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Photosynthetic and wood anatomical responses of *Tamarix africana* Poiret to water level reduction after short-term fresh- and saline-water flooding

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Abstract In the Mediterranean basin, *Tamarix* spp. constitute important populations along rivers and sea coasts, and might be primarily subjected to water level fluctuations and salinization, as a consequence of global climate change. Here, we analyze leaf gas exchange and xylem anatomy during a water level decrease below the soil surface after short-term flooding with fresh- and saline-water (200 mM) in order to predict *Tamarix africana* Poiret responses under future environmental conditions. Fresh-water level reduction negatively affected stomatal conductance (−56.3 %), but only when water decreased to the lowest level (15 cm below the soil surface). No effects on assimilation rates and xylem vessel dimensions occurred. Under saline conditions, the rate of the water level decrease was lower compared to the non-saline treatment, as stomatal conductance was negatively affected by salinity (−59.5 %) and significantly declined over time. Moreover, decreases in mean xylem vessel area (−51.3 %), assimilation rates (−52.2 %) and stomatal conductance (−76.0 %) were also observed compared to the control, indicating both an osmotic stress and a toxic effect of NaCl on leaf gas exchange. These leaf responses were probably induced by greater belowground-root salt absorption and transport compared to previous flooding conditions, as confirmed by the increase in salt excretion (+473.2 %). The results emphasize the survival risk of *Tamarix* spp. to water level variation under both saline and non-saline

conditions, and the need of management practices focused on the conservation of these populations.

Keywords *Tamarix africana* Poiret · Water level reduction · Salinity · Leaf gas exchange · Xylem vessels

Introduction

Coastal and estuarine environments are characterized by unique biodiversity components, constituting niches and refugia for numerous faunal and microbial species, and a high diversity of biogeochemical processes (Day et al. 2008). These environments represent sensitive sites for plant existence, as salinity and flooding, which usually occur in such areas, can be important stress factors for plant growth (Blom and Voesenek 1996; Kozłowski 1997; Mansour 2000; Chen et al. 2005; Rengifo et al. 2005; Fernandez 2006; Flowers and Colmer 2008). In these areas, the augmented frequency of heavy precipitation events as a consequence of global climate change may modify water level alteration, increasing the risk of periodic flooding events (IPCC 2007). Coastal plants have evolved many physiological, morphological and reproductive strategies to survive water level fluctuation; however, their ability to adapt to multiple stressors is lower compared to their tolerance to a moderate increase of a single stressor (Day et al. 2008). At the estuary of temporary streams, which are usually found in the Mediterranean region, where sea-water intrusion takes place during low river discharge and high tides, heavy precipitation events can provoke short-duration flooding with brackish water. Fresh-water inputs into coastal systems can reduce the level of soil salinity (DeLaune et al. 2003); however, waterlogging and salinity together have been proven to provoke wetland vegetation death, assembling the harmful effects of both stresses (Barrett-Lennard 2003; Wang et al. 2010). In fact, salinity may compromise flood tolerance mechanisms (Salter et al. 2010), preventing adventitious root

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formation (Akilan et al. 1997; Salter et al. 2008) and increasing Na^+ and Cl^- concentration in the foliage of plants under flooding conditions (Marcar et al. 2002). Water level reduction after fresh-water flooding, as a result of water evapotranspiration and/or drainage, may allow the reestablishment of the previous soil water status and aeration; contrarily, in the case of saline-water flooding, a water level decrease implies an accumulation of salt in the soil, negatively affecting the plant growth.

The *Tamarix* genus includes about 90 salt-secreting species that show a high adaptability to different environments and a high endurance of adversity (Ginzburg 1967; Bar-Nun and Poljakoff-Mayber 1974; Brotherson and Field 1987; Cleverly et al. 1997; Di Tomaso 1998; Glenn et al. 1998; Horton et al. 2001; Tallent-Halsell and Walker 2002; Zhang et al. 2002; Gries et al. 2003; Xu and Li 2006). In the Mediterranean Basin, *Tamarix* spp. are naturally distributed in coastal areas, salt marshes and riverbanks of temporary and perennial streams, where they often constitute monospecific dense thickets (Arizpe et al. 2008). Although considered halophytic species, a reduction in *Tamarix* growth rates under concentration of 100 mM NaCl or more has been reported in the literature (Waisel 1961; Kleinkopf and Wallace 1974; Glenn et al. 1998). Moreover, contrasting results exist regarding their tolerance to flooding with fresh water and to water level fluctuation (Horton 1960; Sprenger et al. 2001; Vandersande et al. 2001; Tallent-Halsell and Walker 2002; Gries et al. 2005; Stromberg et al. 2007; Merritt and LeRoy 2010), whereas the effects of water level reduction after flooding with saline water on photosynthesis and growth in these species have never been studied.

In order to predict *Tamarix* responses to water level decreases after short-term flooding, which may take place under future climatic conditions in the Mediterranean area, a laboratory experiment was conducted. We analyzed leaf gas exchange, leaf salt excretion and xylem anatomy in *Tamarix africana* Poiret during water level reduction below the soil surface after short-term flooding with fresh and saline water (200 mM). Our hypothesis was that plants would recover from fresh-water level decreases. Saline-water level reduction was expected to initially benefit water transport as a result of root oxygenation. Thereafter, a decrease in leaf gas exchange was foreseen as a consequence of an accumulation of NaCl in the soil, decreasing the osmotic water potential.

Materials and methods

Six *T. africana* Poiret genotypes were collected in November 2008 from southern Italy. The genotypes were replicated six times through cuttings, which were singularly planted in 1.6 dm³ plastic pots (10.5 × 10.5 × 22 cm) containing sand (35 %) and loamy soil (65 %). The

cuttings were grown in a greenhouse for 6 weeks and subsequently transplanted in 2.6 dm³ plastic pots (17.5 × 17.5 × 25 cm) containing the same soil mixture. The pots were inserted into nine plastic boxes (60 × 40 × 40 cm) in groups of four genotypes and grown for 2 months in a growth chamber (200 × 250 × 200 cm) under a photosynthetic photon flux density of 550 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 60 % relative humidity, with a photoperiod of 12 h and a day/night temperature of 25/15 °C. The plants were watered three times a week with fresh water. Two weeks before the beginning of the experiment, a modified half-strength Hoagland solution was supplied. Before the beginning of the flooding treatments, the height and diameter of the plant dominant sprout were on average equal to 49.5 and 0.4 cm, respectively. The nine boxes were divided into three blocks, each composed of three treatments. The treatments were: (1) daily irrigation with fresh water (control, C), (2) flooding with fresh water (F) and (3) flooding with fresh water with 200 mM NaCl added (FS). The two flooding treatments were provided by filling the plastic boxes respectively with tap and saline water. The amount of water used to provide the treatments was the one that determined the complete soil flooding. The dimension of the boxes was chosen in order to have large volumes of water compared to the soil volumes. Flooding levels were kept constant at 20 cm (3 cm above the soil surface) for 44 days by pouring, respectively, fresh and saline water (200 mM), and a modified Hoagland solution (0.5× concentration) in the boxes once a week. After 14 days of continuous flooding, adventitious roots had formed at the shoot base of all plants grown under flooding conditions, independently of water salinity. After 44 days, no more water was added to the F and FS treatments, and the water level in each box decreased by evapotranspiration until poor water (2–5 cm height) was observed at the bottom of the boxes (day 89). On day 44, two plants per treatment per block were cut off for element content determination (Abou Jaoudé et al. 2012). The remaining two plants per treatment per block (six replicates per treatment) grew in the boxes till the end of the experiment. Gas exchange measurements were performed only on four replicates per treatment.

