Effect of salt and drought on growth, physiological and biochemical responses of two *Tamarix* species

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Tamarix **trees are considered of particular interest for afforestation and reforestation of degraded areas prone to salinity and drought. In this study, a comparison of the performance and physiological responses of two species of *Tamarix* grown in saline and dried soils was carried out. Stem cuttings of *T. aphylla* and *T. jordanis* were collected from a location in the Negev desert and the plantlets obtained were subjected to four different soil treatments under semi-controlled conditions for 14 days. The treatments were: fresh water (C); salt (S: 150 mM of NaCl); drought (D: 50% field capacity); and a combined stress (DS: 150 mM of NaCl + 50% FC). Results showed a higher tolerance to salt stress of *T. jordanis* as compared with *T. aphylla*. The maintenance of high amount of cell carbohydrates, the high capacity of carbon assimilation and the active growth were considered as markers of salt tolerance in *Tamarix* spp. *T. aphylla* showed better performances in terms of growth and biomass production than *T. jordanis* in dry conditions. The high accumulation of sugars found in the leaves of *T. aphylla* under mild drought is considered a mechanism of acclimatization. The combined stress (salt+drought) lowered the performance of plants as compared to salt and drought stress applied alone. The possible role of the accumulation of proline observed in the leaves of both species under stressful conditions is discussed.**

Keywords: ***Tamarix*****, Afforestation, Salinity, Drought, Tolerance**

Introduction

Afforestation and reforestation activities have recently received high attention as measures for carbon sequestration and mitigation of climate change (Wildburger 2004). However, such activities may be complex in degraded lands where soil exhibit harsh conditions due to water shortage and salt accumulation, especially in vulnerable Mediterranean areas. The co-occurrence of the decrease in precipitation, the salinization of groundwater, and the overpopulation compelled many Mediterranean countries to look out for additional water resources (reclaimed waste water, desalinized water, etc.) in order to meet the growing demand and maintain high productivity of plants. Therefore, to face the problem of land degradation, there are increasing prospects for the use of salt and drought tolerant species showing high carbon sequestration rate and requiring minimal management practices and low production costs.

Tamarix is currently regarded as one of the species suitable to combat land degradation and mitigate climate change. This genus consists of halophytic shrubs and trees native to an area spanning from southern Europe and North Africa through the Middle East and south Asia to China and Japan (Rodman 1990). *Tamarix* plants are of particular interest for their fast growth, easy vegetative propagation and acclimatability to a wide range of contrasting environmental conditions (Brotherson & Field 1987). *Tamarix* shows various protective mechanisms allowing its survival and growth in harsh environments, including the presence of salt glands on leaves (Waisel 1972, Metcalfe & Chalk 1950, McClintock 1951), which play an important role in regulating ionic balance (Ramadan 1998) and maintaining/stabilizing osmotic and turgor pressure under high salinity (Ding et al. 2009). Indeed, such glands may excrete the salt excess which accumulates in the tissue through transpiration (Scholander et al. 1964, 1965). Previous studies have also investigated the biochemical responses of *Tamarix* spp. under different levels of salt stress (Solomon et al. 1994, Jones et al. 1995, 2005). *Tamarix jordanis* Boiss has been reported to contain proline analogues that can reduce the negative effects of NaCl on the RuBisCO activity (Solomon et al. 1994). In addition, proline has been reported to accumulate in roots of *Tamarix tetragyna* Ehrenb with increasing salinity up to 480 mM (Bar-Nun & Poljakoff 1977).

The ability of *Tamarix* to closely regulate photosynthesis and leaf conductance during drought increases its survivability and competitiveness in arid and semiarid rangelands (Mounsif et al. 2002). According to Frasier & Johnsen (1991), *Tamarix* spp. are well adapted for survival in arid and semi-arid climates, and may tolerate even dramatic changes in soil moisture after establishment, provided that groundwater is available. *Tamarix* has a deep, extensive root system very efficient in accessing limited water supplies, and has higher water-use efficiency than native riparian (Zlatnik 1999). While the role of metabolites such as soluble sugars (sucrose, glucose and fructose) in conferring drought tolerance is well documented for many plants (Crowe et al. 1990, Vertucci & Farrant 1995, Hoekstra et al. 2001), little is known about the accumulation of osmolytes and their role in drought tolerance of *Tamarix* spp.

Approximately 54 species of *Tamarix* spp. have been described worldwide (Baum 1978), which may differ in their tolerance to salt and drought stress. However, few studies compared their performance under different harsh conditions. Thus, understanding how *Tamarix* spp. respond to the combined effect of drought and salt stresses may contribute to the selection of the best material to be used for biomass production and conservation purposes in arid zones. This study aims to evaluate the effects of salinity, drought stress and their combination on growth rate, biomass and gas exchange of two *Tamarix*

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species, as well as on their carbohydrate and proline content.

