short period of intense salinity, and found it to be particularly tolerant to salt stress, although growth was markedly reduced, at least using irrigation water of 23 dS m^{-1} (200 mM NaCl). In addition, Armas et al. (2010) conducted a study to determine species' tolerances to salinity and found that *P. lentiscus* can withstood salinity levels similar to that of groundwater, reached 25.3 dS m^{-1} (220 mM NaCl). However, it is well known that plant response to salinity depends not only on the intensity of salt treatment, but also on the time of exposure to the salt treatment (Álvarez and Sánchez-Blanco, 2015). These important aspects must be considered when saline water is used for irrigation in long-lived species, as the interaction of both parameters will determine the physiological and molecular changes that take place. Since the growing season also seems to affect the response of shrubs to salt or water deficit (Valdez-Aguilar et al., 2011; Álvarez et al., 2013), the present research was carried out during the entire growing season using different deficit irrigation levels and water quality.

The purpose of this work was to study the long-term effects on *P. lentiscus* plants in response to saline water and water deficit. For this, growth, ion uptake, gas exchange, leaf water potential and their components, oxidative damage and photosynthesis responses were evaluated to ascertain the changes that take place in plants exposed to different levels of deficit irrigation and salinity. Understanding the limits and trade-offs between drought and salt tolerance, and the traits that are associated with tolerance to both factors, would provide important insights that would contribute to water management in the Mediterranean area, where deficit irrigation strategies using low quality waters are very often applied in gardening and landscaping projects.

2. Materials and methods

2.1. Plant material and experimental conditions

Seedlings of 1-year-old *Pistacia lentiscus* (mastic) grown in 5 × 5 × 11 cm pots by a specialised nursery were transplanted into 4 L plastic pots (15 × 15 × 20 cm) filled with a 5:4:1 (v/v/v) mixture of coconut fibre, black + sphagnum peat and perlite amended with 2 g L^{-1} of Osmotocote Plus (14:13:13 N, P, K plus microelements). Plants were placed in a plastic greenhouse equipped with a cooling system, located in Santomera, Murcia, Spain (38°06′N, 1°02′W, 110 m a.s.l.). All the plants were watered daily for 4 weeks to field capacity prior to starting the treatments. The micro-climatic conditions, registered with a Hoboware Lite Data Logger (Escort Data Loggers, Inc., Buchanan, Virginia, USA), were 12.9 °C (mean minimum), 25.5 °C (mean maximum) and 20.3 °C (average) temperature; and 42% (mean minimum), 77% (mean maximum) and 62.9% (average) relative humidity.

2.2. Treatments

Pistacia lentiscus plants were subjected to four irrigation treatments (40 plants per treatment) lasting 11 months using a computer-controlled drip irrigation system. The irrigation treatments consisted of a control (C) corresponding to 100% water holding capacity (leaching 15% (v/v) of the applied water), using tap water where the electrical conductivity of the water was 1.0 dS m^{-1}, a saline treatment (S) using tap water with salt added to reach 44 mM NaCl (4.0 dS m^{-1}) and two deficit irrigation treatments: (60% of the control level of irrigation water, 1.0 dS m^{-1} (moderate water deficit; MW) and 40% of the control irrigation water, 1 dS m^{-1} (severe water deficit; SW). One drip nozzle, delivering 2 L h^{-1} per plant, was connected to two spaghetti tubes (one on each side of every pot) and the duration of each irrigation episode was used to vary the amount of water applied, which depended on the treatment and on weather conditions. All the plants were irrigated daily.

2.3. Growth and physiological measurements

At the beginning and at the end of the treatment period ten plants per treatment were separated into shoots (leaves and stem) and roots before being oven-dried al 80 °C until they reached a constant weight to measure the respective dry weights (DW). Leaf area was determined in the same plants, using a leaf area meter (Delta-T Devices Ltd., Cambridge, UK). Leaf succulence was calculated by dividing the fresh by the dry weight and leaf mass per area (LMA) was calculated by dividing the dry weight by the leaf area. Throughout the experiment, plant height was measured periodically in 30 plants per treatment. At the beginning and at the end of the experimental period, ten plants per treatment (separated into leaves, stem and roots) were washed with distilled water and dried at 80 °C, before being stored at room

Table 1
Growth parameters and ion leakage at the end of the experiment in *P. lentiscus* subjected to different irrigation treatments. Values are the mean of ten plants.

Parameters	Treatments				P
	C	S	MW	SW	
Leaf DW (g $plant^{-1}$)	16.5 ± 1.4b	17.0 ± 2.3b	16.9 ± 1.1b	13.0 ± 0.9a	*
Stem DW(g $plant^{-1}$)	23.1 ± 1.1c	25.0 ± 3.0c	16.7 ± 1.4b	12.5 ± 0.8a	***
Root DW (g $plant^{-1}$)	22.2 ± 1.8b	20.3 ± 2.4b	19.8 ± 1.3b	15.8 ± 0.7a	*
Leaf area (cm^2)	1123 ± 131b	1233 ± 239b	1280 ± 99b	959 ± 84a	*
Root to shoot ratio	0.57 ± 0.05a	0.49 ± 0.02a	0.61 ± 0.06a	0.65 ± 0.06a	ns
Leaf succulence (g g^{-1})	2.16 ± 0.11a	2.90 ± 0.07b	2.20 ± 0.07a	2.32 ± 0.08a	***
LMA (g m^{-2})	216.2 ± 23.5b	164.4 ± 12.9b	173.5 ± 9.1b	133.4 ± 6.4a	**
Ion leakage (%)	34.2 ± 0.55a	31.1 ± 0.86a	39.1 ± 1.35a	35.8 ± 0.90a	ns

Means within a row without a common letter are significantly different according to Duncan 0.05 test.
(P; probability level, ns; non significance, *P < 0.05, ** P ≤ 0.01, *** P ≤ 0.001).

