PHOTOSYNTHETICA
International Journal for Photosynthesis Research

DOI 10.32615/ps.2021.007 PHOTOSYNTHETICA 59 (1): 171-184, 2021

Physiological changes of three woody plants exposed to progressive salt stress

Y. LU[*,**,***,+], F.J. ZENG[*,**,***,+], X.Y. LI[*,**,***], and B. ZHANG[*,**,***]

Xinjiang Key Laboratory of Desert Plant Roots Ecology and Vegetation Restoration, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, 830011 Urumqi, China[*]
State Key Laboratory of Desert and Oasis Ecology, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, 830011 Urumqi, China[**]
Cele National Station of Observation and Research for Desert-Grassland Ecosystems, 848300 Cele, China[***]

Abstract

The saline character of water imposes restrictions on plant growth and survival in the Taklamakan Desert, China. Experiments were conducted on woody plant species, *Tamarix ramosissima*, *Populus euphratica*, and *Haloxylon ammodendron* grown under different levels of salinity to elucidate their adaptation to a saline environment. *H. ammodendron* accumulated large amounts of Na^+ and Cl^- in leaves. *P. euphratica* restricted redundant Na^+ and Cl^- transport to the shoots treated with lower NaCl concentrations. Na^+ in leaves of *T. ramosissima* accumulated significantly only under higher NaCl concentrations (≥ 200 mM NaCl). The analyzed plants' exposure to high saline concentrations induced oxidative stress as evidenced by the increase of H_2O_2 and malondialdehyde and changes in photosynthetic pigments, gas-exchange characteristics, and antioxidant enzyme activities. *T. ramosissima* and *H. ammodendron* exhibited a greater ability to adapt to saline-induced oxidative stress due to more efficient antioxidant enzyme system to prevent oxidative damage.

Keywords: antioxidative defense; osmotic adjustment; photosynthesis; water relations.

Introduction

Soil salinization is a major international concern and more than 6% of the world's land suffers from salinity (FAO and ITPS 2015). In China, nearly 35 million hectares have been saline (Pan *et al*. 2013). Soil salinity problem is a major problem for ecology and agriculture worldwide, particularly in arid and semiarid regions, where soil water availability is low, evaporation is high, and precipitation is deficient in terms of salt leaching (Cui *et al.* 2011). Soil degradation resulting from various types of salinity is a major environmental problem, especially in arid and semiarid regions (Min *et al.* 2019). Plants are very sensitive to abiotic stresses, including salinity (Sehar *et al.*

Highlights

- Changes in photosynthetic parameters differed between species exposed to salinity
- The higher oxidative damage was reported in *Populus euphratica*
- More effective ROS scavenging was observed in *Haloxylon ammodendron* and *Tamarix ramosissima*

Received 5 November 2020
Accepted 20 January 2021

[+]Corresponding author
e-mail: zengfj@ms.xjb.ac.cn (F.J. Zeng)
luyanu@126.com (Y. Lu)

Abbreviations: APX – ascorbate peroxidase; CAT – catalase; Car – carotenoids; Chl – chlorophyll; C_i – intercellular CO_2 concentration; DM – dry mass; E – transpiration rate; FM – fresh mass; g_s – stomatal conductance; MDA – malondialdehyde content; P_N – net photosynthetic rate; POD – guaiacol peroxidase; ROS – reactive oxygen species; RWC – relative water content; SOD – superoxide dismutase; WUE – water-use efficiency; ψ_w – water potential.

Acknowledgments: This work was financially supported by Poverty Alleviation Program of Chinese Academy of Sciences (KFJ-FP-201903), National Natural Science Foundation of China (41977050), and Key Program of Joint Funds of the National Natural Science Foundation of China and the Government of Xinjiang Uygur Autonomous Region of China (U1603233, U1903102).

Conflict of interest: The authors declare that they have no conflict of interest.

2019, Rajhi *et al*. 2020). Therefore, in order to meet the demand for rehabilitation of degraded arid lands, a detailed understanding of the mechanisms employed by plants in adaptation to salt stress is of a high priority for land utilization.