Material and methods

Plant material and culture conditions

Stem cuttings of two species of *Tamarix*, *T. aphylla* (L.) Karst and *T. jordanis* Boiss, were collected from a plantation located in the Negev Desert (southern Israel - 31° 30′ 56.01″ N, 34° 26′ 59.62″ E). Average annual rainfall at the sampling site was less than 100 mm yr^{-1} and summer average temperature between 32 and 39 °C. One year old stem cuttings (diameter: 7-9 mm, length: 20 cm) were planted in pots of 24 cm in diameter with drain holes filled with sandy soil. After rooting of cuttings, plants showing homogeneous development and size were selected, transplanted and acclimatized in a greenhouse for 14 days. Each species was then submitted to four different treatments, each including seven replicates, totaling 112 plants (28 individuals per species per treatment). The first treatment was irrigated with fresh water (C), the second with saline water 150 mM (S), the third (D) was irrigated to 50% of field capacity (FC), and the fourth with saline water 150 mM to 50% FC (DS). Pots were covered with plastic film to minimize evaporation. Four pots with no plants were used as a control to monitor the evaporative loss from the soil surface. Plants were then grown in a greenhouse for 14 days. All the plants received periodically the same quantity of nutrients. Water content was monitored gravimetrically every night by weighing the pots, and water level (measured as weight) was adjusted relative to the FC level needed. Electrical conductivity (EC) of the drained liquid phase was measured daily in the morning after irrigation, using a conductimeter (HI9811, Hanna instruments INC, USA) equipped with an electrode probe (HI1285, Hanna instruments INC, USA). Salt concentration in the soil was assessed by collecting a soil core at 20 cm depth, and then measuring the electrical conductivity of the saturated soil extract ECe. Electrical conductivity was expressed as ds m^{-1}, ranging for both species from 0.9 to 1 ds m^{-1} for treatment C, 1-1.33 ds m^{-1} for treatment D 14.8-15.6 ds m^{-1} for treatment S and 15-18 ds m^{-1} for treatment DS.

Growth measurements

Plant growth was monitored at the beginning, at the midpoint and at the end of the experiment by measuring the increment in plant height. At the end of the experiment, plants were divided into leaves, stems and roots and their respective fresh weight (FW) was determined. Dry weight (DW) was obtained by oven drying at 70 °C until a constant weight was reached.

Gas exchange measurements

Net assimilation of CO_2 (A), stomatal conductance (g_s) and transpiration rate (E) were measured at the beginning, midway and at the end of the experiment using a portable photosynthesis system (LI-6400) equipped with a conifer chamber. The intrinsic water use efficiency (*WUE*) was obtained as the ratio between net assimilation and stomatal conductance (A/g_s).

All measurements were made in the morning from 9:30 to 13:00 h, on a green twig of about 5 cm^2 selected in the middle part of the plant. Measurement conditions were as follows: 1800 µmol m^{-2} s^{-1} photosynthetically active radiation (PAR), 400 µmol mol^{-1} ambient CO_2 concentration, and 28 ± 2 °C leaf temperature. Data were automatically recorded after stabilization of the photosynthesis rate.

Carbohydrates analysis

Fresh leaf tissue was finely ground using liquid nitrogen, and 40 mg of ground tissue were extracted with 1.5 ml of 80% ethanol and 20% buffer (Hepes 100 mM, pH 7.1, $MgCl_2$ 10 mM) at 80 °C for 45 min. After cooling and centrifugation at 13 000 *g* for 5 min, the supernatant was collected for quantification of soluble sugars, while the pellet was used for the determination of the starch content. Soluble sugars were measured using enzymatic assays as described by Moscatello et al. (2011). Sugar determination assays were performed using an Anthos plate reader (Anthos Labtec Instruments, Wals, Austria) in dual-wavelength mode (340-405 nm). The endpoint determination for all sugars (glucose, fructose and sucrose) was based on the reduction of NAD^+ to NADH. Glucose was determined by first converting to glucose-6-phosphate in the presence of 100 mM Hepes (KOH - pH 7.1), 10 mM $MgCl_2$, 1 mM DTT, 0.02% BSA, ATP 100 mM, NAD^+ 80 mM and hexokinase 0.5 U, and then measuring its reduction of NAD^+ in the presence of glucose-6-phosphate dehydrogenase 0.3 U. Absorbance by NADH was read at 340 nm. Fructose was phosphorylated as for glucose, converted to glucose-6-phosphate with phosphoglucoisomerase 0.3 U, and then assayed as for glucose. Sucrose was hydrolyzed with invertase 50 U, and glucose and fructose released by hydrolysis were assayed as described above. The pellet was washed three times with sodium acetate 50 mM pH 4.6, autoclaved with 1 ml sodium acetate for one hour at 120 °C, and then hydrolyzed by adding 70 U amyloglucosidase and 4 U α-amylase. The obtained suspension was incubated at 50 °C for one hour with regular shaking. Following centrifugation at 13 000 *g*, the glucose released in the supernatant was used for starch quantification as in the aforementioned assay.

Proline analysis

Free proline content was estimated following the method of Bates (1973). Fresh leaves (0.5 g) were extracted in 3% sulphosalicylic acid, and the homogenates obtained were centrifuged at 10 000 *g* for 10 min. A 2 ml of the supernatant was reacted with 2 ml of acid ninhydrin reagent and 2 ml of glacial acetic acid in a test tube for one hour at 100 °C and the reaction was terminated in an ice bath. The reaction mixture was extracted with 4 ml of toluene and mixed vigorously with a vortex mixture for 15-20 s. The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance measured at 510 nm using toluene as blank. Proline concentration was calculated from a standard curve using 0-100 µg l^{-1} proline (Sigma®).

Greenhouse conditions

Temperature and humidity inside the greenhouse were monitored daily throughout the period of the experiment. The mean temperature ranged between 22.02 and 27.32 °C, while mean relative humidity ranged between 37.8 and 73.9%. The observed lowest values of relative humidity were attained only around the 7th day of the experiment (37.8%).