Water electrical conductivity and water level

The electrical conductivity (EC) of the water contained in each box was measured along the experiment using a conductometer (HI9811, Hanna Instruments, Inc., USA) equipped with an electrode probe (HI1285, Hanna Instruments, Inc., USA). At the same time, the water level (WL) and the volume of water present in each box were determined. The measures were made on days 3, 18, 33, 44, 60, 73 and 89 after the beginning of the experiment under both flooding treatments. The total quantity of salt (SM) present in the boxes at each sampling date during the water level decrease (days 44, 60, 73 and 89) was estimated by multiplying water salinity

(expressed in g salt l⁻¹ water) and the respective water volume.

Gas exchange and chlorophyll fluorescence measurements

As *Tamarix africana*, similarly to *Cupressus* spp., is characterized by the presence of scale-like leaves, gas exchange measurements were not performed on single leaves but on green twigs. Leaf gas exchange was measured on the tenth twig from the apical bud on days 44, 60, 73 and 89 after the onset of the experiment. All twigs were selected from the dominant sprout. Gas exchange was measured using a portable infrared gas analyzer (LI-6400, LI-COR Biosciences, Inc., Lincoln, NE, USA) equipped with a conifer chamber (LI-6400-05). The cuvette temperature was set at 25 °C, while the air flow was adjusted (400–600 µmol s⁻¹) to maintain a constant VPD (1.5 ± 0.2 kPa). With the only objective of determining the saturating photon flux, which was set at 2000 µmol m⁻² s⁻¹, light curves were run before the beginning of the measures. The artificial light was generated by a halogen incandescent lamp and transmitted to the cuvette by an optical fiber (FL-400 with 400-F; Walz, Germany). Net assimilation rate (*A*) change in response to intercellular [CO₂] variation (*C_i*) was determined for ambient CO₂ concentration values (*C_a*) of 400, 300, 250, 200, 150, 50, 400, 500, 650, 750, 850 and 1000 µmol mol⁻¹ as suggested by Long and Bernacchi (2003); the *A* values were recorded as soon as *C_a* was stable (cv < 0.7 %) (Ainsworth et al. 2002). Net assimilation rates (*A*₄₀₀) and stomatal conductance (*g*₄₀₀) measured at a *C_a* value of 400 µmol mol⁻¹ were considered as the assimilation and the stomatal conductance at growth-chamber CO₂ concentration. Intrinsic water use efficiency (*A/g*) was calculated as the ratio between *A*₄₀₀ and *g*₄₀₀. The maximum carboxylation rate (*V_{cmax}*) and the maximum rate of electron transport (*J_{max}*) were estimated according to Farquhar et al. (1980), using the software Prism 4 (GraphPad software, Inc). Following *A–C_i* curves and 5 min of adaptation to dark conditions, dark respiration (*R*_{d400}) was measured at a *C_a* value of 400 µmol mol⁻¹. After gas exchange measurements, the twigs were cut off, and their fresh weight was determined. The twigs were then scanned and the images analyzed by the software Skyroot (Llandrindod Wells, Powys, UK) in order to obtain the total twig length. The twig area was estimated by multiplying the twig length by the measured mean twig diameter (0.7 mm). A small twig portion was dried at 70 °C for dry weight estimation, while the rest of the twig was dark-adapted for 15 min for chlorophyll fluorescence measurements.

Chlorophyll fluorescence was measured with a PAM 2000 fluorimeter (Heinz Walz, Effeltrich, Germany) on the same twig used for gas exchange measurements. Photochemical efficiency was estimated by measuring the quantum yield of PSII (*F_v/F_m*) in dark-adapted twigs.

Twig characteristics

After fluorescence measurements, the twigs were dried in an oven until reaching a constant weight. The twigs were then inserted into plastic tubes containing 25 ml of deionized water and shaken at 500 rpm for 20 min. The liquid phase was separated from the twigs with filter paper. The electrical conductivity of the liquid phase was measured using a conductometer (HI9811, Hanna Instruments, Inc., USA) equipped with an electrode probe (HI1285, Hanna Instruments, Inc., USA). In order to obtain the amount of salt secreted and accumulated outside the twig (ES), the measured electrical conductivity values were converted in g salt g⁻¹ twig dry weight. Twig dry weight without salt was determined after drying in an oven at 70 °C until reaching a constant weight.

Xylem anatomy

At the end of the experiment, the plants were cut, and wood samples were collected at the base of the dominant shoot of each plant. Transverse wood microsections were made with a GLS1 microtome (Schenkung Daples, Zürich, Switzerland). The thickness of the sections varied between 30 and 50 µm. According to Schweingruber et al. (2008), wood cross sections were stained with safranin and astra-blue mixed in equal proportions, then dehydrated with alcohol at 50, 75, 95 and 100 % and embedded in Canada balsam. Two radii per section were digitally photographed under a light microscope (Leica DM4000B, Leica Microsystems AG, Wetzlar, Germany) (Fig. 5a–c). The observation of the obtained images revealed the presence of a band of tiny cells near the pith of all plants that were treated by flooding with both fresh and saline water (see arrows in Fig. 5). This band has never been observed under control conditions and likely represents the initial effect of the flooding treatment before adventitious root emission, thus a period of reduced water absorption. An image analysis was performed using the IMAGEJ software (National Institute of Health, Bethesda, MD, USA), which automatically recognizes and measures the lumen area of each vessel after the creation of a binary image (Fig. 5d). The images were examined in order to analyze the lumen area distribution of the vessels along the radius. Five 200 × 200 µm areas were selected from three wood portions (Fig. 5) of each of the two radii per plant: (1) a portion was located between the pith and the band of tiny cells (P), corresponding to the plant's secondary growth before the beginning of the submergence; (2) a second wood portion was located in the middle of the radius, after the observed band of tiny cells (M), representing the period of growth under continuous flooding conditions; (3) a third area was located near the bark (B), corresponding to the plant growth during water level reduction. For plants subjected to the control treatment, the wood diameter was simply divided into

three equal portions. Inside each selected area, the mean vessel area (MVA) and the total vessel area (TVA) of vessels $> 200 \mu\text{m}^2$ were calculated.

