DOI: 10.1007/s11099-016-0224-8 PHOTOSYNTHETICA 55 (2): 231-239, 2017

Tolerance of *Mitragyna parvifolia* (Roxb.) Korth. seedlings to NaCl salinity

A. BIDALIA[+], M. HANIEF, and K.S. RAO

Department of Botany, University of Delhi, Delhi – 110007, India

Abstract

Increase in salinity is predicted to affect plant growth and survival in most arid and semiarid regions worldwide. *Mitragyna parvifolia* (Roxb.) Korth. is an important medicinal tree species distributed throughout the semiarid regions of India; however, it is facing a threat of its extinction in its natural habitat. We examined the effects of increasing NaCl salinity on two-month-old *M. parvifolia* seedlings grown in an environment-controlled chamber and exposed to soils of different electrical conductivity (EC) caused by NaCl [0–5 (control), 5–10, 10–15, 15–20, and 20–25 dS m^{-1})] for 85 days. Seedlings transferred to soil of EC >15 dS m^{-1} did not survive beyond 1 week. Increase in the Na^+ concentration negatively correlated with their height and positively correlated with their water-use efficiency (WUE). However, leaf area, net photosynthetic rate (P_N), stomatal conductance, and transpiration rate showed varying correlations and an overall decrease in these parameters compared with the control. At EC of 10–15 dS m^{-1}, the seedling height was reduced by 37% and P_N was lowered by 50% compared with those of the control. An increase in the Na^+/K^+ ratio was observed with increasing salinity. The maximum quantum efficiency of PSII significantly decreased with increasing salinity compared with the control. Our results suggest that the increase in salinity reduced the overall performance of the *M. parvifolia* seedlings. However, the maintenance of WUE and maximum quantum efficiency of PSII might help *M. parvifolia* to tolerate NaCl salinity of 15 dS m^{-1}.

Additional key words: chlorophyll fluorescence; gas exchange; growth analysis; Kadam; long-term salinity; water-use efficiency.

Introduction

Salinity affects 954.8 million ha of land worldwide (Pessarakli and Szabolcs 2011) and is expected to increase in most arid and semiarid regions because of the excessive use of ground water and increased evapotranspiration (Bates *et al*. 2008). Furthermore, these ecosystems are located in areas with predominantly low and erratic rainfall, intense solar radiation, and high wind velocity (Shankarnarayan *et al*. 1987). Such rapidly changing environmental conditions are likely to affect particularly the adaptive potential of trees with long reproductive cycles (Schützendübel and Polle 2002). Three possible fates have been predicted for tree populations: (*1*) migration to other areas (because plants follow favorable ecological niches), (*2*) adaptation, and (*3*) extirpation (Aitken *et al*. 2008). Remarkably, most plants possess an adaptive capacity acquired from the variable environment in which they have developed, and this is only revealed under adverse conditions (Sam *et al*. 2003). However, such a survival mechanism reduces the genetic potential of plants in their natural habitats (Bohnert 2007).

Increasing salinity has deleterious effects on plants due to the disproportionate amounts of salts in the soil, which alters the osmotic potential, pH, and carbonate formation in the soil. These changes directly affect several physiological processes in plants (Garg and Gupta 2011). In addition, plants encounter osmotic stress and ion toxicity (Munns 2002).

Received 8 May 2015, *accepted* 8 March 2016, *published as online-first* 6 April 2016.

[+]Corresponding author; e-mail: bidaliaankita@gmail.com

Abbreviations: Chl – chlorophyll; *E* – transpiration rate; F_o – minimal fluorescence yield of the dark-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_v/F_m – maximal quantum yield of PSII photochemistry; g_s – stomatal conductance; S1 – 0–5 dS m^{-1} NaCl (control); S2 – 5–10 dS m^{-1}; S3 – 10–15 dS m^{-1}; S4 – 15–20 dS m^{-1}; S5 – 20–25 dS m^{-1}; LA – leaf area; P_N – net photosynthetic rate; WUE – water-use efficiency (= P_N/E).

Acknowledgments: We are grateful to Govindjee, Professor Emeritus of Biochemistry, Biophysics and Plant Biology, University of Illinois at Urbana-Champaign, for discussions and reading the earlier draft of our manuscript. We thank Dr. Chirashree Ghosh, Department of Environmental Studies, University of Delhi, for providing access to the growth chamber facility. We also acknowledge the Forest Department of the state of Rajasthan (India) for permission to collect seeds from the forest and the University Grants Commission, India for financial support. The authors would like to thank the anonymous reviewers for their constructive suggestions and Enago (www.enago.com) for the English-language review.

Woody plants are less sensitive during germination but more sensitive at emergence and the seedling stage (Kozlowski 1997) and are then stress tolerant at 4–6 months of age (Niknam and McComb 2000). Increase in salinity noticeably inhibits growth in most plants (Maeda and Nakazawa 2008); however, in *Atriplex triangularis*, low salinity stimulates plant growth (Khan and Ungar 1984). The osmotic effect of salt stress can lead to various physiological and morphological changes, such as cell expansion, cell division, and nutrient balance (Hamamoto *et al.* 2015). Furthermore, salt-stressed plants have low specific leaf area (LA) (Parida *et al.* 2005) and stomatal and mesophyll conductance (Chaves *et al.* 2009), decreased biomass (Parida *et al.* 2005), photosynthetic gas-exchange rate (Long and Baker 1986, Pezeshki and Chambers 1986, Moradi and Ismail 2007), PSII activity (Kalaji *et al.* 2011), and increased WUE (Li *et al.* 2013) and protective enzyme activity (Meloni *et al.* 2003). These plants also show changes in the allocation of mineral distribution (Yang *et al.* 2008).