Experimental design and statistical analysis

A completely randomized design was used. Due to destructive nature of the analyses carried out, seven replicates of plant height increment data and four replicates for all other measurements were used. Means were submitted to the analysis of variance and tested using a least significant difference (LSD) test ($\alpha = 0.05$). When a significant difference ($P \leq 0.05$) was detected, mean values were compared using the Duncan's multiple range test. Factorial analysis was used to test for inter-specific differences induced by the treatment, and repeated measures ANOVA to test for the time effect. All statistical analyses and computations were carried out using the software package Statistica® for Windows version 7.0.61 (StatSoft, Tulsa, OK, USA).

Results

Growth measurements

At the end of the experiment, *T. aphylla* and *T. jordanis* showed different growth responses depending the applied stress. Salt had a highly negative effect on plant height increment of *T. aphylla*, which was reduced by 27.3%, while it did not show any significant effect on *T. jordanis* (Tab. 1). The shoot dry weight of both species was not negatively affected by salt stress in the short period of the experiment, yet shoot dry weight of *T. jordanis* under salt stress was even

Tab. 1 - Height increment, shoot, root weight and root/shoot ratio of *T. aphylla* and *T. jordanis* plants exposed to stress. Treatments: (C) Control; (S) salt (150 mM); (D) drought (50% FC); (DS) drought + salt (150 mM + 50% FC). Different letters indicate statistically different means ($P \leq 0.05$) at the 14th day after the Duncan's multiple range test. Data of plant height increment are means of seven replicates ± standard error. Data of shoots dry mass, root dry mass and root/shoot ratio are means of four replicates ± standard error.

Species	Treatment	Plant height increment (cm/14 days)	Shoots dry mass (g plant^{-1})	Root dry mass (g plant^{-1})	Root/shoot ratio
T. aphylla	C	0.73 ± 0.06^{a}	41.45 ± 4.37^{a}	20.37 ± 2.55 ^{a}	0.49 ± 0.02^{b}
	S	0.53 ± 0.05^{b}	42.95 ± 5.62^{a}	21.38 ± 2.35^{a}	0.50 ± 0.03^{b}
	D	0.52 ± 0.05^{b}	33.65 ± 2.94^{ab}	20.92 ± 1.50^{a}	0.63 ± 0.03^{ab}
	DS	0.11 ± 0.01^{c}	26.84 ± 4.45^{b}	21.06 ± 3.68^{a}	0.85 ± 0.17^{a}
T. jordanis	C	1.10 ± 0.16^{a}	66.00 ± 7.74^{a}	30.19 ± 2.13^{a}	0.47 ± 0.04^{b}
	S	1.12 ± 0.25^{a}	77.10 ± 2.46^{a}	29.20 ± 4.47^{a}	0.45 ± 0.05^{b}
	D	0.49 ± 0.08^{b}	44.49 ± 4.57^{b}	35.85 ± 1.22^{a}	0.84 ± 0.10^{a}
	DS	0.26 ± 0.05^{b}	44.96 ± 4.78^{b}	26.98 ± 1.35^{a}	0.61 ± 0.05^{b}

higher than the control (Tab. 1). Similarly, roots of both species were not affected by salt stress.

Contrastingly, drought (50% FC) showed an opposite impact on *Tamarix* spp., determining a severe reduction of plant height increment by 55% in *T. jordanis vs.* only 28.7% in *T. aphylla*. Reduction of shoot dry weight observed in the drought treatment (50% FC) was also severe for *T. jordanis,* (32.5%), and non significant for *T. aphylla* (19% - Tab. 1). Consequently, a higher root/ shoot ratio was recorded in *T. jordanis,* while non-significant changes of root/shoot ratio were detected in *T. aphylla* (Tab. 1).

The combination of both stress (treatment DS) had the most pronounced effect on plant growth of both species. On the third day of experiment, symptoms of leaf yellowness and shedding were detected at the bottom part of plants and were more pronounced for *T. aphylla* than for *T. jordanis.* At the end of experiment, height increment of *T. aphylla* and *T. jordanis* plants was strongly influenced by the combined stress, with a significant decrease by 85% and 76.3%, respectively, as compared with control plants (Tab. 1). In the combined stress treatment (DS), shoot dry weight of *T. aphylla* and *T. jordanis* plants were significantly lower than control plants by 35.4% and 31.8%, respectively. Root dry weight was not affected by the combined stress in both species (Tab. 1). The root/ shoot ratio of *T. aphylla* plants grown under the combined stress was significantly higher (73.5%) than that of control plants, while no significant differences were observed in root/ shoot ratio between *T. jordanis* and control plants.

Gas exchange measurements

Absolute differences in the mean of carbon assimilation capacity (*A*), stomatal conductance (g_s), and transpiration rate (*E*) were observed between *T. aphylla* and *T. jordanis* plants. Generally, the highest values of *A*, g_s and *E* throughout the experiment were recor-