Statistical analysis

An analysis of variance (ANOVA) was performed on all the analyzed photosynthetic, twig and wood anatomical parameters, using the statistical software Systat 12.0 (SPSS Inc.) to evaluate the main effects of treatments, time and their interaction. The post hoc analysis was performed using Fisher's LSD test. Water salinity and level were analyzed by ANOVA to evaluate the effect of treatments and by repeated ANOVA to analyze their variation over time, using the same statistical software. The post hoc analysis was performed using Bonferroni's test. Prism 4 software (GraphPad Software, Inc.) was used to test the correlation between $V_{\text{c}_{\text{max}}}$ and J_{max} .

As after 40 days from the beginning of the treatments one *Tamarix* plant grown under FS conditions died, the number of replicates under FS was three. Under C and F, statistical analysis was made on four replicates. Significance was considered for p values < 0.05 .

Results

Water level and salinity dynamic

Water level (WL) and water electrical conductivity (EC) variations under flooding conditions (from day 0 to day 44) and during a water level decrease (from day 44 to day 89), in both fresh- (F) and saline-water (FS) treatments, are shown in Fig. 1. Fresh- and saline-water levels did not decrease at the same rate: WL under FS was significantly higher on days 73 (11.1 cm; $p < 0.001$) and 89 (5.1 cm; $p < 0.001$) than under F conditions (9.9 and 2.0 cm respectively).

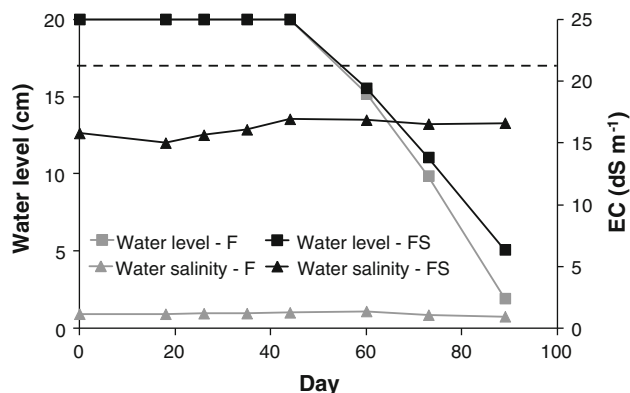


Fig. 1 Water level (WL; closed squares) and electrical conductivity (EC; closed triangles) dynamics under fresh- (grey) and saline-water (black) treatments in both treatments. The dashed line indicates the soil surface. On days 60, 73 and 89, the water level was under the soil surface. The values are mean \pm SE ($n = 4$ under F; $n = 3$ under FS).

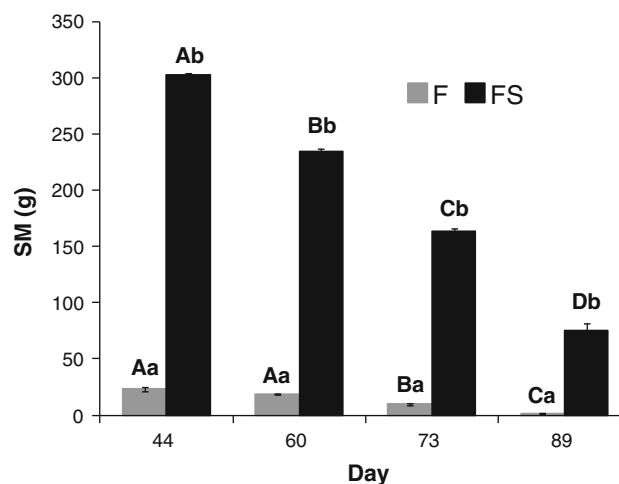


Fig. 2 Salt mass (SM) present in the respective volumes of water on days 44, 60, 73 and 89 under fresh- (grey bars) and saline-water (black bars) treatments. Lowercase letters refer to the comparison between the treatments. Capital letters refer to the comparison among dates. The values are mean \pm SE ($n = 4$ under C and F; $n = 3$ under FS).

The dynamic of water salinity also differed under flooding and during water level reduction. Particularly under FS, EC significantly increased until day 44 ($p < 0.001$), then remained constant in the following days, reaching average values of 16.6 dS m^{-1} .

Conversely, the mass of water-dissolved salt (SM) significantly changed over time during water level reduction ($p < 0.001$; Fig. 2): under FS, SM measured on days 60, 73 and 89 was respectively 23, 46 and 75 % lower compared to day 44 ($p < 0.001$ in all cases). The same trend was observed under F: on day 89, SM was reduced by 93 % compared to day 44 ($p < 0.001$). Water salinity was always significantly higher under saline than under non-saline conditions ($p < 0.001$).

Leaf gas exchange

Leaf gas exchange parameters are shown in Table 1. Net assimilation rates measured at an ambient CO_2 concentration of $400 \mu\text{mol mol}^{-1}$ (A_{400}) were similar in all treatments on days 44, 60 and 73, and did not change over time under C and F. However, on day 89, A_{400} was significantly lower under FS compared the control (C; -52.2% ; $p < 0.05$). Furthermore, A_{400} significantly changed over time under FS, being intermediate on day 44, and higher on days 60 ($p < 0.05$) and 73 ($p < 0.05$) compared to day 89. This decrease was not accompanied by a reduction in the maximum rate of carboxylation ($V_{\text{c}_{\text{max}}}$), which remained unchanged over time and was on average similar in the three treatments ($96.5 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). $V_{\text{c}_{\text{max}}}$ was also linearly correlated with the maximum rate of electron transport (J_{max}), independently of the treatment (Fig. 3). Dark respiration (R_{400}) was not affected by the treatments and did not vary over time, being on average equal to $3.1 \mu\text{mol}$