Mitragyna parvifolia, locally known as Kadam in Hindi, belongs to the family Rubiaceae. It is listed as an endangered species in Rajasthan, India, where the degradation of its habitat and exploitation because of its economic potential are the main reasons for its decline (Panwar and Tarafdar 2006). Middleton (2009) reported a decline in the population of *M. parvifolia* in Keoladeo National Park, Bharatpur, Rajasthan, India. This park is a Ramsar site under the Convention on Wetlands of International Importance and is a United Nations Educational, Scientific, and Cultural Organization world heritage site (Singh *et al.* 2011). This species provides important ecological services in the park, including a habitat for local and migratory birds. The park has a semiarid climate, and in the recent past, frequent droughts and prolonged water scarcity have attributed to increased salinity, which threaten the existence of flora and fauna in the park. In our 2012 field survey of the park, we observed good germination of *M. parvifolia* seeds, but a low rate of seedling survival, and those that survived were mostly under the shade of shrubs and grasses. We also observed a variable distribution of Na^+ concentration ($[Na^+]$) in the park's soil (data not published).

Our observations motivated us to protect the survival and regeneration of this species in its natural habitat. So far, no information regarding the effect of salinity on *M. parvifolia* is available. Here we aimed to examine the tolerance of *M. parvifolia* seedlings to NaCl salinity under controlled conditions and to determine the critical levels of NaCl salinity that enable the seedlings to grow and survive.

Materials and methods

Plant material and growth conditions: Seeds were collected from *M. parvifolia* in Keoladeo National Park (27°7'6"N–27°12'2"N and 77°29'5"E–77°33'9"E) in May 2013 and were grown in a garden at the University of Delhi, India, in July 2013. Two-month-old seedlings were transplanted into 5-L pots containing an autoclaved mixture of sand, garden soil, and organic manure (1:1:4) and were then transferred to a growth chamber. The electrical conductivity of garden soil was 0.07 dS m^{-1}. Each pot was considered as a replicate, and each treatment comprised 15 pots with one seedling each. The average air temperature and relative humidity during the experimental period were 28°C and 82.5%, respectively. On the basis of field observation, the maximum PPFD inside the growth chamber was maintained at 300 μmol(photon) m^{-2} s^{-1} with a 12-h photoperiod. The seedlings were exposed to five different levels of NaCl salinity: S1: 0–5 dS m^{-1} for control treatment with no added NaCl and always watered with solutions of electrical conductivity < 4 dS m^{-1}; S2: 5–10 dS m^{-1}; S3: 10–15 dS m^{-1}; S4: 15–20 dS m^{-1}; and S5: 20–25 dS m^{-1}. All plants were watered with 650 mL of respective stock solution three times per week, and the drainage water was collected and analyzed for electrical conductivity. These values were used to adjust the NaCl salinity of each treatment to the required level for the 85-d experimental period.

Growth: Seedling height was measured each week, and LA was determined at the completion of the experiment by drawing each leaf outline on a graph paper and counting the square grids per leaf.

Biomass: After 85 d of exposure to each treatment, the seedlings were harvested, briefly rinsed with deionized water, oven dried at 60°C to a constant mass, and weighed to determine the dry biomass.

Nutrient analysis: The oven-dried leaves were ground to a fine powder. The powdered leaves (0.05 g) were digested in a nitric acid:perchloric acid (1:5) solution (Allen *et al.* 1974) prior to Na^+ and K^+ concentration analysis using a flame photometer (*Flame Photometer 128*, *Systronics*, India).

Leaf gas exchange and chlorophyll (Chl) *a* fluorescence measurements: Leaf gas exchange was measured weekly throughout the experimental period between 8:00 and 12:30 h. All measurements were conducted on mature and fully expanded leaves. The net photosynthetic rate (P_N), stomatal conductance to water vapor (g_s), and transpiration rate (E) were measured using a 2-cm^2 chamber *Li-Cor 6400XT* with a red–blue light-emitting diode light source (*Li-Cor Inc.*, Lincoln, Nebraska, USA). All these measurements were performed at PPFD of 1,000 μmol(photon) m^{-2} s^{-1}, which was the estimated maximum light efficiency determined by the light-saturated rates of photosynthesis based on a light-response curve at 400 ppm CO_2 (Iwanaga and Yamamoto 2007, Debez *et al.* 2008). Gas exchange was calculated using the equations by von Caemmerer and Farquhar (1981), and WUE was calculated as P_N/E (Houle *et al.* 2001).

Chl *a* fluorescence was measured using a *Li-Cor 6400XT* leaf chamber fluorometer (*Li-Cor* Inc., USA) on the same leaves used for gas-exchange measurements. The leaves were dark-adapted for 30 min, and then, a pulse of far-red light was applied to the leaf sample to assure full oxidation of the primary quinone electron acceptor Q_A. Next, measuring light (630 nm) of very low light intensity [< 1 μmol(photon) m^{-2} s^{-1}] which does not induce any significant electron transfer from PSII, was applied to measure the minimal fluorescence (F_o). The maximal (F_m) fluorescence of the dark-adapted leaf sample was measured by applying a saturating flash of light [630 nm; >7,000 μmol(photon) m^{-2} s^{-1}] that induced maximum fluorescence by PSII. The maximal quantum yield of PSII photochemistry [$F_v/F_m = (F_m - F_o)/F_m$] provided information regarding the photochemical efficiency (Briantais *et al.* 1986, Maxwell and Johnson 2000, Govindjee 2004, Papageorgiou and Govindjee 2004, Baker 2008).

Statistical analyses: A completely random experimental design was used, and the position of the pots was changed every second day. We used one-way analysis of variance (*ANOVA*; $\alpha = 0.05$) to compare the mean values with *Tukey*'s post-hoc test. To determine the linear relationship among the variables, correlations were conducted on the last measurements of the experiment at both $\alpha = 0.05$ and $\alpha = 0.01$ levels of significance. The statistical tests were conducted using *SPSS version 16.0* (*IBM SPSS*, NY, USA).

Results

Effect of NaCl salinity on the morphological parameters: When the growing medium exceeded 15 dS m^{-1}, the seedlings did not survive beyond one week. After 85 d of treatment, the seedling height in S3 was significantly lesser (almost 37%) than that in S1, whereas that in S2 did not significantly differ from those in S1 and S3 (Fig. 1, Table 1). LA was significantly reduced by 45% in S3 and by 21% in S2 compared with 100% in S1 (Fig. 2, Table 1). The total seedling biomass was significantly lower in S3 than in S1, whereas the biomass in S2 did not differ compared with that in S1 or S3 (Fig. 2).