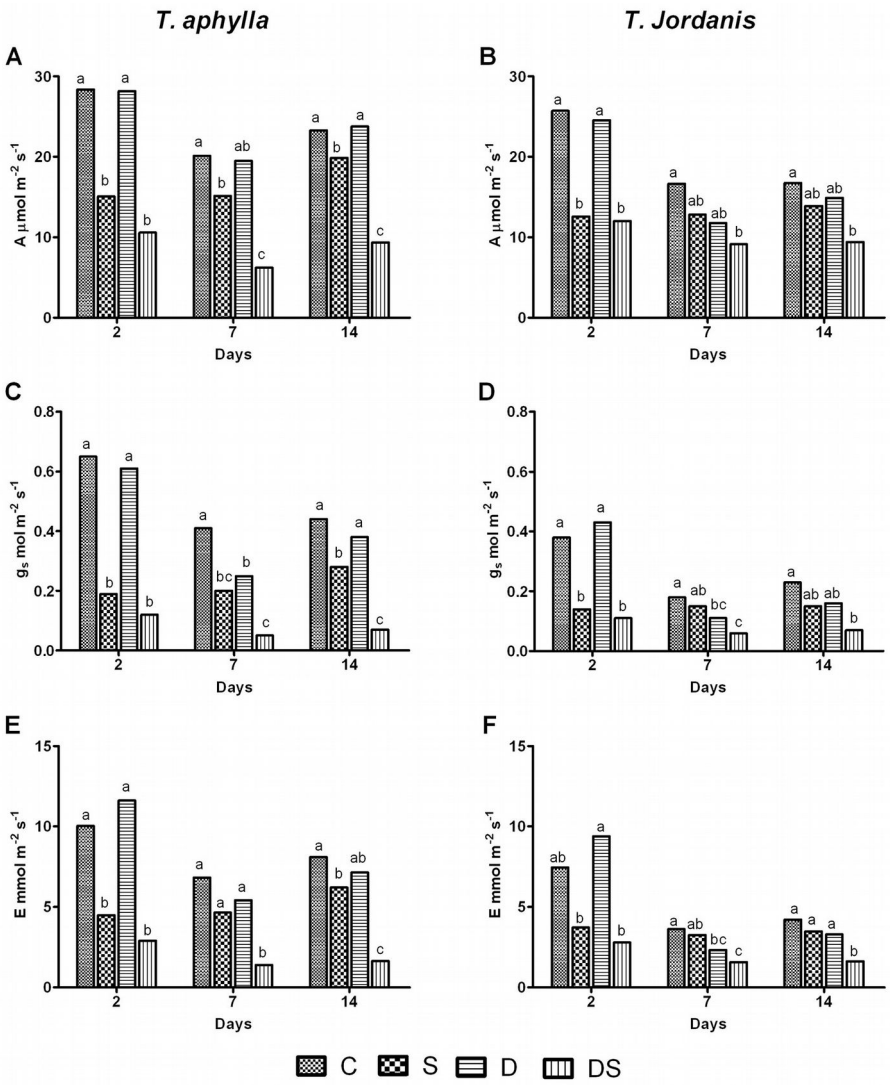

Fig. 1 - Gas exchange measurements of *T. aphylla* (A, C, E) and *T. jordanis* (B, D, F) plants exposed to different stress treatments. (*A*): assimilation rate (panels A-B); (g_s): stomatal conductance (panels C-D); (*E*): transpiration rate (panels E-F); (C): Control; (S): salt (150 mM); (D): drought (50% FC); (DS): drought + salt (150 mM +50% FC). Data are means of four replicates. Different letters at the top of bars indicate statistically different means ($P \leq 0.05$) after the Duncan's multiple range test.

ded for *T. aphylla*, with values higher by 21%, 43.7% and 25.2%, respectively, as compared with *T. jordanis* plants (Fig. 1). Salinity (S) and the combination of drought and salt stress (DS) did affect A, g_s and E in both species to a higher extent than drought alone (D).

On the 2nd day, salt (150 mM) reduced significantly A, g_s and E relative to the control ($p \leq 0.05$) by 46.9%, 70.3%, 55% in *T. aphylla*, and by 51.1%, 62.6%, 50% in *T. jordanis*, respectively. Afterwards, both species showed an improvement of such parameters on the 7th day and a full restoration on the 14th day as compared with untreated plants. However, a better improvement of these parameters was achieved by *T. jordanis* plants. Indeed, reductions of A, g_s and E in *T. jordanis* plants were no longer significantly different from controls on day 14, while *T. aphylla* plants showed a significant reduction of A, g_s and E (by 14.7%, 36%, 23.3%, respectively - Fig. 1).

Drought (50% FC) effects on A, g_s and E were minimal over the experiment in both species, as compared with salt stress. Only on the 7th day, water deficit (50% FC) determined some alterations of g_s and E (Fig. 1C-F), while had no significant effect on A (Fig. 1A-B).

Plants subjected to the combined salt and drought stress (DS) had the most affected values of gas exchange among all treatments and throughout the 14 days of the experiment (Fig. 1). Unlike plants exposed to the salt stress alone (treatment S), both species grown under the combined stress (DS) did not achieve any significant improvement during the experiment. At the end of the experiment, A was significantly lower than control in *T. aphylla* (59.8%) and *T. jordanis* (43.7%, $p \leq 0.05$ - Fig. 1A-B).

Water use efficiency

Factorial analysis revealed that the intrinsic water use efficiency ($WUE = A/g_s$) was generally higher in *T. jordanis* than in *T. aphylla*, regardless of the stress applied ($p \leq 0.05$ - Fig. 2), due to its relatively low values of g_s (Fig. 1C-D). Overall, a certain increase in the ratio A/g_s was recorded in all treatments for both *Tamarix* species, as compared with the control plants.

As for the salt stress treatment (S), *WUE* increased during the experiment in both species. *T. aphylla* plants showed a significant increase (44.15%) in *WUE* since the second day, while in *T. jordanis* *WUE* increased only at the end of the experiment (Fig. 2).

Under drought stress (treatment D), *T. aphylla* plants showed some fluctuations of *WUE* over the experiment, with an observed increase of 31.3%, while *WUE* values in *T. jordanis* increased only in the last experimental stage (Fig. 2). Contrastingly, both species had the highest *WUE* values in the combined treatment (Fig. 2), with higher increments in *T. aphylla* than in *T. jordanis* over the trial period ($p \leq 0.05$).