temperature for inorganic solute analyses. The concentration of Cl^- was analysed by chloride analyzer (Chloride Analyser Model 926, Sherwood Scientific Ltd.) in the aqueous extracts obtained when mixing 100 mg of dry vegetable powder with 40 mL of water and shaking for 30 min before filtering. The concentrations of Na^+ were determined in a digestion extract with HNO_3:$HClO_4$ (2:1, v/v) by Inductively Coupled Plasma optical emission spectrometer (ICP-OES IRIS INTREPID II XDL Thermo, England). The absorption rate of Na^+ and Cl^- ions by the root system (J) was calculated by considering the total salt content of ten plants per treatment at harvest and expressed as mmol Na^+ and Cl^-, and the mean root DW, using the formula described by Pitman (1975).

$$J = \frac{(M_2 - M_1)}{(WR \times t)}$$

where M_1 and M_2 correspond to a concentration in mmol of Na^+ or Cl^- in the total plant at the beginning and at the end of experimental period, respectively, t corresponds to time in days and WR is the logarithmic mean root biomass, calculated as $\frac{WR_2 - WR_1}{Ln\left(\frac{WR_2}{WR_1}\right)}$ with WR_1 and WR_2 are the root DW at the beginning and at the end of experimental period, respectively.

Chlorophyll fluorescence and relative chlorophyll content (RCC) measurements were taken in eight plants per treatment at midday, on the adaxial leaf surface. The values of maximum photochemical efficiency of photosystem II (F_v/F_m) were obtained using a portable fluorometer Opti-Sciences (Model OS 30; Opti-Sciences Inc., Tyngsboro, MA, USA) on leaves re-darkened for 20 min before starting the measurements (Camejo et al., 2005) and RCC was measured with a Minolta SPAD-502 chlorophyll meter.

The rate of passive ion leakage from stress-sensitive plant tissue can be used as a measure of alterations in membrane permeability. In our case, ion leakage was estimated at the end of the experiment with ten replicates per treatment in mature leaves, according to the method described by Lafuente et al. (1991).

The substrate water content was periodically determined using time domain reflectometry (TDR) equipment (Tektronic, model. 1502B) in eight plants per treatment. Throughout the experimental period, in May, June, July and October (6, 7, 8 and 10 months since beginning the treatments) the diurnal patterns of leaf water potential (Ψ_l), relative water content (RWC), stomatal conductance (g_s) and net photosynthetic rate (P_n) were measured from sunrise to sunset, at 2 h intervals, in eight plants per treatment. Ψ_l was estimated according to the method described by Scholander et al. (1965), using a pressure chamber (Soil Moisture Equipment Co, Santa Barbara, CA, USA), for which leaves were placed in the chamber within 20 s of collection and pressurised at a rate of 0.02 MPa s^{-1} (Turner, 1988) while the RWC of leaves was calculated according to Barrs (1968). Gas exchange parameters (g_s and P_n) were determined using a gas exchange system (LI-6400, Li-cor Inc., Lincoln, NE, USA). Estimates of the bulk modulus of elasticity (ε), leaf osmotic potential at full turgor (Ψ_{100s}), leaf water potential at turgor loss point (Ψ_{tlp}) and relative water content at turgor loss point (RWC_{tlp}) were obtained at the end of the different irrigation treatments in three leaves per plant and five plants per treatment, via pressure-volume (P-V) analysis of leaves, as outlined by Wilson et al. (1979).

2.4. Statistical analyses of data

In the experiment 40 plants were randomly assigned to each treatment. The data were analyzed by one-way ANOVA using SPSS 17.0 software (SPSS Inc., 2002). Ratio and percentage data were subjected to an arcsine square-root transformation before statistical analysis to ensure homogeneity of variance. Treatment means were separated with Duncan's Multiple Range Test. Statistical comparisons were considered significant at $P \leq 0.05$.

3. Results

3.1. Growth analysis and mineral concentration

At the end of the experimental period, salinity and water deficit affected the growth and size of the *Pistacia lentiscus* plants. However, the effect depended on the treatment and the studied parameter. Water deficit affected biomass production of the *P. lentiscus* plants at the end of the experimental period although no significant differences were observed between saline and control treatments (Table 1). Both water deficit treatments (MW and SW) significantly reduced stem dry weight (DW) compared with the control, the effect being more pronounced in SW plants, while root and leaf growth (leaf DW and leaf area) was only reduced in SW plants. As regard the root to shoot ratio, no differences between treatments were observed (F = 1.75, P = 0.174). The reductions in total DW were around 0, 14 and 33% for S, MW and SW treatments, respectively, compared with the control. Plants submitted to saline irrigation treatment showed an increase in leaf succulence at the end of the experimental period (F = 10.75, P = 0.000), while this parameter was not modified in plants submitted to a water deficit. SW treatment significantly reduced leaf mass per area (LMA) compared with the rest of the treatments (Table 1; F = 5.45, P = 0.003).

Salinity and, especially, water deficit reduced plant height, the effect of the latter constraint being particularly marked in SW plants (Fig. 1). At the end of the experimental period, the reductions in height in plants submitted to salinity were similar to those found in plants submitted to MW, although differences between them were evident throughout the experimental period. Plant height began to be inhibited in deficit irrigated plants 18 weeks after beginning deficit irrigation (March; F = 3.90, P = 0.012), while in saline plants it began to be inhibited 37 weeks after beginning the saline treatment (July; F = 23.54, P = 0.000). Throughout the experiment, control plants were the tallest and the smallest plants were those subjected to severe water stress. At the end of the experiment the reductions in plant height were around 20, 21 and 34% for S, MW and SW, respectively, compared with the

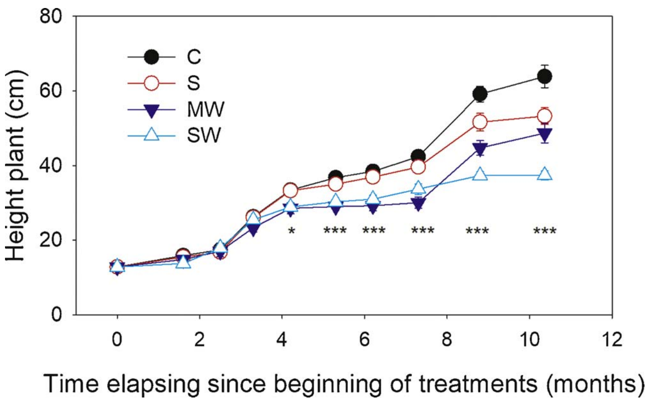

Fig. 1. Evolution of plant height in *P. lentiscus* plants submitted to different irrigation treatments. Values are means ± s.e., n = 30. Symbols represent the different treatments: Control (filled circles), saline (open circles), moderate water deficit (filled triangles) and severe water deficit (open triangles). For each studied day, asterisks indicate significantly different between treatments ($^*P < 0.05$, $^{***} P \leq 0.001$).

control (Fig. 1).