Table 1 Gas exchange and fluorescence parameters

Days											
44			60			73			89		
	Mean	SE		Mean	SE		Mean	SE	Mean	SE	
<hr/>											
<i>A</i> ₄₀₀ (μmol CO ₂ m ⁻² s ⁻¹)											
C	19.8	1.2		19.7	1.3		18.7	2.4	a	20.7	4.1
F	16.7	3.7		22.0	1.2		24.7	6.3	ab	14.5	1.6
FS	13.6	1.5	A	19.8	2.5	A	20.0	2.4	Bb	9.9	2.5
<i>V</i> _{cmax} (μmol CO ₂ m ⁻² s ⁻¹)											
C	93.9	6.0		116.5	12.0		104.7	8.4		110.1	19.3
F	90.1	12.2		109.7	11.4		112.1	28.6		73.5	5.9
FS	68.6	4.7		87.9	28.3		99.3	16.8		74.2	10.7
<i>R</i> _{d400} (μmol CO ₂ m ⁻² s ⁻¹)											
C	2.0	0.5		3.3	0.7		2.7	0.5		2.5	0.6
F	4.0	1.0		3.4	0.8		2.4	0.8		2.3	0.5
FS	2.7	1.5		2.8	0.7		3.6	0.5		2.4	0.0
<i>E</i> ₄₀₀ (mmol H ₂ O m ⁻² s ⁻¹)											
C	5.7	1.1	a	3.9	0.4		4.2	0.3		4.4	1.4
F	5.0	1.3	b	6.0	0.3		7.7	2.6		3.9	0.9
FS	5.8	2.4	ab	4.6	1.3		4.9	1.2		1.7	0.5
<i>g</i> _{l400} (mol H ₂ O m ⁻² s ⁻¹)											
C	0.39	0.03	a	0.31	0.04	ab	0.37	0.07	a	0.36	0.11
F	0.42	0.14	AB b	0.49	0.04	A a	0.64	0.18	B ab	0.28	0.07
FS	0.23	0.04	AB c	0.14	0.09	A b	0.29	0.05	B b	0.09	0.03
<i>A/g</i> _l (μmol CO ₂ /mol H ₂ O)											
C	49.3	6.0		57.3	7.6	a	51.8	3.7		63.5	12.4
F	47.0	7.8	AB	56.6	6.9	A b	39.3	1.9	B	83.8	13.0
FS	61.8	6.3		60.5	14.3	c	71.1	6.3		86.6	29.5
<i>F</i> _v / <i>F</i> _m											
C	0.78	0.03		0.80	0.01		0.82	0.02		0.79	0.01
F	0.80	0.02		0.82	0.02		0.84	0.01		0.80	0.03
FS	0.80	0.01	A	0.81	0.00	A	0.80	0.01	B	0.75	0.01

Net assimilation rates (A_{400}), maximum carboxylation rate ($V_{c_{\max}}$), dark respiration (R_{d400}), transpiration (E_{400}), stomatal conductance (g_{l400}), intrinsic water use efficiency (A/g_l) and quantum yield of PSII in the dark (F_v/F_m), measured on the four dates (days 44, 60, 73 and 89) after the water level decrease on the tenth leaf from the apical bud

Lowercase letters refer to the comparison among the treatments. Capital letters refer to the comparison among dates. The values are mean \pm SE ($n = 4$ under C and F; $n = 3$ under FS)

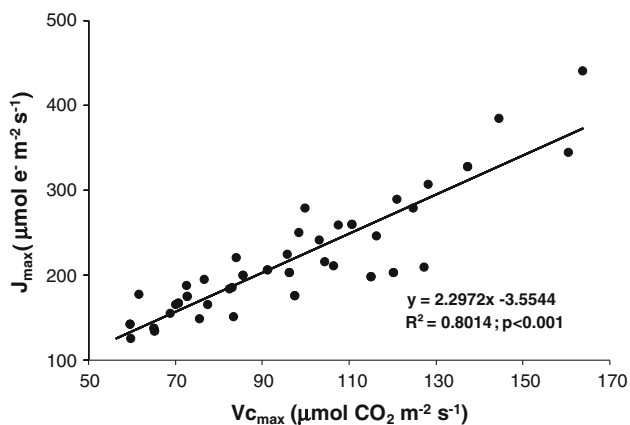


Fig. 3 Correlation between maximum carboxylation rate ($V_{c_{\max}}$) and maximum rate of electron transport (J_{\max}) calculated from net assimilation rate (A) versus intercellular CO_2 concentration (C_i) curves on days 44, 60, 73 and 89, under control, and under both fresh- and saline-water treatments. The equation, R^2 and p values are referred to the global fit

$\text{CO}_2 \text{ m}^{-2} \text{ s}^{-1}$. Transpiration rates (E_{400}) measured on days 44, 73 and 89 were similar in all treatments and did not vary over time. Under F, E_{400} was 35.2 % higher on day 60 ($p < 0.05$) compared to C. The stomatal conductance (g_{l400}) was negatively affected by water level reduction under both saline and non-saline conditions: g_{l400} significantly decreased in the last measuring day ($p < 0.05$) compared to the previous date (day 73). Moreover, a higher g_{l400} was measured under F on day 60 compared to C ($p < 0.05$) and FS ($p < 0.01$), and on day 73 compared to FS ($p < 0.05$). On day 89, g_{l400} was significantly higher under C than under FS ($p < 0.05$). Intrinsic water use efficiency (A/g_l) was similar in all treatments on days 44, 60 and 89, while after 29 days from the beginning of the water level decrease (day 73), A/g_l was significantly higher under FS treatment than under C ($p < 0.05$) and F ($p < 0.01$). Furthermore, A/g_l measured under F conditions increased during the last sampling date compared to day 44 ($p < 0.05$) and 73 ($p < 0.01$). The quantum yield of PSII measured in the dark (F_v/F_m) was similar under all treatments on days 44, 60, 73 and 89. Under FS, F_v/F_m was significantly

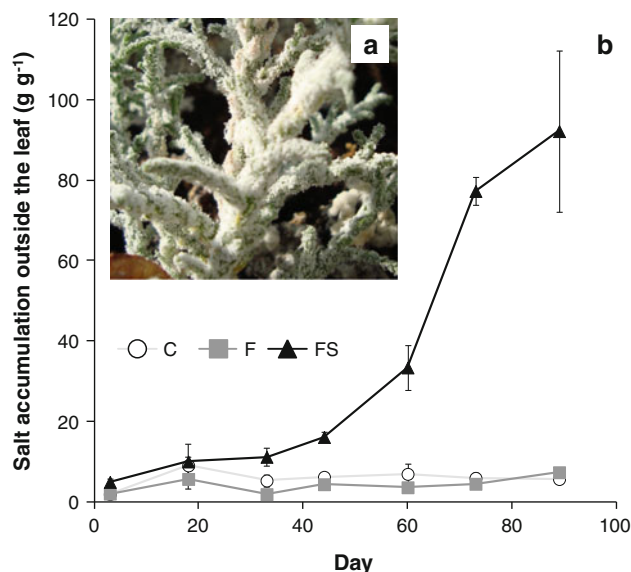


Fig. 4 Leaves covered by the secreted salt (a) and mass of the salt (ES) secreted by salt glands and accumulated on the twig surface, expressed on a twig dry weight basis (b). The measures were performed on days 3, 18, 33, 44, 60, 73 and 89 after the onset of the treatments, under control (white symbols), fresh- (grey symbols) and saline-water (black symbols) conditions. The values are mean \pm SE ($n = 4$ under C and F; $n = 3$ under FS)

lower on day 89 compared to day 44 ($p < 0.01$), 60 ($p = 0.001$) and 73 ($p < 0.01$).