Sugars

Leaf sugar content was affected by stress in both species, showing similar sugar trend responses with slight differences and a marked response in *T. aphylla.*

Under salt stress (S), a drastic reduction of glucose (51%) and fructose (41.5%) content was detected on the 2nd day in *T. aphylla* plants. Afterwards, glucose and fructose content recovered in the leaves of *T. aphylla* on the 7th day. Nonetheless, at the end of the experiment the content of both sugars resulted significantly lower in treated plants than in control plants. On the other hand, from the 2nd to the 14th day, the reduction in content of glucose and fructose was not significant for *T. jordanis* plants subjected to salt stress (150 mM - Fig. 3A-D).

Salt stress treatment (S) did not affect sucrose content in both species (Fig. 3E-F), while caused a decline of starch content in both species. In *T. aphylla*, starch was highly affected by salt stress, decreasing sharply by 49.7% on the 2nd day, while *T. jordanis* showed a non significant reduction of starch content. However, a recovery in starch content in *T. aphylla* and *T. jordanis* was noticed in the following days (Fig. 4).

Under drought stress (treatment D), the substantial difference in soluble sugar content was evident only at the 7th day. Accumulation of all soluble sugars (glucose, fructose and sucrose) was significantly higher than the control only for *T. aphylla* leaves (Fig. 3). Drought (50% FC) provoked an initial significant decrease in starch content of *T. jordanis* leaves and did not influence starch level in *T. aphylla* leaves. However, a lower starch content was detected on the 7th day in both species (37% in *T. aphylla* and 44% in *T. jordanis*) as compared with control plants, partly balanced by an increase in soluble sugars in the leaves of both species. Afterwards, starch content fairly increased in both species under drought (D), equaling the control plant values (Fig. 4).

Combined stress (DS) initially induced a reduction in glucose and fructose in both species. Glucose was reduced by 56% in *T. aphylla* and by 46% in *T. jordanis,* while fructose was reduced by 44% in *T. aphylla* and by 31% in *T. jordanis*, as compared with the control plants (Fig. 3A-D). At the end of treatment, the combined stress treatment (DS) showed the same effect of the salt treatment (S) on glucose and fructose (Fig. 3A-D). Unlike hexose sugars, sucrose was stable throughout the whole experiment, being values for all treated plants not significantly different from those of the control plants (Fig. 3E-F). The combined stress significantly reduced the starch content relative to the control in both species, with an estimated reduction at the 2nd day between 49.7% and 61.3% in *T. jordanis* and *T. aphylla*, respectively. The last day of experiment, starch content of plants grown under combined stress (DS) was significantly lower than the control 64.2%, 74.2% in *T. jordanis* and *T. aphylla*, respectively (Fig. 4). The starch response under the combined stress was similar to that described for the salt stress treatment at the beginning, though at the end of the experiment clear differences from S and D treatments were observed (Fig. 4).

Proline

In general, *T. aphylla* accumulated larger quantity of proline than *T. jordanis*. Salt stress induced an accumulation of proline in the second day only in *T. aphylla*. Neither species showed any significant response in

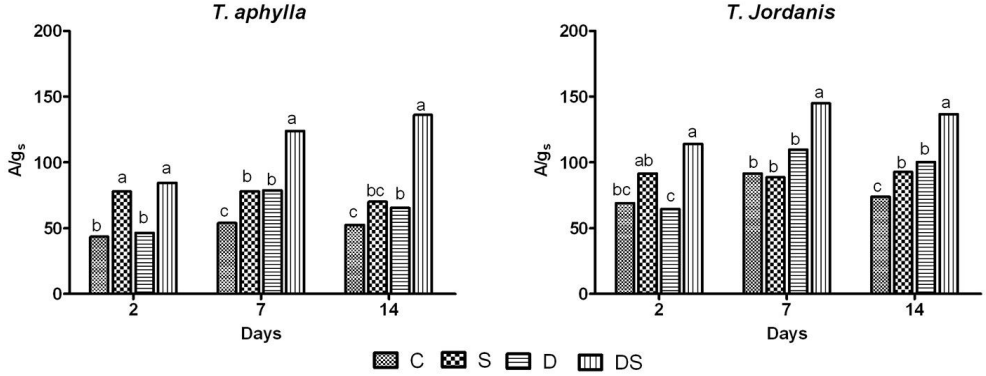

Fig. 2 – Water use efficiency (A/g_s) of *T. aphylla* and *T. jordanis* plants under different stress treatments. (C): Control; (S): salt (150 mM); (D): drought (50% FC); (DS): drought + salt (150 mM + 50% FC). Data are means of four replicates. Different letters on the top of the error bars indicate statistically different means ($P \leq 0.05$) after the Duncan's multiple range test.