Salinity increased the rates of both Na^+ and Cl^- absorption by roots (J) compared with the rest of treatments (Fig. 2). In the saline treatment the absorption rates of both ions by the root system reached values 4.3 and 3.0 fold higher than in control plants for Na and Cl, respectively, while the relative concentrations in the irrigation water in the saline treatment was 17 fold higher than in the control water. The ability of these plants to restrict the entry of Na or Cl through the roots was investigated by calculating the slope of the linear regression between the increasing Na and Cl concentrations in the water and their relative absorption rate by the root system (Fig. 2). In the saline treatment the absorption rates of Na showed a higher slope than Cl, which means that the plants were able to restrict Cl^- uptake by roots to a greater extent than Na.

While no accumulation of Cl^- and Na^+ was observed in the plants subjected to the water deficit treatments (MW and SW), the concentrations of both ions increased with salinity in all parts of the plants (Table 2). As regards Na^+ distribution in plants irrigated with water of low EC, the highest values for Na were found in the roots, regardless of the amount of water (C, MW and SW) (Table 2). In saline conditions (S), higher values were found in leaves and roots than in the stem. The leaf Na concentration in plants of the S treatment increased sharply, reaching a value 30-fold higher than in control plants. In the case of Cl^-, plants irrigated without added salt (C, MW and SW) showed the highest values in leaves, while under saline conditions (S) the highest values were found in the root system. The tendency of the plants to preferentially accumulate Na and Cl in a given part of the plant (leaves, stem or roots) was investigated by calculating the slope of the linear regression between the Na^+ and Cl^- concentration in plant tissue and their relative concentrations in the irrigation water (Table 3). In S plants, the accumulation of Na in the root system and, especially, in the leaves showed a higher slope than that obtained for the stem (Table 3). In the case of Cl accumulation, higher slopes were found for the root system, compared to stem and leaves. This means that under saline conditions the transport of ions from the roots to the leaves was only restricted in the case of Cl ion, and that each toxic ion was distributed differently.

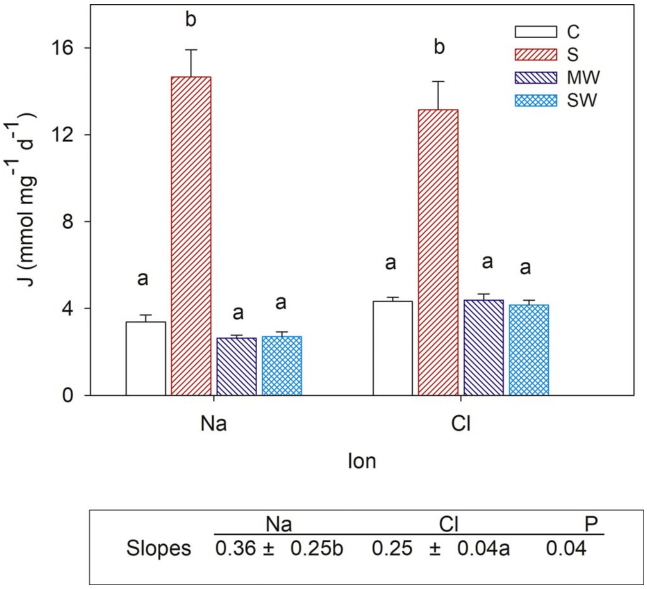

Fig. 2. Absorption rate of Na^+ and Cl^- ions by the root system (J) measured at the end of the experimental period in *P. lentiscus* plants submitted to different irrigation treatments. Values are the mean of ten plants. Means within an ion without a common letter are significantly different by Duncan $_{0.05}$ test. The slopes of the linear regressions between Na^+ and Cl^- concentration in the irrigation water and rate of absorption rate by the root system in plants subjected to saline treatment (S) are shown in the bottom part of the figure.

3.2. *Plant water relations, stomatal conductance and photosynthetic parameters*

The volumetric water content of the substrate was highest in the saline plants and decreased proportionally with the deficit level imposed with respect to the control (Fig. 3; $P < 0.01$). This pattern was reflected in the seasonal values of the leaf water potential (Ψ_l) at predawn, which decreased in the water deficit treatments, especially in SW plants in June ($F = 12.37$, $P = 0.000$), while no significant differences were observed between the saline and control treatments (Fig. 4). However, in July and October the values of Ψ_l at predawn for water deficit treatments were higher than in previous months, being very similar between them. The Ψ_l values decreased in all treatments as the evaporative demand of the atmosphere increased, reaching values at midday of −2.3 and −2.5 MPa for the saline and SW treatments, respectively in October. In general, the highest Ψ_l values were found in the early morning and the lowest at midday, after which the values recovered, although this did not occur in the case of SW plants (May and June), in which Ψ_l remained lower than the other treatments (Fig. 4a and b).

RWC values showed a similar behavior to that observed for Ψ_l, with SW plant having the lowest values, especially in May and June, when RWC values remained lower than the other treatments during all time of the day (from predawn to afternoon) (Fig. 4e–h). In July and October the values of RWC for SW treatment were higher than in previous months and significant differences between SW and control only were observed at the end of the day. No pronounced differences in RWC were observed between the control and saline treatment plants during most of the experimental period, although salinity affected RWC at the end of the experiment, when lower values were observed in saline-treated plants compared with the control.