Salt accumulation outside the twig

Salt accumulation on the twig surface and its dynamic (ES) are shown in Fig. 4a, b. A higher amount of secreted salt was measured on days 33, 60, 73 and 89 in plants grown under FS compared to plants grown under control ($p < 0.05$ for day 33, $p < 0.001$ for all the other dates) and F conditions ($p < 0.01$ for day 33, $p < 0.001$ for all the other dates). Under FS, salt accumulation measured on days 3, 18, 33 and 44 (on average equal to $10.5 \text{ g NaCl g}^{-1}$ twig) was significantly different from that measured on days 73 ($77.3 \text{ g NaCl g}^{-1}$ twig; $p < 0.05$ in all cases) and 89 ($92.2 \text{ g NaCl g}^{-1}$ twig; $p < 0.01$, in all cases). Moreover, the quantity of salt accumulated on the FS twig surface on day 89 was significantly higher than that measured on day 60 ($33.4 \text{ g NaCl g}^{-1}$ twig; $p < 0.05$).

Wood anatomical characteristics

An example of the lumen area distribution of the vessels along the stem radius under the three treatments is shown in Fig. 5. The mean vessel area (MVA; Fig. 6a) significantly differed along the radius and among the treatments. Particularly, while no variations were observed in the wood portions located near the pith

(P) and at the center of the section (M) under the different treatments, MVA was significantly lower near the bark (B) of plants grown under FS compared to C (-51.3% ; $p < 0.01$). Accordingly, the total vessel area (TVA; Fig. 6b) was lower in the B portion of plants grown under FS conditions compared to those grown under C (-55.1% ; $p < 0.01$). Moreover, under FS, a significant lower TVA was measured in the B portion, compared to the P ($p < 0.05$) and the M ones ($p < 0.01$).

Discussion

Water level reduction, water salinity and salt extrusion

A higher rate of water level reduction was observed under fresh-water flooding compared to saline water, indicating a negative effect of salt on evapotranspiration processes. In fact, salt presence affected plant transpiration as demonstrated by the reduced stomatal conductance measured at the different dates under FS compared to F.

Although under saline conditions water salinity remained constant after the beginning of the water level decrease, the mass of salt dissolved in the solution decreased by 227 g from day 44 to day 89. Moreover, the quantity of salt accumulated outside the twigs of *Tamarix* plants grown under saline conditions was significantly higher compared to previous flooding conditions. In the saline treatment, the lower salt accumulation on the twig surface observed under flooding compared to post-flooding conditions was probably due to the presence of adventitious roots, which may exclude Na^+ (Saqib et al. 2005), or to their potential as reservoir for this ion, as already observed by Boursier and Lauchli (1990), reducing the overall quantity of salt present in photosynthetic organs. At the end of the experiment the quantity of secreted salt was equal to 92.2 g per g of twig biomass, and twig biomass was on average equal to 4.3 g (under review); thus, the quantity of salt secreted by this twig biomass was equal to 204 g , which is similar to the dissolved salt mass reduction. These results bring us to two conclusions: (1) *Tamarix* spp. are able to absorb, translocate and secrete large amounts of salt; (2) the salt absorbed by roots is not stored in other plant organs but is almost all secreted by leaf salt glands and accumulated on the leaf surface.

Tamarix responses to fresh-water level decrease

Stomatal closure, induced by the increase in phytohormones concentration (especially ABA) in response to anoxic root conditions (Kozłowski 1997), is a common effect observed in shoots of flooding intolerant species (Pezeshki et al. 1996; Braendle and Crawford 1999; Rengifo et al. 2005). In this study, no significant

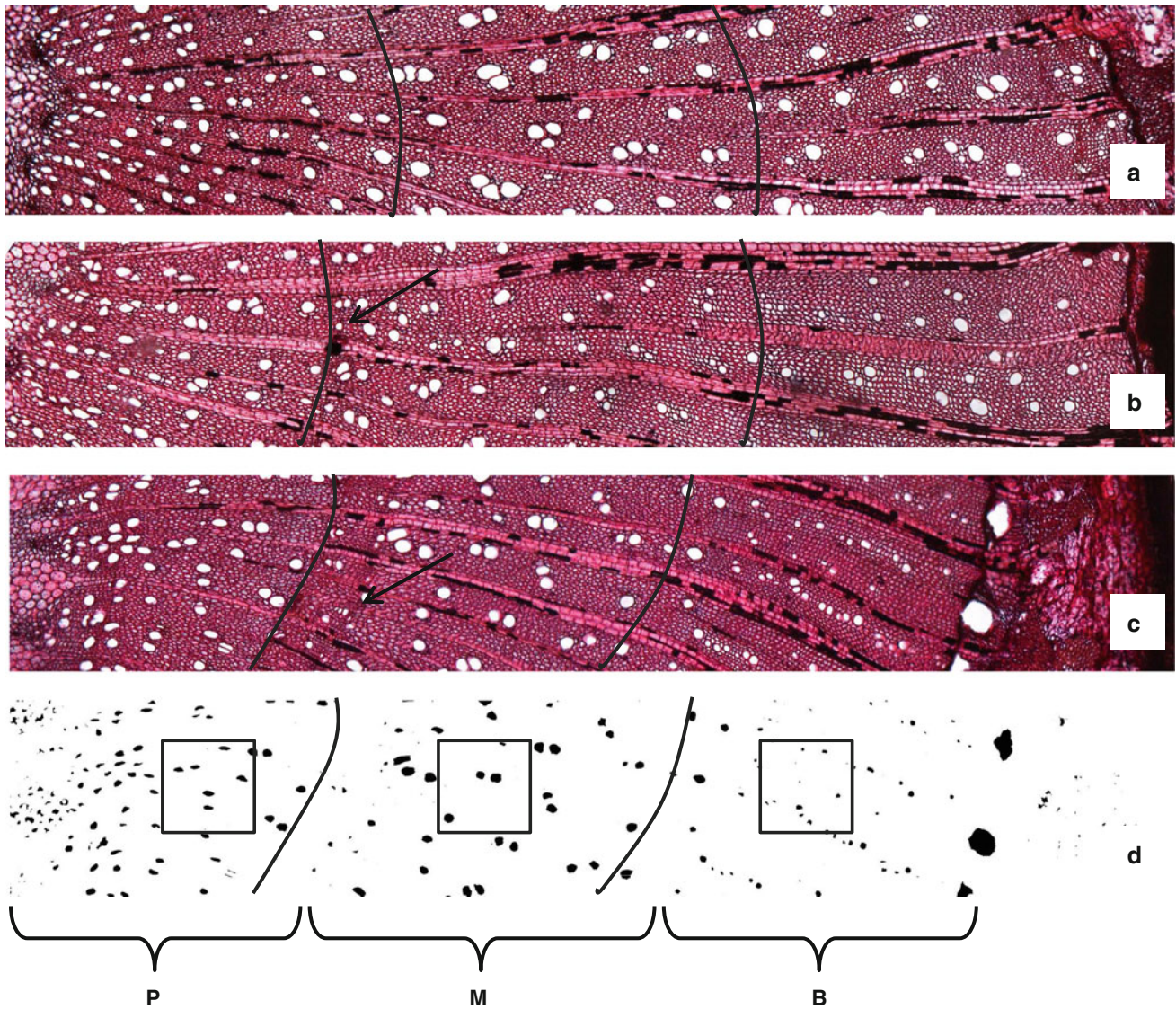


Fig. 5 Transverse wood microsections of plants grown under control (a), flooding (b) and flooding with saline water (c). The black lines indicate the edges of the three identified wood portions per section located: near the pith (P), at the center of the