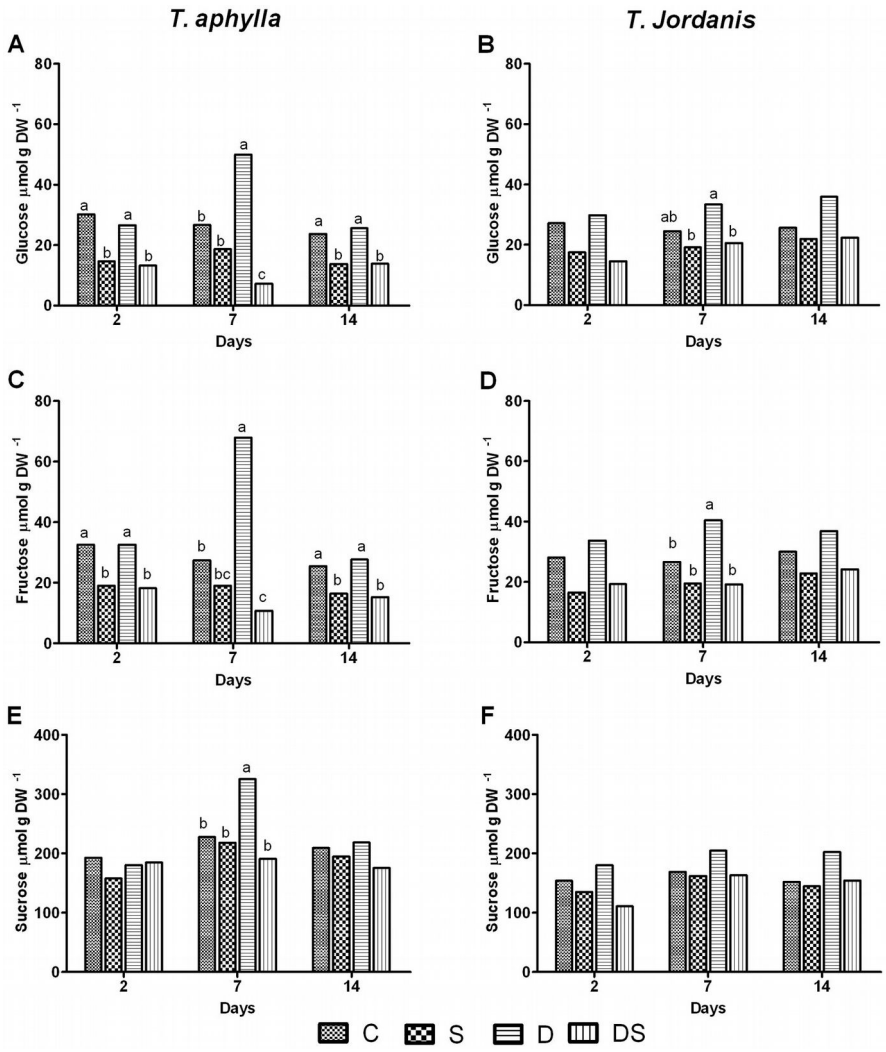

Fig. 3 - Soluble sugar content (glucose: A-B, fructose: C-D; and sucrose: E-F) of *T. aphylla* and *T. jordanis* plants exposed to different stress treatments. (C): Control; (S): salt (150 mM); (D): drought (50% FC); (DS): drought + salt (150 mM + 50% FC). Data are means of four replicates. Different letters on the top of bars indicate statistically different means ($P \leq 0.05$) after the Duncan's multiple range test.

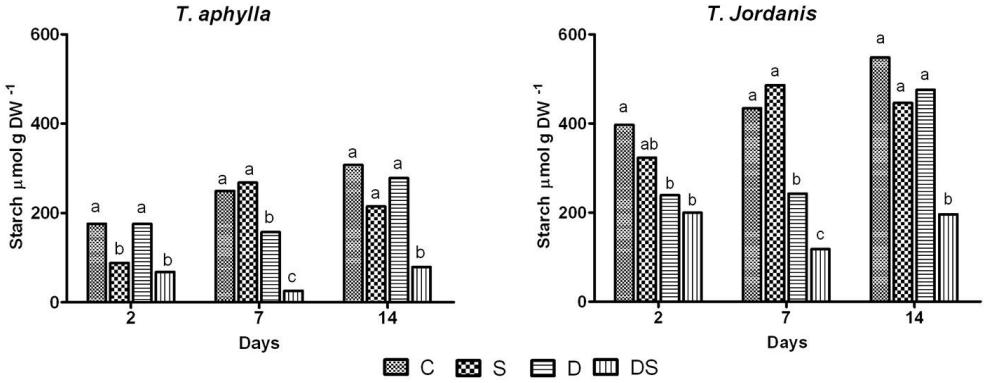

Fig. 4 - Starch content of *T. aphylla* and *T. jordanis* plants exposed to different stress treatments. (C): Control; (S): salt (150 mM); (D): drought (50% FC); (DS): drought + salt (150 mM + 50% FC). Data are means of four replicates. Different letters on the top of bars indicate statistically different means ($P \leq 0.05$) after the Duncan's multiple range test.

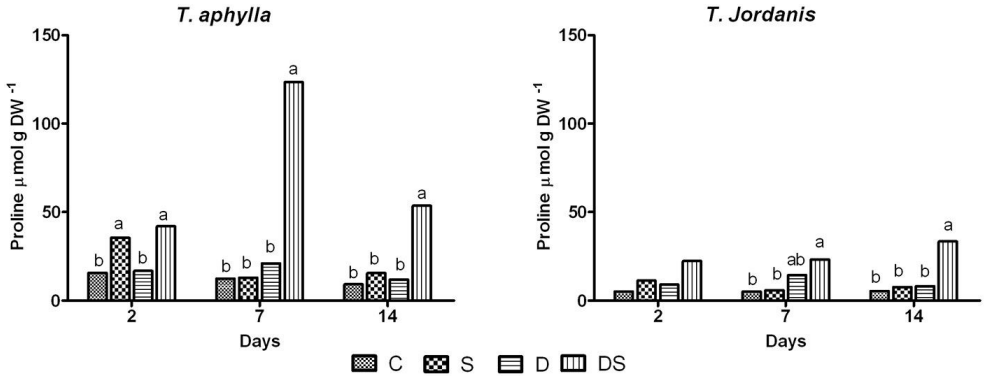

Fig. 5 - Proline content of *T. aphylla* and *T. jordanis* plants exposed to different stress treatments. (C): Control; (S): salt (150 mM); (D): drought (50% FC); (DS): drought + salt (150 mM + 50% FC). Data are means of four replicates. Different letters on the top of bars indicate statistically different means ($P \leq 0.05$) after the Duncan's multiple range test.

proline accumulation under water deficit. For both species proline accumulation was higher for plants under treatment DS (combined stress), with the free proline content increasing approximately 5-9 fold as compared with the control plants (Fig. 5).