Effect of NaCl salinity on [Na^+] **and** [K^+]: In *M. parvifolia* leaves, [Na^+] increased by 51.8% in S3 and by 23% in S2, (Table 1). In S3, it was significantly higher than that in S1 (Fig 3). However, [K^+] did not significantly differ between the treatments (Fig. 3). The ratio of Na^+/K^+ also increased with S2 (123.1%) and S3 (161.5%) compared with S1 (100%) (Fig. 3, Table 1).

Effect of NaCl salinity on photosynthetic performance: NaCl salinity significantly affected the photosynthetic parameters (Fig. 4). P_N declined by >50% in S3 and >17%

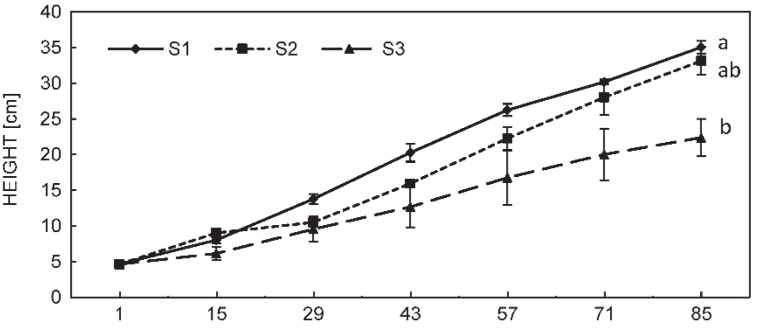

Fig. 1. Seedling height in response to the long-term salinity where each value represents mean replicates ± SE ($n = 5$). Values followed by *different letters* are statistically significant ($\alpha = 0.05$).

Table 1. Difference in seedlings height, leaf area (LA), total biomass, leaf Na^+ and K^+, net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), water-use efficiency (WUE), and maximal quantum efficiency of PSII (F_v/F_m) in the seedlings after 85 days of treatment. S1 – 0–5 dS m^{-1} NaCl (control); S2 – 5–10 dS m^{-1}; S3 – 10–15 dS m^{-1}.

Salinity	Height [%]	LA [%]	Biomass [%]	Na^+ [%]	K^+ [%]	Na^+/K^+ [%]	P_N [%]	g_s [%]	E [%]	WUE [%]	F_v/F_m [%]
S1	100	100	100	100	100	100	100	100	100	100	100
S2	94.51	78.59	94.84	123.70	97.85	123.0	83.37	65.65	66.88	103.76	99.17
S3	63.84	55.89	66.87	151.77	96.14	161.53	49.09	37.85	37.54	124.48	96.20

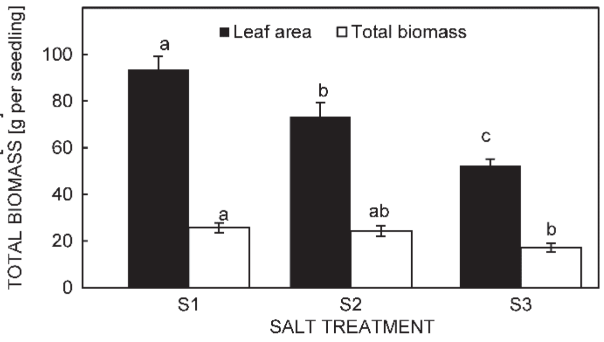

Fig. 2. Leaf area and total biomass after 85 days of salt treatments [S1 = 0–5 dS m^{-1} (control); S2 = 5–10 dS m^{-1}; S3 = 10–15 dS m^{-1}]. Data given are mean ± SE ($n = 5$). Values followed by *different letters* are statistically significant ($\alpha = 0.05$).

in S2, and g_s and E decreased by 60% in S3 and 35% in S2 (Table 1). P_N significantly declined with increasing NaCl salinity (Fig. 4*A*), and g_s and E showed a similar trend ($\alpha = 0.05$), except in S2, which did not differ significantly from that in S1 or S3 (Fig. 4*B*,*C*). WUE was 24.5% higher in S3 than in S1 (Table 1); however, there were no significant differences between the treatments (Fig. 4*D*). F_v/F_m was significantly lower in S3 than in S1 and S2 (Fig. 4*E*).

Correlation analyses: The result of correlation analysis between the variables in S1 (control) showed that [Na$^+$] positively and significantly correlated with [K$^+$], P_N, g_s, and E; and negatively with WUE, whereas height, LA, P_N, g_s, and E positively correlated with each other (Table 2).

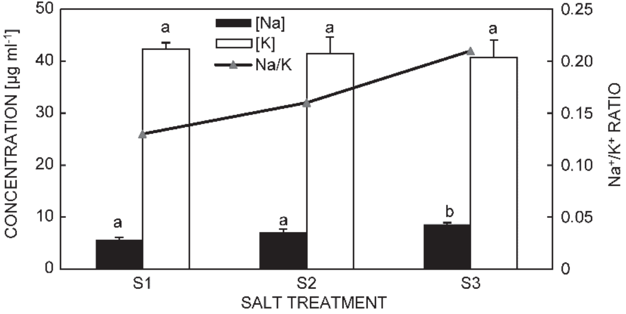

Fig. 3. Leaf Na$^+$ and K$^+$ concentrations and Na$^+$/K$^+$ ratio after 85 days of salt treatments [S1 = 0–5 dS m^{-1} (control); S2 = 5–10 dS m^{-1}; S3 = 10–15 dS m^{-1}]. Data given are mean ± SE ($n = 5$). Values followed by *different letters* are statistically significant ($\alpha = 0.05$).

In S2, correlation analysis showed that an increase in [Na$^+$] negatively and significantly correlated with LA, P_N, g_s, and E, whereas biomass positively correlated with [Na$^+$] and negatively with LA. LA showed positive correlation with the height of the plants. In S3, the results of the correlation analysis showed that with more increase in [Na$^+$] (values less dispersed) most of the parameters, such as LA and biomass, showed a positive correlation, and P_N, g_s, E, and WUE showed a statistically positive correlation. However, height was negatively correlated with all the parameters, including [Na$^+$], LA, biomass, P_N, g_s, E, and WUE in S3 (Table 2).