The parameters derived from the pressure-volume curve at the end of the experiment are shown in Table 4. Leaf osmotic potential values at full turgor (Ψ_{100s}) decreased in S plants, which was indicative of the osmotic adjustment that occurred due to saline irrigation. The difference between the values obtained in the control and saline plants were taken as an estimate of this adjustment (−0.38 MPa) in the S treatment. However, no consistent effect of water deficit on Ψ_{100s} was observed, while leaf water potential at the turgor loss point (Ψ_{tlp}) was similarly reduced in both S and SW treatments. The relative water content at the turgor loss point was only affected by the SW treatment, for which the point of zero turgor occurred at a lower relative water content than in the control. The bulk modulus of elasticity increased in plants submitted to the S treatment and decreased in the SW plants, both compared with controls.

Table 2
Concentrations of Na^+ and Cl^- at the end of experimental period in *P. lentiscus* subjected to different irrigation treatments. Values are the mean of ten plants.

(mmol Kg^{-1} DW)		Treatments				P
		C	S	MW	SW	
Na	Leaves	25.0 ± 2.1aA	736.5 ± 78.8bB	20.6 ± 2.7aA	10.4 ± 1.7aA	***
	Stem	22.6 ± 2.5aA	165.8 ± 16.8bA	25.0 ± 4.0aA	10.0 ± 1.3aA	***
	Root	368.9 ± 33.7aB	748.5 ± 60.7bB	297.1 ± 17.8aB	358.2 ± 27.1aB	***
		***	***	***	***	
Cl	Leaves	245 ± 25.5aB	490.3 ± 52.6bB	264.6 ± 32.1aB	244.0 ± 15.7aB	***
	Stem	157.7 ± 17.5aA	337.1 ± 29.3bA	168.0 ± 17.5aA	173.7 ± 19.3aA	***
	Root	186.9 ± 16.4aA	654.6 ± 30.0bC	192.0 ± 22.7aA	227.1 ± 19.7aAB	***
		*	***	*	*	

Means within a row without a common lower case letter are significantly different according to Duncan 0.05 test. Means within a column without a common capital letter are significantly different according to Duncan 0.05 test.
(P; probability level, $*P < 0.05$, $*** P \leq 0.001$).

Table 3
Slopes of the linear regressions between Na^+ and Cl^- concentration in the irrigation water and plant concentration at the end of the experimental period in *P. lentiscus* plants subjected to saline treatment(S). Values are the mean of ten plants.

Part of the plant	Na^+	Cl^-	P
Leaf	21.89 ± 2.36cB	7.44 ± 0.82bA	***
Stem	4.40 ± 0.45aA	5.19 ± 0.40aA	ns
Root	11.67 ± 0.85bA	13.53 ± 0.46cA	ns
	***	***	

Means within a column without a common lower case letter are significantly different according to Duncan0.05 test. Means within a row without a common capital letter are significantly different according to Duncan 0.05 test.
(P; probability level, ns; non significance, $*** P \leq 0.001$).

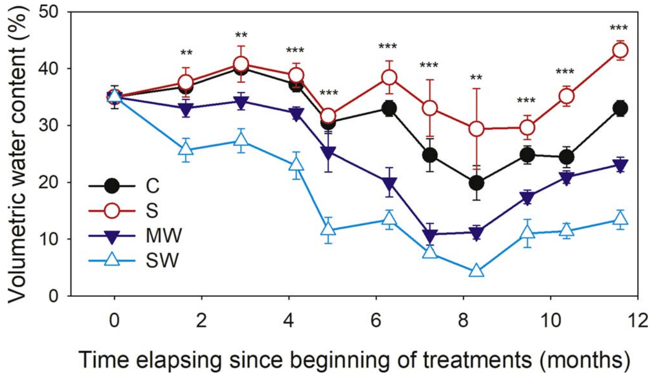

Fig. 3. Evolution of the volumetric substrate water content in *P. lentiscus* plants submitted to different irrigation treatments. Values are means ± s.e., n = 8. Symbols represent the different treatments: Control (filled circles), saline (open circles), moderate water deficit (filled triangles) and severe water deficit (open triangles). For each studied day, asterisks indicate significantly different between treatments (** $P \leq 0.01$, *** $P \leq 0.001$).

The relative chlorophyll content values did not change during the experiment in any of the treatments (Fig. 5a). In contrast, the chlorophyll fluorescence values (F_v/F_m) were around 0.80–0.75 for control plants, 0.70 for MW and 0.62 for SW plants in the early summer (June), after which the values for water deficit plants recovered, showing similar values of F_v/F_m for all treatments. No significant differences were observed between saline and control treatments throughout the experiment (Fig. 5b). Membrane damage, assessed by ion leakage was not affected by salinity or water deficit (Table 1).

The highest values of g_s were recorded during the early hours of the day, followed by a gradual decline thereafter in all irrigation regimes (Fig. 6). The plants subjected to both water deficit treatments showed lower stomatal conductance (g_s) and photosynthesis net rate (P_n) than the control from the beginning of the experiment (Fig. 6a–h), especially in the early summer (May and June), when plants of the SW treatment reached very low g_s values of below 50 mmol $m^{-2} s^{-1}$. Control plants had the highest P_n and g_s values during most of the experiment, except in autumn (at the end of the experimental period; Fig. 5d and h), when plants from both deficit irrigation treatments had similar g_s values to control plants and even higher P_n values than the control plants. The S treatment produced a slight reduction in g_s with respect to the control, a reduction that was more marked at the end of the experiment. The g_s and P_n values fell later in the saline treatment than in both water stress treatments, although at the end of the experiment the effect on P_n and g_s was more marked in saline than in the water stressed plants, S plants reaching g_s values, below 50 mmol $m^{-2} s^{-1}$ at this time.