microsection (M) and near the bark (B). After the creation of a binary image, randomly selected areas were used for mean and total vessel area measurements (d)

decrease of stomatal conductance was observed after 44 days of continuous soil flooding with fresh water, indicating a high tolerance of *T. africana* to short-term anoxia conditions (Abou Jaoudé et al. 2012). Alleviation of anoxic stress is usually achieved by aerenchyma and adventitious root formation (Visser et al. 1995; Blom and Voesenek 1996; Drew et al. 2000; Colmer 2003; Evans 2004). Although the presence of aerenchyma was not investigated, adventitious root development was observed after 14 days from the beginning of the treatment (unpublished data), allowing the re-establishment of a contact between the air and the root apparatus, enabling stomatal opening and gas exchange. Adventitious roots had formed at the base of the shoots, floating on the water surface. Thus, their ability to absorb water decreased simultaneously with the water level decrease.

On day 60, when the water level was reduced by 5 cm compared to day 44, adventitious roots were no longer able to achieve their function. Consequently, water absorption during the water level decrease was carried out by belowground roots. High stomatal conductance and transpiration rates were observed on days 60 and 73, indicating an adequate water supply, probably because of an increased water transport through belowground roots and to a beneficial effect of soil oxygenation after the water level decrease. In contrast, stomatal conductance was significantly reduced at the end of the experiment compared to the previous measuring dates, while intrinsic water use efficiency significantly increased. As also proposed by Kozłowski (1997), previously flooded plants might be more sensitive to drought because of a reduced root growth that cannot supply the adequate

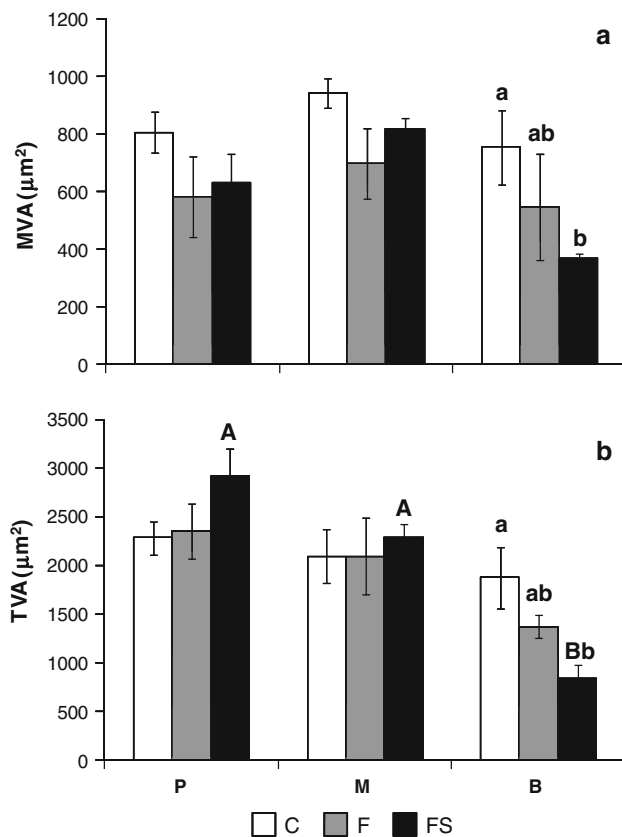


Fig. 6 Mean vessel area (MVA; **a**) and total vessel area (TVA; **b**) measured under control (white bars), F (grey bars) and FS (black bars) in the three wood portions: near the pith (P), at the center of the microsection (M) and near the bark (B). Lowercase letters refer to the comparison among the treatments. Capital letters refer to the comparison among wood portions. The values are mean \pm SE ($n = 4$ under C and F; $n = 3$ under FS)

quantity of needed water. In fact, after 44 days of flooding, plants were characterized by a reduced root/shoot ratio compared to the control (unpublished data).

Tamarix responses to saline water level decrease

During the saline-water level decrease, *T. africana* showed a reduced stomatal conductance compared to non-saline conditions. This decrease did not cause any variation in the assimilation rates on days 60 and 73, as the maximum rate of carboxylation increased (although not significantly) compared to day 44. A significant decrease in the photosynthetic rate was only observed on day 89. This reduction was not induced by a decline in carboxylation activity or electron transport, as $V_{c_{max}}$ and J_{max} both did not vary by an increase in dark respiration. A decrease in the plant photosynthesis and growth has been reported in both glycophytes (Bongi and Loreto 1989; McLeod et al. 1999; Centritto et al. 2003; Loreto et al. 2003) and halophytes (Ueda et al. 2003; Parida et al. 2004; Nandy Datta et al. (2007), and

is often the result of reduced stomatal conductance. An increase in stomatal resistance can be caused by feedback controls in response to the increase of intermediates or products of the Calvin cycle that accumulate because of the reduced growth under saline conditions (Lovelock and Ball 2002), or by an increase in the cellular concentration of Na^+ and/or Cl^- . In fact, below-ground roots are generally characterized by a higher efficiency in ion absorption compared to adventitious roots (Saqib et al. 2005); in our study, this is proved by the increased salt extrusion through the salt glands during water level reduction, which implies higher ion absorption compared to previous flooding conditions. Further resistance to water loss can be the result of the physical obstruction of the stomata by salt particles accumulated on the leaf surface, as usually occurs with dust (Hirano et al. 1995), which can also have an hygroscopic effect, trapping water losses. Although a decrease in the efficiency of photosystem II over time was observed in this study, no direct effects of the salt treatment were detected on day 89 compared to the control, indicating that this reduction is a secondary cause of the decrease in the photosynthetic activity.