Discussion

NaCl salinity reduced the growth and survival of *M. parvifolia* seedlings. The parameters used to measure the various physiological mechanisms that respond to the surroundings are widely used to assess stress tolerance in plants (Niknam and McComb 2000, Chelli-Chaabouni *et al.* 2010). Our results showed that *M. parvifolia* seedlings could not survive under NaCl treatments that exceeded 15 dS m^{-1}, indicating ion toxicity. Liu *et al.* (2010) obtained similar results for sunflower seedlings. Visible signs, such as wilting and curling, were observed in *M. parvifolia* seedlings that did not survive above 15 dS m^{-1}. During long-term exposure to NaCl, plants settle down to a reduced state or show visible injuries such as yellowing or death of older leaves (Munns 2002). Reduced growth is linked to (*1*) osmotic stress, (*2*) ion toxicity, and (*3*) nutritional imbalance (Díaz-López *et al.* 2012). It was observed that with the duration of stress, plants decrease its investments into structural components, such as height and LA. At high NaCl concentrations, a significant reduction in height was shown in *M. parvifolia* seedlings (Fig. 1), which agrees with studies supporting a salt-induced decrease in the growth of woody plants, except for a few species. For example, a reduction in growth was observed in *Jatropha curcas* (Díaz-López *et al.* 2012) and *Arbutus unedo* (Navarro *et al.* 2007), whereas an increase was observed in *Suaeda salsa* (Lu *et al.* 2003) and *Plantago coronopus* (Koyro 2006). In our study, it was observed that LA was first affected under NaCl salinity in *M. parvifolia* seedlings. The decrease in LA could be explained by a decrease in leaf epidermal and cross-sectional areas as shown in *Hibiscus cannabinus* (Curtis and Läuchli 2014). LA was negatively and significantly correlated with [Na$^+$] in S2, whereas LA negatively but not significantly correlated with [K$^+$] in S3 (Table 2); this suggests that LA was affected by [K$^+$] in S3. Further, reduction in LA also suggests a decline in the ability of seedlings to use sunlight under salt stress (Sinclair 1984).

Assessing percentage of biomass production in saline and control conditions over a long time period may provide an important criterion for salt tolerance in plants (Munns 2002). A salt-induced reduction in biomass has been reported for many tree species, such as *Solanum quitoense* L. (Ebert *et al.* 1999), *Cordia rothii* (Ramoliya 2003), *Delonix regia* (Patel *et al.* 2009), and *Acacia longifolia* (Morais *et al.* 2012). Our results indicate that the biomass production of *M. parvifolia* decreased by approximately 34% in S3 compared with that in control S1 (Table 1). Total biomass negatively correlated with height and LA in both S2 and S3 (Table 2). These parameters are considered to be directly associated with biomass reduction. However,