4. Discussion

The response of species to stresses in terms of growth is the ultimate expression of several interacting physiological and biochemical parameters and has often been used to characterize salt or water deficit tolerance (Sidari et al., 2008; Cassaniti et al., 2009; Álvarez et al., 2012; Gómez-Bellot et al., 2013a; Álvarez and Sánchez-Blanco, 2015). Biomass accumulation in *Pistacia lentiscus* plants was affected more by deficit irrigation than by salinity. According to Armas et al. (2010) salinity level applied to *P. lentiscus* does not seem to have constrained performance of plant, since it is known to be a relatively salt-tolerant species. In contrast in *C. laevis* plants, grown under similar conditions and irrigated with the same levels of deficit irrigation and salinity as used in our trial, plant growth was more sensitive to salinity than to soil drying (Álvarez and Sánchez-Blanco, 2015). The inhibition of leaf growth under deficit irrigation is seen as an adaptation, because it allows plants to reduce water losses by lowering transpiration and delaying the onset of more severe stress (Ruiz-Sánchez et al., 2000). However, it has been suggested that under salt stress the main reason for growth reduction is associated with ion toxicity, ion imbalance, and/or cell wall properties (Munns, 2005; Rodríguez et al., 2005). In our experiment, saline water irrigation had no effect on *P. lentiscus* biomass and, although it slightly inhibited the plant height, such a reduction was only noticeable a long time after the beginning of the treatments, confirming that the duration of the salt stress is an important factor, since salts take time to accumulate inside plants before the concentrations reach toxic levels and affect plant function (Munns and Tester, 2008). In this way, ionic stress affects growth later than osmotic stress, when salt reaches toxic concentrations in the old leaves. In the presence of salt, leaf succulence was greater than the other treatments. This response has previously been reported by Slama et al. (2008) and by Suárez (2011). Under conditions of saline stress, increased succulence tends to lower intracellular ion concentrations, and prevent the excessive accumulation of ions in the leaf sap. Contrarily, under water stress, succulence may dilute organic ion concentration,

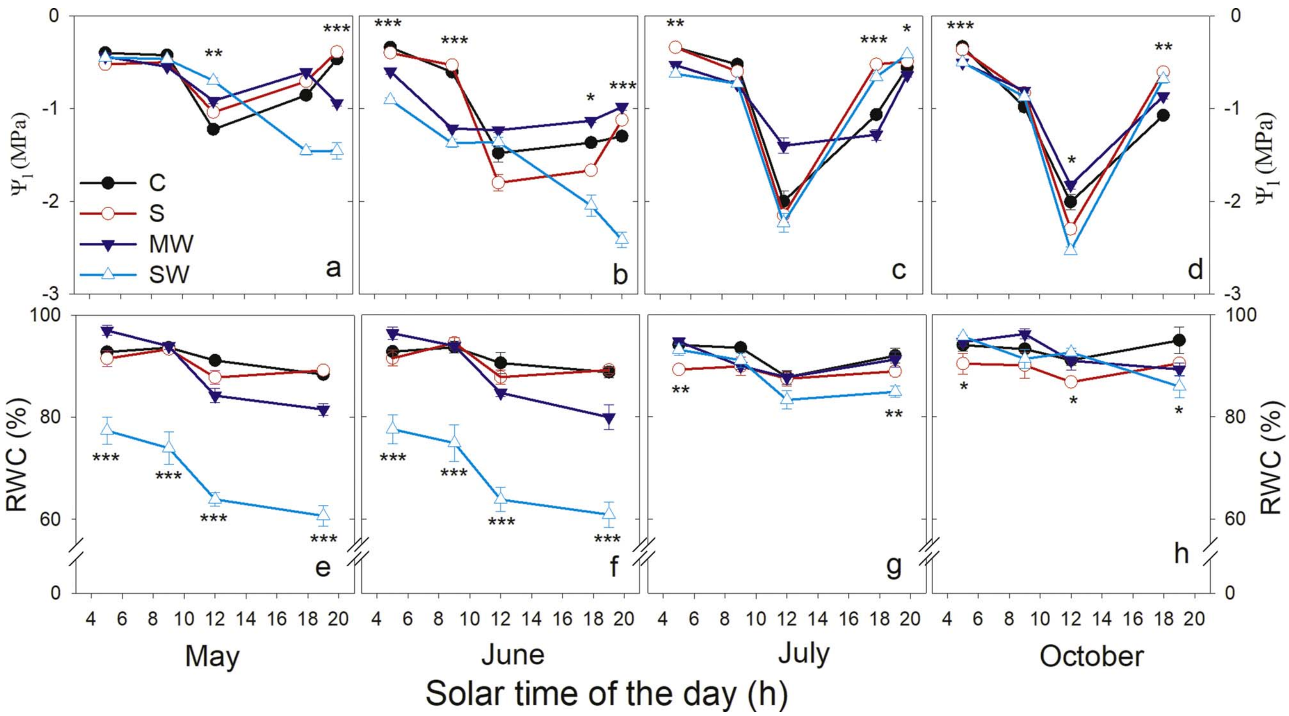

Fig. 4. Evolution of the leaf water potential (Ψl; a–d) and relative water content (RWC; e–h) in *P. lentiscus* plants submitted to different irrigation treatments. Values are means ± s.e., n = 8. Symbols represent the different treatments: Control (filled circles), saline (open circles), moderate water deficit (filled triangles) and severe water deficit (open triangles). For each studied time of the day, asterisks indicate significantly different between treatments (*$P < 0.05$, ** $P \le 0.01$, *** $P \le 0.001$).

increasing the cost of osmotic adjustment (Flowers and Yeo, 1986; Sucre and Suárez, 2011), which did not occur in our assay in plants submitted to water deficit. In our experiment, plants submitted to severe water deficit showed significant decreases in LMA, which potentially confer an advantage in resource-uptake efficiency, by increasing the absorption surface per unit of tissue biomass, as demonstrated in several Mediterranean woody species (de la Riva et al., 2016). In spite of being a morphological trait, LMA is widely used as a good indicator of plant functioning, as it is highly correlated with processes such as maximum photosynthetic rate, potential growth rate, chemical composition and resistance to herbivory.