Meyer and Boyer (1972) reported that salt stress can limit cellular expansion, which is more sensitive to low water potentials than cellular division. Moreover, excess salinity causes osmotic stress and thus increases the risk of cavitation in a similar way to drought (Munns 2002). As a result, vessel characters should be adapted to prevent the loss of conductive area upon embolism (Schmitz et al. 2007). Safe hydraulic structures are concerned with the construction of vessels characterized by a small diameter (Junghans et al. 2006; Sobrado 2007), a small wall area and, thus, a relatively low pit area per vessel (Hacke et al. 2006). Further studies are therefore needed to assess whether the decrease in mean vessel area and total vessel area in the wood portion formed during the saline-water level decrease is the result of salt-induced drought stress and wood structure responses to increasing risk of cavitation, or the response to a direct effect of salt on cell division processes.

Although field experiments are necessary to assess the responses of *Tamarix* to water level fluctuation in natural ecosystems, this study emphasizes the survival risk of these species in coastal and estuary environments under future climatic conditions. Particularly a water level decrease after saline-water flooding is expected to negatively affect photosynthetic activity and water transport in *Tamarix africana* as a result of the increased salt transport to the photosynthetic organs. In fact, salt did not accumulate in the soil solution as we first hypothesized. Thus, the reduction in assimilation rates was rather due to a direct sodium chloride effect on stomata than to an osmotic stress per se. Under non-saline conditions, a higher sensitivity to soil drying was observed. As a worldwide increase of the sea-water level is expected as a consequence of global warming (IPCC 2007), management practices focused on reducing sea-water intrusion and flooding events seem to be essential

for the preservation of coastal area populations, as higher salinity may further increase plant stress.

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References

- Abou Jaoudé R, de Dato G, Palmegiani M, De Angelis P (2012) Impact of fresh and saline water flooding on leaf gas exchange in two Italian provenances of *Tamarix africana* Poir. *Plant Biol*. doi:10.1111/j.1438-8677.2012.00597.x
- Ainsworth EA, Davey PA, Hymus GJ, Drake BG, Long SP (2002) Long-term response of photosynthesis to elevated carbon dioxide in a Florida scrub-oak ecosystem. *Ecol Appl* 12:1267–1275
- Akilan K, Marshall JK, Morgan AL, Farrell RCC, Bell DT (1997) Restoration of catchment water balance: responses of clonal river red gum (*Eucalyptus camaldulensis*) to waterlogging. *Restor Ecol* 5:101–108
- Arizpe D, Mendes A, Rabaça JE (2008) Sustainable riparian zones. A management guide. Generalitat Valenciana, Spain
- Bar-Nun N, Poljakoff-Mayber A (1974) Some aspects of protein metabolism in *Tamarix tetragyna* roots grown in a saline environment. *Aust J Plant Physiol* 1:237–246
- Barrett-Lennard EG (2003) The interaction between waterlogging and salinity in higher plants: causes, consequences and implications. *Plant Soil* 253:35–54
- Blom CWPM, Voessen LACJ (1996) Flooding: the survival strategies of plants. *Trends Ecol Evol* 11:290–295
- Bongi G, Loreto F (1989) Gas-exchange properties of salt-stressed olive (*Olea europaea* L.) leaves. *Plant Physiol* 90:1408–1416
- Boursier P, Läuchli A (1990) Growth responses and mineral nutrient relations of salt stressed sorghum. *Crop Sci* 30:1226–1233
- Braendle R, Crawford RMM (1999) Plants as amphibians. *Perspect Plant Ecol Evol Syst* 2:56–78
- Brotherson JD, Field D (1987) *Tamarix*: impacts of a successful weed. *Rangelands* 9:110–112
- Centritto M, Loreto F, Chantzoulakis K (2003) The use of low [CO₂] to estimate olive saplings. *Plant Cell Environ* 26:585–594
- Chen HJ, Qualls RG, Blank RR (2005) Effect of soil flooding on photosynthesis, carbohydrate partitioning and nutrient uptake in the invasive exotic *Lepidium latifolium*. *Aquat Bot* 82:250–268
- Cleverly JR, Smith SD, Sala A, Devitt DA (1997) Invasive capacity of *Tamarix ramosissima* in a Mojave Desert floodplain. The role of drought. *Oecologia* 111:12–18
- Colmer TD (2003) Long-distance transport of gases in plants: a perspective on internal aeration and radial oxygen loss from roots. *Plant Cell Environ* 26:17–36
- Day JW, Christian RR, Boesch DM, Yáñez-Arancibia A, Morris J, Twilley RR, Naylor L, Schnaffner L, Stevenson C (2008) Consequences of climate change on the ecogeomorphology of coastal wetlands. *Estuar Coast* 31:477–491
- DeLaune RD, Jugsujinda A, Peterson G, Patrick W (2003) Impact of Mississippi River freshwater reintroduction on *Spartina patens* marshes: responses to nutrient input and lowering of salinity. *Wetlands* 25:155–161
- Di Tomaso JM (1998) Impact, biology, and ecology of saltcedar (*Tamarix* spp.) in the Southwestern United States. *Weed Tech* 12:326–336
- Drew MC, He CJ, Morgan PW (2000) Programmed cell death and aerenchyma formation in roots. *Trends Plant Sci* 5:123–127
- Evans DE (2004) Aerenchyma formation. *New Phytol* 161:35–49
- Farquhar GD, von Caemmerer S, Berry JA (1980) A biochemical model of photosynthetic CO₂ assimilation in leaves of C₃ species. *Planta* 149:78–90
- Fernandez MD (2006) Changes in photosynthesis and fluorescence in response to flooding in emerged and submerged leaves of *Pouteria orinocoensis*. *Photosynthetica* 44:32–38
- Flowers TJ, Colmer TD (2008) Salinity tolerance in halophytes. *New Phytol* 179:945–963
- Ginzburg C (1967) Organization of the adventitious root apex in *Tamarix aphylla*. *Am J Bot* 54:4–8
- Glenn E, Tanner R, Mendez S, Kehret T, Moore D, Garcia J, Valdes C (1998) Growth rates, salt tolerance characteristics of native and invasive riparian plants from the delta of Colorado River, Mexico. *J Arid Environ* 40:271–294
- Gries D, Zeng F, Arndt SK, Bruelheide H, Thomas FM, Zhang X, Runge M (2003) Growth and water relations of *Tamarix ramosissima* and *Populus euphratica* on Taklamakan desert dunes in relation to depth to a permanent water table. *Plant Cell Environ* 26:725–736
- Gries D, Foetzki A, Arndt SK, Bruelheide H, Thomas FM, Zhang X, Runge M (2005) Production of perennial vegetation in an oasis-desert transition zone NW China—allometric estimation, and assessment of flooding and use effects. *Plant Ecol* 181: 23–43
- Hacke UG, Sperry JS, Wheeler JK, Castro L (2006) Scaling of angiosperm xylem structure with safety and efficiency. *Tree Physiol* 26:689–701
- Hirano T, Kiyota M, Aiga I (1995) Physical effects of dust on leaf physiology of cucumber and kidney bean plants. *Environ Pollut* 89:255–261
- Horton JA (1960) The ecology of saltcedar. *Proc Arizona Watershed Symp* 4:19–21
- Horton JL, Kolb TE, Hart SC (2001) Leaf gas exchange characteristics differ among Sonoran Desert riparian tree species. *Tree Physiol* 21:233–241
- IPCC (2007) Climate change 2007: the physical science basis. Contribution of Working Group I to the fourth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge
- Junghans U, Polle A, Dücking P, Weiler E, Kuhlman B, Gruber F, Teichmann T (2006) Adaptation to high salinity in poplar involves changes in xylem anatomy and auxin physiology. *Plant Cell Environ* 29:1519–1531
- Kleinkopf GE, Wallace A (1974) Physiological basis for salt tolerance in *Tamarix ramosissima*. *Plant Sci Lett* 3:157–163
- Kozłowski TT (1997) Responses of woody plants to flooding and salinity. *Tree Physiol Monogr* 1:1–29
- Long SP, Bernacchi CJ (2003) Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. *J Exp Bot* 54:2393–2401
- Loreto F, Centritto M, Chantzoulakis K (2003) Photosynthetic limitations in olive cultivars with different sensitivity to salt stress. *Plant Cell Environ* 26:595–601
- Lovelock CE, Ball MC (2002) Influence of salinity on photosynthesis of halophytes. In: Läuchli A, Lüttge U (eds) *Salinity: environment, plants, molecules*. Kluwer Academic Publishers, The Netherlands, pp 315–339
- Mansour MMF (2000) Nitrogen containing compounds and adaptation of plants to salinity stress. *Biol Plant* 43:491–500
- Marcar NE, Crawford DF, Saunders A, Matheson AC, Arnold RA (2002) Genetic variation among and within provenances and families of *Eucalyptus grandis* W. Hill and *E. globosus* Labill. subsp. *globosus* seedlings in response to salinity and waterlogging. *Forest Ecol Manag* 162:231–249
- McLeod KW, McCarron JK, Conner WH (1999) Photosynthesis and water relations of four oak species: impact of flooding and salinity. *Trees Struct Funct* 13:178–187
- Merritt DM, LeRoy Poff N (2010) Shifting dominance of riparian *Populus* and *Tamarix* along gradients of flow alteration in western North American rivers. *Ecol Appl* 20:135–152