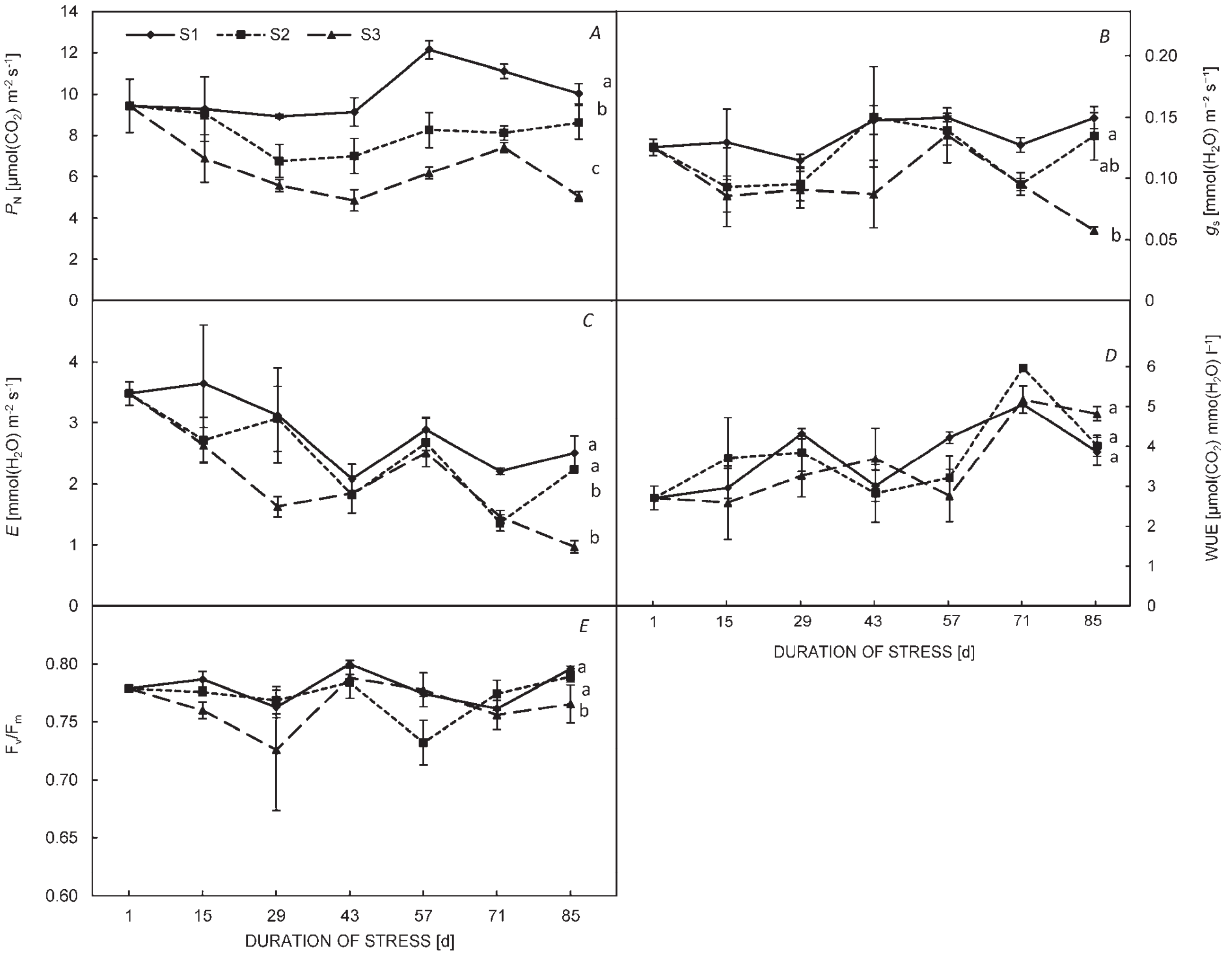

Fig. 4. Effect of salinity on the (*A*) net photosynthetic rate (P_N), (*B*) stomatal conductance (g_s), (*C*) transpiration rate (*E*), (*D*) water-use efficiency (WUE), and (*E*) maximum quantum efficiency of PS II (F_v/F_m) during 85 days (in weeks). Each value represents the mean ± SE (*n* = 4). Values followed by *different letters* are statistically significant (α = 0.05).

a decline in LA, which reduces carbon assimilation, results in decreased plant biomass under high salinity; this is explained by an increased demand for plant metabolism under high salinity in sorghum (Netondo *et al.* 2004).

Under saline conditions, a steep thermodynamic gradient occurs across the plasma membrane in plants, which allows the passive influx of Na$^+$ (Niu *et al.* 1995), thus resulting in a competition between the chemically the similar Na$^+$ and K$^+$ for binding sites in transport systems that mediate K$^+$ uptake (Hasegawa *et al.* 2000, Borsani *et al.* 2003). It is therefore essential for plants to use predominant ions present in the environment for osmotic adjustment and to control cytosolic [Na$^+$] to reduce ion toxicity, which is an important mechanism for salt adaptation (Niu *et al.* 1995). Our results showed that in *M. parvifolia* seedlings, [K$^+$] did not significantly differ, while [Na$^+$] significantly increased in the leaves (Fig. 3), although most studies have reported an increase in [Na$^+$] and a decrease in [K$^+$] under high NaCl concentrations (Vicente *et al.* 2004, Sekmen *et al.* 2012). A low Na$^+$/K$^+$ ratio is considered to be beneficial for plants (Sun *et al.* 2014) to maintain cellular homeostasis in the presence of high salt concentrations and osmolarity (Hamamoto *et al.* 2015). However, the Na$^+$/K$^+$ ratio can be used as an indicator for salt tolerance and should be less than 1 in nonhalophytic plants for optimal protein synthesis (Greenway and Munns 1980, Imada *et al.* 2009). In our study, we found an increase in the Na$^+$/K$^+$ ratio in S2 (123.1%) and S3 (161.5%) (Fig. 3, Table 1). Hence, our results showed that the ability of seedlings to maintain K$^+$ uptake decreased with the increase in NaCl salinity; however, the ability to maintain K$^+$ is often related to salt tolerance in plants (Ebert 1998).

Munns and Termaat (1986) and Cramer *et al.* (1994) have shown that long-term salinity inhibits plant function by limiting carbon supply rather than by limiting leaf

Table 2. Correlation (*Pearson*, r^2) between seedlings height, leaf area (LA), biomass, leaf Na^+ and K^+, net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), water-use efficiency (WUE), and maximal quantum yield of photosystem PSII (F_v/F_m) after 85 days of salt treatment. *, ** – significance at 0.05 and 0.01 level, respectively. S1 – 0–5 dS m^{-1} NaCl (control); S2 – 5–10 dS m^{-1}; S3 – 10–15 dS m^{-1}.

Correlation (S1 and S3)	$[Na^+]$	$[K^+]$	Height	LA	Total biomass	P_N	g_s	E	WUE	F_v/F_m
S1										
$[Na^+]$	1									
$[K^+]$	0.95^{*}	1								
Height	0.12	0.42	1							
LA	0.01	0.32	0.99^{**}	1						
Total biomass	0.69	0.88	0.79	0.72	1					
P_N	0.97^{*}	0.99^{**}	0.34	0.23	0.84	1				
g_s	0.99^{**}	0.97^{*}	0.23	0.12	0.77	0.99^{**}	1			
E	0.99^{**}	0.97^{*}	0.22	0.12	0.77	0.99^{**}	0.99^{**}	1		
WUE	-0.97^{*}	–0.85	0.10	0.21	–0.51	–0.90	–0.94	–0.94	1	
F_v/F_m	–0.47	–0.17	0.81	0.87	0.30	–0.26	–0.36	–0.37	0.65	1
S2										
$[Na^+]$	1									
$[K^+]$	0.12	1								
Height	–0.84	0.42	1							
LA	-0.98^{*}	0.07	0.93	1						
Total biomass	0.99^{**}	0.12	–0.84	-0.97^{*}	1					
P_N	–0.10	-0.99^{**}	–0.43	–0.09	–0.11	1				
g_s	–0.69	–0.80	0.20	0.53	–0.69	0.79	1			
E	–0.66	–0.82	0.17	0.50	–0.67	0.81	0.99^{**}	1		
WUE	0.99^{**}	0.021	–0.89	-0.99^{**}	0.99^{**}	–0.005	–0.61	–0.58	1	
F_v/F_m	0.97^{*}	–0.08	–0.93	-0.99^{**}	0.97^{*}	0.09	–0.53	–0.50	0.99^{**}	1
S3										
$[Na^+]$	1									
$[K^+]$	0.43	1								
Height	–0.86	0.08	1							
LA	0.16	–0.82	–0.63	1						
Total biomass	0.73	0.93	–0.28	–0.55	1					
P_N	0.96^{*}	0.66	–0.69	–0.12	0.89	1				
g_s	0.99^{**}	0.49	–0.82	0.08	0.77	0.97^{*}	1			
E	0.88	0.79	–0.53	–0.30	0.96^{*}	0.98^{*}	0.92	1		
WUE	0.99^{**}	0.55	–0.78	0.02	0.81	0.98^{*}	0.99^{**}	0.94	1	
F_v/F_m	–0.59	-0.98^{*}	0.11	0.69	-0.98^{*}	–0.79	–0.65	–0.89	–0.70	1