In general, an increase in external NaCl concentrations induces an increase of Na^+ and Cl^- in roots and leaves of different ornamental plants (Navarro et al., 2007; Cassaniti et al., 2009; Álvarez et al., 2012). Our findings are consistent whit those of higher xylem sap osmolatity as salinity increases, as in Armas et al. (2010), who reported that sap osmolality in lentisc xylem increased as result of salty groundwater uptake. In a saline environment, controlling Na^+ and/or Cl^- concentrations of the aerial parts of plants, restricting entry through the roots and limiting transport to the shoots (retaining these ions in the root and lower stem) is an important tolerance mechanism that allows plants to survive and grow under salt stress conditions (Pérez-Alfocea et al., 2000; Colmer et al., 2005; Murillo-Amador et al., 2006). Our results for *P. lentiscus* did not confirm this finding in the case of the Na^+ ions, although the plants did show an ability to differentiate between Na^+ and Cl^- retention and transport, as other authors have verified (Romero et al., 1997). Whatever the case, in our conditions the accumulation of Na^+ in plant tissues did not induce any symptoms of necrosis, suggesting a certain degree of salt compartmentalisation and exclusion from the cytoplasm (Sánchez-Blanco et al., 2004; Rodríguez et al., 2005). According to Tattini et al. (2006), ***Pistacia lentiscus*** can be classified as salt-including, based on the rates of Na transported to the leaf. They noted that a massive allocation of Na to the leaf tissue together with a leaf Na concentration dramatically greater than in stem tissue and in soil solution and Hasegawa et al., 2000 suggested that an active secretion of Na in the leaf vacuole operates in *P. lentiscus*. This can be supported by observing how salts had little effect on PSII photochemistry in our conditions, despite the massive accumulation of potentially toxic ions in the leaves (Tattini et al., 2006; Tattini and Traversi, 2008). Indeed, *P lentiscus* does not have specialized organs to excrete toxic ions from the leaf (Brosse, 1979). The effectiveness of the compartmentalization process protects the cytoplasmic organoids from the toxic effect of salt and supports the osmotic adjustment (Martinez et al., 2005; Koyro, 2006; Slama et al., 2008; Sucre and Suárez, 2011).

In our experiment, salt tolerance in *P. lentiscus* was also associated with the restricted uptake of Cl- and its storage in roots, as Cl^+ was

Table 4
Parameters derived from the pressure–volume curves at the end of the experiment in *P. lentiscus* subjected to different irrigation treatments. Values are the mean of five plants.

Parameters	Treatments				P
	C	S	MW	SW	
Ψ_{100s} (MPa)	−2.2 ± 0.1b	−2.5 ± 0.1a	−2.2 ± 0.1b	−2.2 ± 0.1b	*
Ψ_{tlp} (MPa)	−2.7 ± 0.1b	−2.9 ± 0.1a	−2.8 ± 0.1b	−3.0 ± 0.0a	*
ε (MPa)	19.8 ± 0.6b	23.7 ± 1.3c	19.1 ± 0.4b	14.8 ± 0.5a	***
RWC_{tlp} (%)	89.7 ± 0.3b	90.2 ± 0.5b	89.5 ± 0.4b	86.2 ± 0.5a	**

Means within a row without a common letter are significantly different according to Duncan 0.05 test.
(P; probability level, *$P < 0.05$, ** $P \le 0.01$, *** $P \le 0.001$).

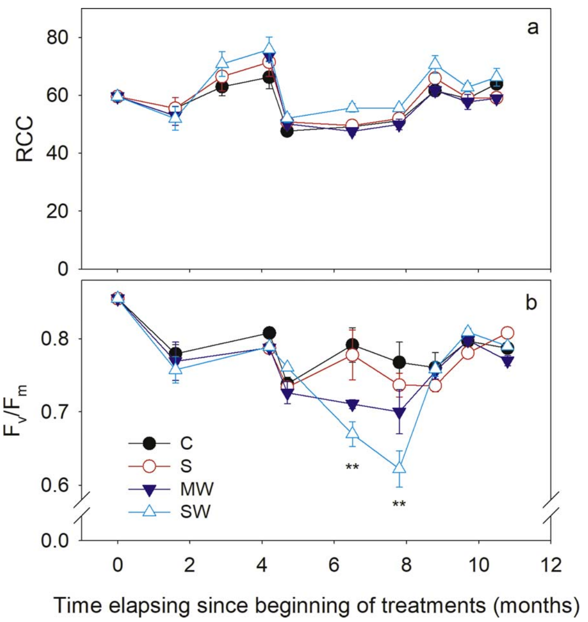

Fig. 5. Evolution of relative chlorophyll content (RCC; a) and chlorophyll fluorescence values (F_v/F_m; b) in *P. lentiscus* plants submitted to different irrigation treatments. Values are means ± s.e., n = 8. Symbols represent the different treatments: Control (filled circles), saline (open circles), moderate water deficit (filled triangles) and severe water deficit (open triangles). For each studied day, asterisks indicate significantly different between treatments (** $P \leq 0.01$).

effectively held in the woody roots and little reached the leaves. However, the cumulative effect of irrigating with saline water for long periods (11 months) was an over-accumulation of Na^+ and Cl^- in leaves, which probably contributed to the pronounced decrease in photosynthesis, confirming the importance of the time of salt stress exposure (Álvarez and Sánchez-Blanco, 2013).

Common responses in species exposed to saline or drought stress are an increase in osmotic adjustment and changes in cell wall elasticity, which result in the turgor loss point being reached at a lower leaf water potential and at a lower relative water content (Zheng et al., 2010; Suárez, 2011; Álvarez and Sánchez-Blanco, 2015). In our conditions, at the end of the experimental period, plants exposed to saline stress (S) showed significant increases in ε. Similarly, in many species exposed to saline stress, tissues became less elastic and the tissue water deficit associated with decreases in leaf Ψ was reduced, mostly through a decrease in Ψ_{tlp}, facilitating the continued uptake of water from saline soils (Navarro et al., 2007; Sassi et al., 2010). However, plants submitted to severe water deficit did not show osmotic adjustment and increases in cell wall elasticity (decreases in ε) were observed as a tolerance mechanism to drought in order to maintain turgor, as demonstrated in several ornamental species (Sánchez-Blanco et al., 2009; Álvarez et al., 2009; Álvarez et al., 2011). In fact, in our conditions RWC at zero turgor of SW was significantly lower under severe water deficit, suggesting that SW leaves were able to maintain turgidity with a lower water content than control leaves. Long periods of water stress can produce significant modifications in the morphofunctional characteristics of the plants, as if a preconditioning process had occurred during the experiment. Species like *P. lentiscus* are very sensitive to preconditioning and one of these modifications related to this process (acclimation) is a higher elasticity of cell membranes (Rubio et al., 2001). Under both stresses a reduction in the leaf osmotic potential in order to maintain the water flux and leaf cell turgor pressure may occur due to the net accumulation of solutes in the cells, or through cell dehydration (Sucre and Suárez, 2011; Acosta-Motos et al., 2017). Compared to the control, dehydration was more pronounced under severe water stress, especially in SW (Dichio et al., 2005; Pérez-Pérez et al., 2007; Slama et al., 2008). The maintenance of turgor by the passive concentration of solutes (increased solute concentration through reduced cell volume) under drought stress is an advantageous mechanism in arid environments to avoid energetic costs a result of the synthesis of organic solutes (Evans et al., 1992). In addition, this salinity level (4.0 dS m^{-1}) pointed to a limited degree of osmotic adjustment, which has been reported in other studies on Mediterranean ornamental plants