Discussion

Tamarix plants are halophytes that can acclimate and grow under high salinity levels, surviving in areas where the groundwater concentration of dissolved solids reaches 15 000 ppm (Waisel 1972, Carman & Brotherson 1982). This tolerance to saline soil is achieved mainly by special salt excreting glands (Waisel 1961, Kozlowski 1997).

In this study, submission of two different *Tamarix* species to salt stress (150 mM) induced notable changes on physiological responses and growth of both species relative to plants grown under control conditions. The yellowing and shedding of old leaves observed in both species during the experiment suggest that toxic ions could accumulate in the oldest leaves, preventing the death of younger ones. Such symptoms appeared earlier and more severely in *T. aphylla*, indicating its lower ability in partitioning toxic ions in the vacuoles, and therefore a lower salt tolerance as compared with *T. jordanis*. Storage of salt in old leaves probably occurs *via* the absorption of incoming ions from the xylem or retranslocation through the phloem from young to older leaves. This mechanism has been reported in many halophytes plants, and limit the transportation of salt to active younger leaves and reduce their toxic effect on such organs. The shedding of old leaves may then eliminate the excess of salt (Mian et al. 2011, Albert 1975). Likely, the initial decrement of gas exchange measures observed in this study for both species was generated by the salt osmotic stress, and was followed by a better acclimation and performances of *T. jordanis* than *T. aphylla.* The greater reduction of g_s observed under salt stress in *T. aphylla* (Fig. 1C-D) is consistent with the findings of Abbruzzese (2011), who reported a higher control of stomatal conductance in *T. aphylla* under salt stress. Differences in the recovery of stomatal opening may also account for the higher salt tolerance observed in *T. jordanis* as compared with *T. aphylla.* Stomata closure in the latter species may contribute to limit the damage under salt stress, thus reducing the assimilation rate, which increased the intrinsic water use efficiency (A/g_s), thereby maintaining a high biomass in the short period (Tab. 1). Contrastingly, *T. jordanis* showed a higher water use efficiency in absolute term.

The remarkable reduction of hexoses and starch in the leaves of *T. aphylla* under salt stress on the 2nd day (Fig. 3A-C, Fig. 4) was associated with a decrease in CO_2 assimilation, though the recover of the starch content to the same level of control plants was observed afterward. This could be related to both a partial recover of photosynthesis and a reduction of plant sinks demands, slowing down the plant height increment (Tab. 1), and decreasing sugar export according to the source/sink equilibrium under salt stress (150 mM). The observed reduction of sugar content in *T. aphylla* confirm the results of Parida et al. (2004) who reported that salinity decreased soluble sugars in the salt tolerant mangrove *Aegiceras corniculatum* L., as well as those of Gadallah (1999) who observed a reduction of soluble sugars in *Vicia faba* L. On the contrary, the content of sugars in the leaves of *T. jordanis* was not significantly reduced under salt stress (Fig. 3B-D, Fig. 4). The maintenance of sugars in *T. jordanis* and the higher content of starch under control and salty conditions may have a protective role on membranes. Carbohydrates such as soluble sugars (glucose, fructose, sucrose) affects osmoregulation, osmoprotection, carbon storage, and radical scavenging (Parida et al. 2002). Consistently with many other halophytes species (Flowers et al. 1986), *T. jordanis* growth was stimulated by salinity, while *T. aphylla* showed a growth reduction in the same experimental conditions (Tab. 1). Accordingly, Waisel (1961) reported a reduced growth of saplings of *T. aphylla* under irrigation with 0.1M and 0.2 M of NaCl. Such growth depression is probably due to the osmotic and toxic effect of the NaCl solution (Waisel 1961), which accumulated at higher concentration in *T. aphylla* than in *T. jordanis* (Abbruzzese 2011). Growth was also possibly reduced as a consequence of the increase in cost energy associated with salt pumping, increased respiration (Kleinkopf & Wallace 1974) and decreased photosynthetic rates (Jackson et al. 1990).

In contrast to salt, drought (50% FC) never induced appreciable effects on carbon assimilation in any of the studied species in the short time (Fig. 1). The maintenance of optimal levels of photosynthesis under drought conditions may be related to the high efficiency of *Tamarix* twigs to excrete calcium carbonate in non-saline soils, thereby trapping the CO_2 from the atmosphere. According to Waisel (1991), a leaf chalk crust may have a conspicuous adaptive value in terms of optimization of photosynthesis by concentrating the atmospheric CO_2. Despite its small surface area, *T. aphylla* showed a higher capacity of assimilation under control and drought stress (Fig. 1A-B). *T. aphylla* excrete more calcium than *T. jordanis* (Abbruzzese 2011), therefore it may benefit from an extra source of carbon trapped by calcium, and exploit it for optimal assimilation during early morning (Waisel 1991).