expansion. In *M. parvifolia* seedlings, we observed that leaf gas-exchange behavior tended to increase or decrease with time (Fig. 4*A*); this is in agreement with the findings of Musyimi *et al.* (2007), who explained this trend as an increase in vapor pressure deficit and growth behavior in avocado seedlings. A positive correlation between P_N and height indicates that P_N is a growth-limiting factor (Navarro *et al.* 2007). Furthermore, P_N positively correlated with g_s, which in turn correlated with E, and these parameters positively correlated with height in S1 (Table 2). However, with an increase in salinity, the correlation was disrupted in S2 (negative with $[Na^+]$) and S3 (positive with $[Na^+]$ and negative with height) (Table 2), leading to a decrease in the seedling height. The decrease in g_s and E are considered to be an important adaptive mechanism for salt tolerance that reduces water loss in rice plants (Moradi and Ismail 2007). However, a decrease in photosynthesis has been correlated with stomatal inhibition (Fang *et al.* 2006), which disrupts CO_2 influx (Nandy *et al.* 2007). Transpiration plays an essential role in controlling ion accumulation in the aerial parts of plants under saline conditions (Hasegawa *et al.* 2000). We observed no significant increase in WUE (Fig. 4*D*); in addition, $[Na^+]$ positively correlated with WUE in S2 and S3 in contrast to S1 (Table 2). Wu and Zuo (2009) and Díaz-López *et al.* (2012) observed similar results for *Pyrus betulaefolia* and *Jatropha curcas* seedlings, respectively. Our results also suggest that an increase in WUE is a selfregulatory strategy for plants to control water loss, which has been reported in other species such as *Populus euphratica* (Li *et al.* 2013). Ashraf (2001) also observed that high salt tolerance is associated with high WUE in *Brassica carinata*; however, in *Avicennia marina*, WUE remained constant across the salinity gradient (Nguyen *et al*. 2015).

Chl *a* fluorescence measurements on dark-adapted leaves have been used as a rapid tool to detect salt tolerance, and thus, it can indicate stress in plants including barley and wheat (Monneveux *et al.* 1990, Belkhodja *et al.* 1994). This technique has been widely used previously because it is rapid and noninvasive and can provide detailed information on the state of PSII (Papageorgiou and Govindjee 2011, Stirbet and Govindjee 2011, Murchie and Lawson 2013). We observed a significant decrease in F_v/F_m with an increase in NaCl salinity (Fig. 4*E*) although F_v/F_m was within the range for healthy plants (0.75–0.85), suggesting that there was no damage to PSII machinery (Maxwell and Johnson 2000, Percival *et al.* 2003). This may be because plants could maintain PSII photochemistry as an adaptation to high salinity as suggested in *Suaeda salsa* (Lu *et al*. 2003).

Conclusions: In this study, we demonstrated that the survival of *M. parvifolia* seedlings is threatened on exposure to NaCl concentrations exceeding 10–15 dS m^{-1}. In *M. parvifolia* seedlings, no visible symptoms of injury were observed with increasing NaCl salinity, although a decline in plant performance was observed as a decrease in height, biomass, LA, P_N, g_s, and E and as an increase in leaf [Na^+] and Na^+/K^+ ratio. Seedlings were not able to maintain [K^+] with an increase in NaCl salinity. Increase in WUE and maintenance of maximal quantum efficiency of PSII might be strategies by plants for tolerance to NaCl salinity up to 15 dS m^{-1} (S3). However, seedlings acclimatized to the NaCl salinity by decreasing their investments into the structural components, such as height and LA. Tolerance to NaCl salinity by *M. parvifolia* seedlings may contribute to their survival in the Keoladeo National Park. Our findings suggest that other stressors, such as waterlogging, drought, herbivory, and inter- and intraspecific competition between species, could be additional reasons for the decline. Both *in situ* and *ex situ* studies are required to explain the decline of *M. parvifolia* in its natural habitat. Moreover, *M. parvifolia* could be used for afforestation programs in regions with moderate salt conditions. Our findings could also aid land managers and policy planners in formulating strategies for the restoration, plantation, and protection of species across regions with similar environmental conditions.

References

Aitken S.N., Yeaman, S., Holliday J.A. *et al.*: Adaptation, migration or extirpation: climate change outcomes for tree populations. – Evol. Appl. **1**: 95-111, 2008.

Allen S.E., Davison W., Grimshaw H. *et al.*: Chemical Analysis of Ecological Materials. Pp. 46-60. Blackwell Sci. Publ., Oxford 1974.

Ashraf M.: Relationships between growth and gas exchange characteristics in some salt-tolerant amphidiploid *Brassica* species in relation to their diploid parents. – J. Exp. Bot. **45**: 155-163, 2001.

Baker N.R.: Chlorophyll fluorescence: a probe of photosynthesis *in vivo*. – Annu. Rev. Plant Biol. **59**: 89-113, 2008.

Bates B., Kundzewicz Z.W., Wu S. *et al.*: Climate change and water. Pp. 103. Intergovernmental Panel on Climate Change (IPCC), Geneva 2008.

Belkhodja R., Morales F., Abadia A. *et al.*: Chlorophyll fluorescence as a possible tool for salinity tolerance screening in barley (*Hordeum vulgare* L.). – Plant Physiol. **104**: 667-673, 1994.

Bohnert H.J.: Abiotic stress. – In: Hetherington A.M. (ed.): Encyclopedia of Life Sciences. Pp. 1-9. John Wiley & Sons Ltd, London 2007.

Borsani O., Valpuesta V., Botella M.: Developing salt tolerant plants in a new century: a molecular biology approach. – Plant Cell Tiss. Org. **73**: 101-115, 2003.

Briantais J.M., Vernotte C., Krause G.H.: Chlorophyll *a* fluorescence of higher plants: chloroplast and leaves. – In: Govindjee, Amesz J., Fork D.C. (ed.): Light Emission by Plants and Bacteria. Pp. 539-583. Academic Press, Orlando 1986.

Chaves M.M., Flexas J., Pinheiro C.: Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. – Ann. Bot.-London **103**: 551-560, 2009.