- Meyer RF, Boyer JS (1972) Sensitivity of cell division and cell elongation to low water potentials in soybean hypocotyls. *Planta* 108:77–87
- Munns R (2002) Comparative physiology of salt and water stress. *Plant Cell Environ* 25:239–250
- Nandy Datta P, Das S, Ghose M, Spooner-Hart R (2007) Effects of salinity on photosynthesis, leaf anatomy, ion accumulation and photosynthetic nitrogen use efficiency in five Indian mangroves. *Wetl Ecol Manag* 15:347–357
- Parida AK, Das AB, Mittra B (2004) Effects of salt on growth, ion accumulation, photosynthesis and leaf anatomy of the mangrove, *Bruguiera parviflora*. *Trees Struct Funct* 18:167–174
- Pezeshki SR, Pardue JH, DeLaune RD (1996) Leaf gas exchange and growth of flood-tolerant and flood-sensitive tree species under low soil redox conditions. *Tree Physiol* 16:453–458
- Rengifo E, Tezara W, Herrera A (2005) Water relations, chlorophyll a fluorescence, and contents of saccharides in tree species of a tropical forest in response to flood. *Photosynthetica* 43:203–210
- Salter J, Morris K, Boon PI (2008) Does salinity reduce the tolerance of two contrasting wetland plants, the submerged monocot, *Vallisneria australis* and the woody shrub *Melaleuca ericifolia*, to wetting and drying? *Mar Freshw Res* 59:291–303
- Salter J, Morris K, Read J, Boon PI (2010) Impact of long-term, saline flooding on condition and reproduction of the clonal wetland tree, *Melaleuca ericifolia* (Myrtaceae). *Plant Ecol* 206:41–57
- Saqib M, Akhtar J, Qureshi RH (2005) Na⁺ exclusion and salt resistance of wheat (*Triticum aestivum*) in saline-waterlogged conditions are improved by the development of adventitious nodal roots and cortical root aerenchyma. *Plant Sci* 169:125–130
- Schmitz N, Jansen S, Verheyden A, Kairo JG, Beeckman H, Koedam N (2007) Comparative anatomy of the intervessel pits in two mangrove species growing along a natural salinity gradient in Gazi Bay, Kenya. *Ann Bot* 100:271–281
- Schweingruber FH, Börner A, Schulze ED (2008) Atlas of woody plant stems: evolution, structure, and environmental modifications. Springer, Heidelberg
- Sobrado MA (2007) Relationship of water transport to anatomical features in the mangrove *Laguncularia racemosa* grown under contrasting salinities. *New Phytol* 173:584–591
- Sprenger MD, Smith LM, Taylor JP (2001) Testing control of saltcedar seedlings using fall flooding. *Wetlands* 21:437–441
- Stromberg JC, Lite SJ, Marler R, Paradzich C, Shafroth PB, Shorrock D, White JM, White MS (2007) Altered stream-flow regimes and invasive plant species: the *Tamarix* case. *Global Ecol Biogeogr* 16:381–393
- Tallent-Halsell NG, Walker LR (2002) Responses of *Salix gooddingii* and *Tamarix ramosissima* to flooding. *Wetlands* 22:776–785
- Ueda A, Kanechi M, Uno Y, Inagaki N (2003) Photosynthetic limitations of a halophyte sea aster (*Aster tripolium* L.) under water stress and NaCl stress. *J Plant Res* 116:65–70
- Vandersande MW, Glenn EP, Walworth JL (2001) Tolerance of five riparian plants from the lower Colorado River to salinity drought and inundation. *J Arid Environ* 49:147–159
- Visser EJW, Heijink CJ, Vanhout KJGM, Voesenek LACJ, Barendse GWM, Blom CWPM (1995) Regulatory role of auxin in adventitious root-formation in 2 species of *Rumex*, differing in their sensitivity to waterlogging. *Physiol Plant* 93:116–122
- Waisel Y (1961) Ecological studies on *Tamarix aphylla* (L.) Karst. III. The salt economy. *Plant Soil* 13:356–363
- Wang CH, Lu M, Yang B, Yang Q, Zhang XD, Hara T, Li B (2010) Effects of environmental gradients on the performances of four dominant plants in a Chinese saltmarsh: implications for plant zonation. *Ecol Res* 25:347–358
- Xu H, Li Y (2006) Water-use strategy of three central Asian desert shrubs and their responses to rain pulse events. *Plant Soil* 28:5–17
- Zhang D, Yin L, Pan B (2002) Biological and ecological characteristics of *Tamarix* L. and its effect on the ecological environment. *Sci China* 45:18–22