In this study, the stomatal conductance of plants grown under drought conditions was substantially maintained in both species over the whole experiment, except for day 7 (Fig. 1C-D), when an increase in soluble sugars and a concomitant decrease of relative humidity (37.9%) was observed (Fig. 3A-F). Despite the short duration of the experiment carried out, the high content of soluble sugars and their increase mainly in *T. aphylla* suggest that soluble sugars accumulation is one of the possible mechanisms adopted by this species as a response to drought. Accumulation of soluble carbohydrates in leaves is common under water stress (Chaves 1991). Moreover, it has been reported that plants growing in dry habitats contain more soluble sugars than those living in moist habitats. lljin (1957) reported values of sugar content (on a dry weight basis) ranging from 1.3% in herbaceous mesophytes to 6.9% in xerophytic trees and shrubs. In this study, an increase of sucrose and a simultaneous decrease of starch content in plants grown under drought stress was detected on the 7th day of the experiment (Fig. 3E-F, Fig. 4). It is also a common observation that water stress conditions induce a severe decrease in starch and simultaneously an accumulation of soluble sugars (lljin 1957, Turner et al. 1978). Such a shift in carbon partitioning may have an adaptive value, since it could contribute to osmotic adjustment and protection (Daie 1996, Lawlor & Cornic 2002).

Despite the limited effect of drought on photosynthesis, growth of *T. jordanis* plants was severely limited, with height increment lower by 55% than that of control plants. This reduced growth may be due to other factors not analyzed in this study, such as hormones or precursors (Fricke et al. 2006, Munns et al. 2000). Contrastingly, the slight effect of drought on height increment and biomass of *T. aphylla* plants was probably due to the high and early increase of intrinsic water use efficiency in this species, allowing plants under stress conditions to produce a quantity of dry matter similar to control plants with less water consumption. Also, the increase in the root/shoot ratio observed in *T. jordanis* under drought conditions was probably due to a significant decrease in the shoot biomass (Tab. 1), that is considered an adaptive mechanism allowing to reduce the transpiration surface and to increase the water absorption from the soil (Joly et al. 1989).

Controversial results are reported in the literature about the combined effect of salt and drought stress. In this study, the presence of both constraints in the soil (salt 150 mM + drought 50% FC) showed the most severe impact on both species, as compared with water and salt stress applied separately. Symptoms of yellowing and defoliation in plants under such combined stress were much more evident than in leaves of plants grown under salt alone, appearing on the

third day of the experiment. Such symptoms suggest a high accumulation of salt in older tissues to prevent the death of young leaves. Adverse effects on all measured parameters were reported. Photosynthesis, transpiration, stomatal conductance, sugars and starch content were significantly reduced in response to the combined stress. The combination of drought and salt stress induced effects similar to salt stress on the reduction of hexoses sugars. In addition, both species showed a marked decline of the starch content, which was lower than that of salt and drought applied alone. This confirms the positive interaction between salt and drought in the reduction of the starch content in the species analyzed. However, the ability of plants of both species to survive the whole experiment may be associated with a high production of proline content and with an increase of their water use efficiency. Proline may act as an osmoregulator, a ROS (reactive oxygen species) scavenger and a molecule chaperone reinforcing the structure of proteins, thereby protecting plant cells from damage caused by stress (Delauney & Verma 1993, Krasensky & Jonak 2012). Moreover, it has been suggested that proline may represent a stock of nitrogen available for the plant after a period of suffering from water deficiency (Dib et al. 1992). In this study, a high proline content was observed in the two *Tamarix* species grown under the combined stress (Fig. 5). Likely, proline accumulation was induced by NaCl ion stress rather than drought, because plants grown under salt stress alone also showed an initial increase in proline content (Fig. 5). However, proline usually accumulates in the cytosol, which is only 5 to 10% of the cell volume (Lee et al. 1990, Winter et al. 1993). Based on our results, the contribution of proline to the cell osmotic potential (0.028-0.031 MPa at the 7th day) was too low for the osmotic adjustement of the cytosol (0.2-0.4 MPa in osmotic potential). Therefore, we may hypothesize that proline acts mainly as osmoprotector and storage of nitrogen and carbon for future use under stressful conditions. These considerations are supported by Wikqiang et al. (2010) and Koyro (2006), who found that proline contributed to the protection of *T. chinensis* against high salinity in the soil by scavenging the ROS generated by salt stress, which may cause oxidative damage to membrane lipids, proteins and nucleic acids. Furthermore, the accumulation of proline was suggested to be related to a decrease or suppression of gene encoding for the proline dehydrogenase (PDH), or due to the expression of gene *P5CS* for the key enzyme regulating the proline synthesis *via* glutamate pathway under environmental stresses (Krasensky & Jonak 2012).

In this study, the severe reduction of plant growth observed in both *Tamarix* species grown under high salinity and drought could be attributed to more than one factor: hindrance in cell division and cell elongation, reduction in assimilation rate and increase in ion toxicity due to reduction of water availability. All the above factors may retard plant elongation, but also hinder dry matter accumulation causing a decrease in biomass production.

Conclusion

The two species analyzed in this study, *Tamarix aphylla* and *T. jordanis*, proved their ability to cope with salt and drought stress to a different extent, through common acclimatization mechanisms. *T. aphylla* showed better performances and growth than *T. jordanis* under drought stress, due to higher photosynthesis, sugar accumulation and water use efficiency. The maintenance of an adequate sugar content under salt stress reflects the important role of these osmolytes in cell protection of salt tolerant species like *T. jordanis*. From an agronomic and environmental perspective, the two species studied seems to acclimatize fairly well to a moderate salinity (150 mM) without any loss of biomass. In particular, *T. jordanis* seems to maintain a high efficiency in the carbon sequestration over a relatively short period of time. However, a reduction in biomass could be expected at moderate drought (50% FC), particularly in *T. jordanis*. In soils with high salinity (0.1-0.2 M of NaCl) and in the absence of irrigation, a short drought period might causes a severe damage to these trees.

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