Chelli-Chaabouni A., Mosbah A.B., Maalej M. *et al.*: *In vitro* salinity tolerance of two pistachio rootstocks: *Pistacia vera* L. and *P. atlantica* Desf. – Environ. Exp. Bot. **69**: 302-312, 2010.

Cramer G., Alberico G., Schmidt C.: Leaf expansion limits dry matter accumulation of salt-stressed maize. – Funct. Plant Biol. **21**: 663-674, 1994.

Curtis P.S., Läuchli A.: The effect of moderate salt stress on leaf anatomy in *Hibiscus cannabinus* (kenaf) and its relation to leaf area. – Am. J. Bot. **74**: 538-542, 1987.

Debez A., Koyro H., Grignon C. *et al.*: Relationship between the photosynthetic activity and the performance of *Cakile maritima* after long-term salt treatment. – Physiol. Plantarum **133**: 373-385, 2008.

Díaz-López L., Gimeno V., Lidón V. *et al.*: The tolerance of *Jatropha curcas* seedlings to NaCl: An ecophysiological analysis. – Plant Physiol. Bioch. **54**: 34-42, 2012.

Ebert G., Casierra-Posada F., Lüdders P.: Influence of NaCl salinity on growth and mineral uptake of lulo (*Solanum quitoense* L.). – Angew. Bot. **73**: 31-33, 1999.

Ebert G.: Growth, ion uptake and gas exchange of two *Annona* species under salt stress. – J. Appl. Bot. **72**, 61-65, 1998.

Fang S.Z., Song L.Y., Fu X.X.: Effects of NaCl stress on seed germination, leaf gas exchange and seedling growth of *Pteroceltis tatarinowii*. – J. Forest. Res. **17**: 185-188, 2006.

Garg B.K., Gupta I.C.: Salinity Tolerance in Plants: Methods, Mechanisms and Management. Pp. 159-196**.** Sci. Publ., Jodhpur, 2011.

Govindjee.: Chlorophyll *a* fluorescence: A bit of basics and history. – In: Papageorgiou G.C., Govindjee (ed.): Chlorophyll *a* Fluorescence: A Signature of Photosynthesis. Pp. 2-42.

Springer, Dordrecht 2004.

Greenway H., Munns R.: Mechanisms of salt tolerance in nonhalophytes. – Annu. Rev. Plant Physio. **31**: 149-190, 1980.

Hamamoto S., Horie T., Hauser F. *et al*.: HKT transporters mediate salt stress resistance in plants: from structure and function to the field. – Curr. Opin. Chem. Biol. **32**: 113-120, 2015.

Hasegawa P.M., Bressan R.A., Zhu J.K. *et al*.: Plant cellular and molecular responses to high salinity. – Annu. Rev. Plant Physiol. **51**: 463-499, 2000.

Houle G., Morel L., Reynolds C.E. *et al*.: Effect of salinity on different developmental stages of an endemic annual plant *Aster laurentianus* (Asteraceae). – Am. J. Bot. **88**: 62-67, 2001.

Imada S., Yamanaka N., Tamai S.: Effects of salinity on the growth, Na partitioning, and Na dynamics of a salt-tolerant tree, *Populus alba* L. – J. Arid Environ. **73**: 245-251, 2009

Iwanaga F., Yamamoto F.: Growth, morphology and photosynthetic activity in flooded *Alnus japonica* seedlings. – J. Forest. Res. **12**: 243-246, 2007.

Kalaji H.M., Govindjee., Bosa K. *et al*.: Effects of salt stress on photosystem II efficiency and CO_2 assimilation of two Syrian barley landraces. – Environ. Exp. Bot. **73**: 64-72, 2011.

Khan M.A., Ungar I.A.: The effect of salinity and temperature on the germination of polymorphic seeds and growth of *Atriplex triangularis* Willd. – Am. J. Bot. **71**: 481-489, 1984.

Koyro H.W.: Effect of salinity on growth, photosynthesis, water relations and solute composition of the potential cash crop halophyte *Plantago coronopus* (L.). – Environ. Exp. Bot. **56**: 136-146, 2006.

Kozlowski T.T.: Responses of woody plants to flooding and salinity. – Tree Physiol. Monogr. **1**: 1-29, 1997.

Li J., Zhao C., Li J. *et al*.: Growth and leaf gas exchange in *Populus euphratica* across soil water and salinity gradients. – Photosynthetica **51**: 321-329, 2013.

Liu J., Guo W.Q., Shi D.C.: Seed germination, seedling survival, and physiological response of sunflowers under saline and alkaline conditions. – Photosynthetica **48**: 278-286, 2010.

Long S., Baker N.: Saline terrestrial environments. – In: Baker N, Long S. (ed.): Photosynthesis in Contrasting Environments. Pp. 63-102, Elsevier, New York 1986.

Lu C., Qiu N., Wang B. *et al*.: Salinity treatment shows no effects on photosystem II photochemistry, but increases the resistance of photosystem II to heat stress in halophyte *Suaeda salsa*. – J. Exp. Bot. **54**: 851-860, 2003.

Maeda Y., Nakazawa R.: Effects of the timing of calcium application on the alleviation of salt stress in the maize, tall fescue, and reed canary grass seedlings. – Biol. Plantarum **52**: 153-156, 2008.

Maxwell K., Johnson G.N.: Chlorophyll fluorescence – a practical guide. – J. Exp. Bot. **51**: 659-668, 2000.

Meloni D.A., Oliva M.A., Martinez C.A. *et al*.: Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. – Environ. Exp. Bot. **49**: 69-76, 2003.

Middleton B.A.: Vegetation status of the Keoladeo National Park, Bharatpur, Rajasthan, India (April 2009). US Geological Survey Science Investigation Report, 5193, 2009.

Monneveux P., Mekkaoui M., Xu X.: Physiological basis of salt tolerance in wheat. Chlorophyll fluorescence as a new tool for screening tolerant genotypes. – Wheat Breeding Prospects and Future Approaches Conference Proceedings. Pp. 1-33. Toshevo 1990.

Moradi F., Ismail A.M.: Responses of photosynthesis, chlorophyll fluorescence and ROS-scavenging systems to salt stress during seedling and reproductive stages in rice. – Ann. Bot.-London **99**: 1161-1173, 2007.