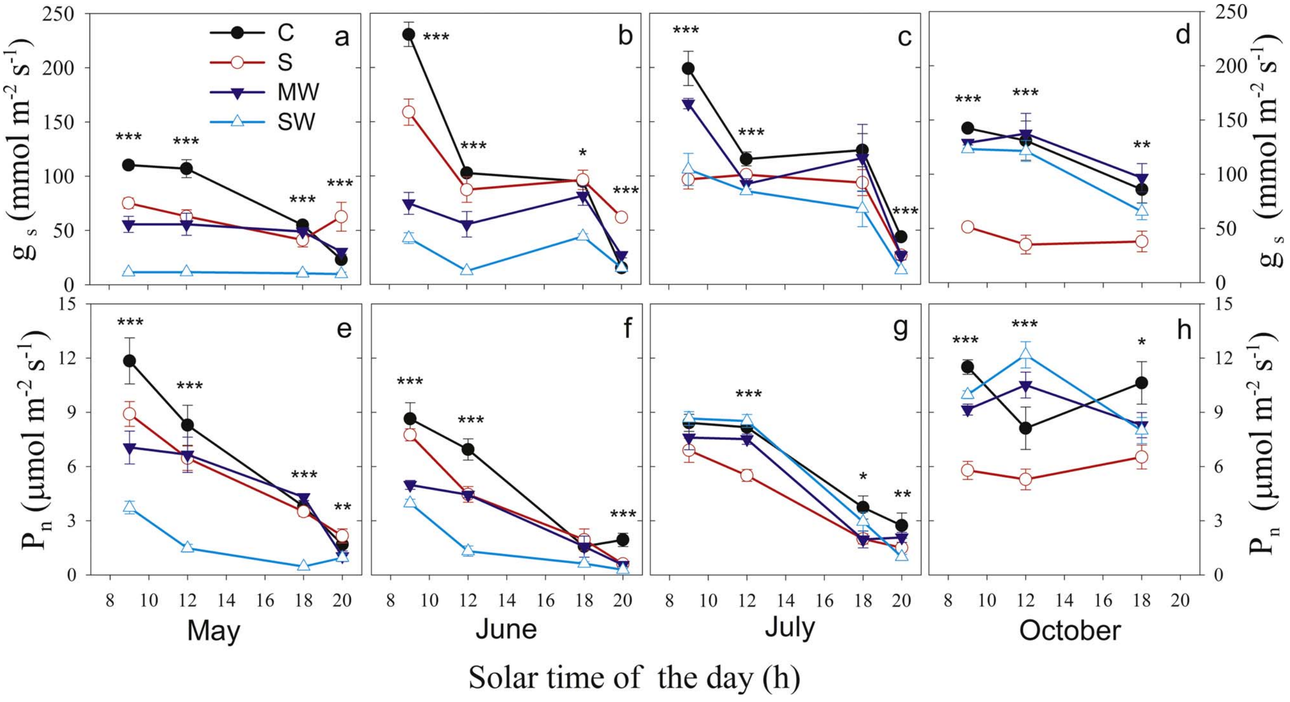

Fig. 6. Evolution of stomatal conductance (gs; a–d) and net photosynthesis rate (P_n; e–h) in *P. lentiscus* plants submitted to different irrigation treatments. Values are means ± s.e., n = 8. Symbols represent the different treatments: Control (filled circles), saline (open circles), moderate water deficit (filled triangles) and severe water deficit (open triangles). For each studied time of the day, asterisks indicate significantly different between treatments (*$P < 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$).

(Navarro et al., 2007; Tognetti et al., 2000, Álvarez et al., 2012). Similarly, in many species that exposed to both saline and drought stress, osmotic adjustment was seen to be lower in drought stress than in saline stress, indicating that osmotic adjustment through the uptake of inorganic ions (Na^+ and Cl^-) that are readily available in the soil solution is more efficient than adjustment through the production of organic solutes (Liu et al., 2008; Slama et al., 2008; Sucre and Suárez, 2011; Álvarez et al., 2012).

As regard water status, plants exposed to saline stress or severe water deficit exhibited slight dehydration throughout the experiment, as indicated by the lower leaf water potential and RWC, due to the low availability of substrate water and difficulty in taking up water from the substrate (Navarro et al., 2007; Sánchez-Blanco et al., 2002; Álvarez et al., 2012). However, as water stress progresses over time, a mechanism of acclimatization could occur in the SW treatment with values of Ψ_l at predawn and RWC higher than those previously obtained. These changes allowed plants to maintain higher net CO_2 assimilation rates and an elevated photosystem II status, suggesting that acclimation to drought through changes in cell water relations (Vilagrosa et al., 2003b, 2010). The absence of significant changes in leaf relative water content in salinized plants during the initial period of osmotic stress agrees with the observations of Ramani et al. (2006) and Sucre and Suárez (2011), who observed that in salt tolerant species, the RWC did not change after days or months of exposure to salinity, while under severe water stress a reduction in RWC is a common response (Pérez-Pérez et al., 2007). Leaf water potential values below the value of Ψ_{tlp} were not found for plants at any sampling time during the experiment. The maintenance of turgor permits cell elongation, stomatal opening and other processes dependent on turgor pressure (Munns, 2002; Dichio et al., 2005). The diurnal course in Ψ_l reflect the stress conditions and even the watered plants were mildly stressed, which may have been the consequence of high air temperature and irradiance producing low Ψ_l and stomata conductance at midday (Navarro et al., 2009; Bacelar et al., 2007; Gratani et al., 2013).

The decrease in g_s observed in our study suggests an adaptive and efficient control of transpiration by this species, limiting water loss (Hessini et al., 2008) or by reducing the salt load of leaves (Koyro, 2006). *P. lentiscus* has been classified as a plant that regulates stomata closure before reaching critical leaf water potential, which would cause cavitation events (Filella et al., 1998; Vilagrosa et al., 2010).

As a result, net photosynthesis is unavoidably reduced due to decreased CO_2 availability at the chloroplast level (Chaves et al., 2009), as seen in many other ornamental species submitted to water deficit and saline conditions (Navarro et al., 2009; Gómez-Bellot et al., 2013b). Eleven months after the beginning of saline treatment, P_n was seen to be negatively affected in plants subjected to salinity. The fact that such a reduction occurred later in saline plants than in deficit irrigation plants would be due to the salts taking time to accumulate inside plants before the concentrations reached toxic levels and affected plant functioning (Munns and Tester, 2008). The close association between P_n and g_s in salt stressed plants suggests that under these conditions a decline in net photosynthesis is largely a consequence of stomatal limitation (Bacelar et al., 2007; Flexas et al., 2004, Chaves et al., 2009). A severe water deficit also had a negative effect on the photosynthetic rate and stomatal conductance of *P. lentiscus* plants, but these parameters were more affected, particularly in early summer, when these plants had very low g_s values- a type of response that has been interpreted as evidence of a gradual increase in the non-stomatal limitation of photosynthesis during stress (Pérez-Pérez et al., 2007). The results of this study are consistent with the finding of Álvarez and Sánchez-Blanco in *C. citrinus* and *C. laevis* (2014, 2015), who reported that if plants show g_s values below $100\,mmol\,m^{-2}\,s^{-1}$ for long periods, intrinsic water use efficiency is sharply reduced and non-stomatal limitations to P_n are predominant, which could delay plant recovery or even cause permanent damage. A reduction in stomatal conductance was one of the causes of lower photosynthesis, although photoinhibition or increased mesophyll resistance may have played a role later when stress was more severe or prolonged (Flexas et al., 2004; Pérez-Pérez et al., 2007; Sucre and Suárez, 2011).

In addition, the analysis of chlorophyll fluorescence rates supports the idea that decreased biochemical factors in the mesophyll (non-stomatal factors) were also responsible for the decline in photosynthesis (Pérez-Pérez et al., 2007). Lower F_v/F_m in water stressed leaves during the early summer at midday can indicate photoinhibitory damage, and a decrease in Ψ_l can lower electron transport to carbon fixation (Maxwell and Johnson, 2000). Flexas et al. (2004) made a convincing argument that g_s and internal mesophyll conductance can be regulated together during salt and drought stress and mesophyll conductance can influence the extent to which leaves can recover photosynthetic capacity after stress. In this sense, the subsequent recovery in F_v/F_m that occurred in *P. lentiscus* at the end of the experiment suggests that severe drought did not cause irreversible damage to leaf tissue, indicating that the maximal PSII primary photochemistry was not permanently affected by the stressful conditions previously experienced by the plants. The changes in the F_v/F_m ratio indicated that under severe drought stress (SW), leaves maintained less active photochemical reactions (photosynthetic reaction centers) than control leaves, but they were able to restore F_v/F_m to the values of control plants at the end of the experiment, when environmental conditions became less stressful, which seemed to be effective in protecting the photosynthetic apparatus from the risk of photodamage under field conditions (Souza et al., 2004). A process of acclimatization to drought could have occurred in these plants, facilitating more efficient mechanisms for PSII repair, which was reflected in higher net photosynthetic rates and stomatal conductance at the end of the experiment (Vilagrosa et al., 2003b). On the other hand, the non-significant changes in RCC in *P. lentiscus* plants subjected to saline or both water stresses during the experimental period suggest that there was no oxidative damage, and, although F_v/F_m values decreased in summer in SW plants, this was not due to alterations in the leaf chlorophyll content. In this sense, chloroplasts in Mediterranean species under drought and high light conditions have been shown to possess several strategies to avoid photoinhibitory processes, e.g., mechanisms to consume the reducing power generated by PSII (Flexas and Medrano, 2002; Demmig-Adams and Adams, 2006). These results agree with those of Vilagrosa et al. (2010), who reported a high resistance at chloroplast level for *P. lentiscus* under drought conditions. Additionally, our data about the unchanged chlorophyll fluorescence in S plants are consistent with the previous study of Armas et al. (2010) in which *P. lentiscus* did not show significant decreases in chlorophyll fluorescence with salinity increasing.

5. Conclusions

The above results show that *Pistacia lentiscus* is highly resistant to water stress and salinity, in which agrees with previous reports on this species. The mechanism of *P. lentiscus* to avoid water deficit was related to its ability to reduce leaf area, to increase cell wall elasticity and to modify leaf gas exchange. This is also clear from an unchanged cell membrane permeability and the decline and subsequent recovery of F_v/F_m values. The salinity tolerance of *P. lentiscus* was related to osmotic adjustment, enhanced leaf tissue rigidity, limited Cl uptake from the substrate and the higher Cl concentration in roots than in leaves, accompanied by effective toxic ion compartmentation and the absence of membrane damage. The response of plants to severe water stress involved a marked decrease in plant height and growth due to leaf tissue dehydration, which resulted in stomatal closure and a decrease in CO_2 absorption, but it allowed conditioning plants to maintain higher water potential and PSII status during an extended and subsequent drought period. Salt induced a slight growth reduction and no toxicity symptoms. Based on its observed behaviour, *P. lentiscus* can be regarded an interesting ornamental species for gardening projects in arid and saline areas.

Acknowledgments

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