Morais M.C., Panuccio M.R., Muscolo A. *et al*.: Salt tolerance traits increase the invasive success of *Acacia longifolia* in Portuguese coastal dunes. – Plant Physiol. Bioch. **55**: 60-65, 2012.

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

Munns R.: Comparative physiology of salt and water stress. – Plant Cell Environ. **25**: 239-250, 2002.

Murchie E.H., Lawson T.: Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. – J. Exp. Bot. **64**: 3983-3998, 2013.

Musyimi D., Netondo G., Ouma G.: Effects of salinity on growth and photosynthesis of avocado seedlings. – Int. J. Bot. **3**: 78-84, 2007.

Nandy P., Das S., Ghose M. *et al.*: Effects of salinity on photosynthesis, leaf anatomy, ion accumulation and photosynthetic nitrogen use efficiency in five Indian mangroves. – Wetl. Ecol. Manage. **15**: 347-357, 2007.

Navarro A., Bañon S., Olmos E. *et al.*: Effects of sodium chloride on water potential components, hydraulic conductivity, gas exchange and leaf ultrastructure of *Arbutus unedo* plants. – Plant Sci. **172**: 473-480, 2007.

Netondo G.W., Onyango J.C., Beck E.: Sorghum and salinity. – Crop Sci. **44**: 797- 805, 2004.

Nguyen H.T., Stanton D.E., Schmitz N. *et al*.: Growth responses of the mangrove *Avicennia marina* to salinity: development and function of shoot hydraulic systems require saline conditions. – Ann. Bot.-London **115**: 397-407, 2015.

Niknam S.R., McComb J.: Salt tolerance screening of selected Australian woody species – a review. – Forest Ecol. Manage. **139**: 1-19, 2000.

Niu X., Bressan R.A., Hasegawa P.M. *et al*.: Ion homeostasis in NaCl stress environments. – Plant Physiol. **109**: 735-742, 1995.

Panwar J., Tarafdar J.C.: Arbuscular mycorrhizal fungal dynamics under *Mitragyna parvifolia* (Roxb.) Korth. in Thar Desert. – Appl. Soil Ecol. **34**: 200-208, 2006.

Papageorgiou G.C., Govindjee.: Chlorophyll *a* Fluorescence: A Signature of Photosynthesis. Pp. 818. Springer, Dordrecht 2004.

Papageorgiou G.C., Govindjee.: Photosystem II fluorescence: Slow changes – Scaling from the past. – J. Photoch. Photobio. B. **104**: 258-270, 2011.

Parida A.K., Das A.B.: Salt tolerance and salinity effects on plants: a review. – Ecotoxicol. Environ. Safe. **60**: 324-349, 2005.

Patel A.D., Bhensdadia H., Pandey A.N.: Effect of salinisation of soil on growth, water status and general nutrient accumulation in seedlings of *Delonix regia* (Fabaceae). – Acta. Ecol. Sin. **29**: 109-115, 2009.

Percival G.C., Fraser G.A., Oxenham G.: Foliar salt tolerance of *Acer* genotypes using chlorophyll fluorescence. – J. Arboric. **29**: 61-65, 2003.

Pessarakli M., Szabolcs I.: Soil salinity and sodicity as particular plant/crop stress factors. – In: Pessarakli M (ed.): Handbook of Plant and Crop Stress. Pp. 3-21. CRC Press, Boca Raton 2011.

Pezeshki S., Chambers J.: Effect of soil salinity on stomatal conductance and photosynthesis of green ash (*Fraxinus pennsylvanica*). – Can. J. Forest Res. **16**: 569-573, 1986.

Ramoliya P.J., Pandey A.N.: Effect of salinization of soil on emergence, growth and survival of seedlings of *Cordia rothii*. – Forest Ecol. Manage. **176**: 185-194, 2003.

Sam O., Ramírez C., Coronado M. J. *et al.*: Changes in tomato leaves induced by NaCl stress: leaf organization and cell ultrastructure. – Biol. Plantarum **47**: 361-366, 2003.

Schützendübel A., Polle A.: Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. – J. Exp. Bot. **53**: 1351-1365, 2002.

Sekmen A.H., Turkan I., Tanyolac Z.O. *et al.*: Different anti-oxidant defense responses to salt stress during germination and vegetative stages of endemic halophyte *Gypsophila oblanceolata* Bark. – Environ. Exp. Bot. **77**: 63-76, 2012.

Shankarnarayan K., Harsh L., Kathju S.: Agroforestry in the arid zones of India. – Agroforest. Syst. **5**: 69-88, 1987.

Sinclair T.: Leaf area development in field-grown soybeans. – Agron. J. **76**: 141- 146, 1984.

Singh R., Agarwal R., Tiwari A.: Ecophysiological observations on Keoladeo National Park, Bharatpur (India). – J. Wetland. Ecol. **4**: 43-68, 2011.

Stirbet A., Govindjee.: On the relation between the Kautsky effect (chlorophyll *a* fluorescence induction) and Photosystem II: Basics and applications of the OJIP fluorescence transient. – J. Photoch. Photobio. B. **104**: 236-257, 2011.

Sun J., Zou D.T., Luan F.S. *et al.*: Dynamic QTL analysis of the Na^+ content, K^+ content, and Na^+/K^+ ratio in rice roots during the field growth under salt stress. – Biol. Plantarum **58**: 689-696, 2014.

Vicente O., Boscaiu M., Naranjo M.Á.: Responses to salt stress in the halophyte *Plantago crassifolia* (Plantaginaceae). – J. Arid Environ. **58**: 463-481, 2004.

von Caemmerer S., Farquhar G.D.: Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. – Planta **153**: 376-387, 1981.

Wu Q., Zou Y.: Adaptive responses of birch-leaved pear (*Pyrus betulaefolia*) seedlings to salinity stress. – Not. Bot. Horti. Agrobo. **37**: 133-138, 2009.

Yang C., Jianaer A., Li C. *et al.*: Comparison of the effects of salt-stress and alkali-stress on photosynthesis and energy storage of an alkali-resistant halophyte *Chloris virgata*. – Photosynthetica **46**: 273-278, 2008.