Salinity influences almost every aspect of physiology and biochemistry of plants, and therefore significantly limits plant growth and productivity. Plants cope with salinity in two ways; first, additional accumulation of Na^+ in root results in osmotic stress, which reduces water potential, and secondly damage to nutrient balance leads to ionic stress (Munns 2005). Na^+ being a nonessential mineral nutrient for most of the plants gets accumulated in plant tissues under saline environment, therefore, changes in ratios of Na^+/Ca^{2+}, Na^+/K^+, Na^+/Mg^{2+}, and Cl^-/NO^{3-}, thus affect plant growth and productivity (Singh *et al*. 2014, Sofy *et al*. 2020). Furthermore, excessive salt accumulation changes electron transport systems occurring in the chloroplast, mitochondria, and plasma membranes resulting in the formation of reactive oxygen species (ROS) as byproducts of oxygen metabolism (Singh *et al*. 2015). Especially, salinity-evoked inhibition of the photosynthetic electron transport causes overaccumulation of toxic ROS, such as superoxide radical ($O_2^{\bullet-}$), hydroxyl radical ($^{\bullet}HO$), and hydrogen peroxide (H_2O_2). Excessive accumulation of ROS induces degradation of chlorophyll (Chl) and decreases photochemical efficiency of PSII developing a vicious cycle (Allakhverdiev *et al*. 2008). ROS is also able to promote oxidative damage to many cellular constituents, such as membrane lipids, proteins, and nucleic acids (Mittler 2002, Gechev and Petrov 2020). One of the most negative effects of oxidative damage is the peroxidation of membrane lipids, which results in the accompanying production of malondialdehyde (MDA) (Lu *et al*. 2017, López-Serrano *et al*. 2020). In order to regulate the content of ROS, plants employ a well-formed and complicated antioxidant defense system including enzymatic and nonenzymatic antioxidant processes (Blokhina *et al*. 2003, Asrar *et al*. 2020). The antioxidant enzymes involve superoxide dismutases (SOD, EC 1.15.1.1), catalases (CAT, EC 1.11.1.6), guaiacol peroxidase (POD, EC 1.11.1.7), and ascorbate peroxidase (APX, EC 1.11.1.11), which can clean and/or neutralize ROS (Blokhina *et al*. 2003, Rattan *et al*. 2020).

The Taklamakan Desert in Northwest China has one of the world's most extreme environmental habitats for plants. The natural vegetation is distinguished by sparse coverage, low diversity, and dominated by perennial plant species (Arndt *et al.* 2004). The available water resource is groundwater that can reach the surface and be recharged by snowmelt water from the Tian-Shan and Kunlun ranges (Shi *et al.* 1989). All surficial waters in the Taklamakan have high pH and are rich in sulfate and sodium chloride (Gibert *et al.* 1996). Hence, it is an adverse environment in which merely a few perennial plant species can settle and grow (Arndt *et al.* 2004). Accordingly, the physiology of the vegetation that allows thriving under extremely hostile conditions should be studied. *Tamarix ramosissima* and *Populus euphratica*, two perennial woody species, are native to the Cele river oases at the extremely arid south rim of the Taklamakan Desert. In recent years, *Haloxylon ammodendron*, a perennial woody species, is introduced in the south rim of the Taklamakan Desert as an excellent economic sand-fixing species, owing to its high endurance to saline-alkaline and drought (Fan *et al.* 2018) and being taken as the host of medicinal plant *Cistanche*. In this study, we analyzed the effects of progressively increased salinity exposure on *H. ammodendron*, *T. ramosissima*, and *P. euphratica*. This study aimed to (*1*) determine ion concentrations (Na^+, K^+, Ca^{2+}, Mg^{2+}, and Cl^-) in leaves, stems, and roots of the three species; (*2*) compare the distribution of ion concentrations among the three species; (*3*) evaluate the stress caused by salinity and the capacity of endurance and detoxification employed by measuring photosynthetic pigments, gas-exchange characteristics, water status, compatible solutes, membrane injury, and enzymatic antioxidant system; and (*4*) elucidate the relationships among all tested physiological and biochemical parameters and evaluate which parameters can be used as valuable screening criteria for the improvement and selection of salt tolerance. Consequently, the results from this study could not only help estimate the suitability of the three species for revegetation of salt-influenced soils but also for choosing the suitable physiological and biochemical parameters as screening criteria.

Materials and methods

Plant materials and experimental treatments: A pot experiment was conducted from 15 April to 25 August 2011 at the site of the Cele Research Station of the Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, located in the Cele Oasis (80°03'24"–82°10'34"E; 35°17'55"–39°30'00"N; 1340–1380 m a.s.l.). During the experiment, the precipitation was only 5.6 mm, the temperature ranged from 10 to 40°C by day and 5 to 20°C by night, and the relative humidity (RH) varied from 20 to 60%, maximum values of PAR rarely exceeded 1,600 μmol(photon) m^{-2} s^{-1} (Zeng *et al*. 2006). *T. ramosissima* cut seedlings, one-year-old *H. ammodendron*, and *P. euphratica* seedlings, with an initial height of 25, 35, and 55 cm, respectively for each plant species, were planted on 15 April into plastic pots (diameter of 50 cm, height of 42 cm) filled with 30 kg of soil from a desert–oasis ecotone (sampling depth of 0–30 cm) and previously passed through a sieve of 2 mm. Soil characteristics were shown by Lu *et al.* (2017). In order to prevent plants from being hurt by extremely high temperatures, all of the pots were embedded underground.

Forty days after transplanting, salinity growth environments were simulated by adding NaCl to deionized water at five concentrations (0, 50, 100, 200, and 400 mM). To avoid saline shock, the NaCl was applied stepwise in aliquots of 50 mM daily till the final concentrations were accomplished. After 90 d, plants were ready for analysis.

Ion concentration: Four individual plants for each treatment were cleaned in deionized water, and rinsed three times with deionized water. After washing, plants were separated into leaves, stems, and roots, and dried in an oven at 70°C for 72 h. Then the dried materials were ashed in a muffle furnace at 550°C for 24 h. The ash (0.1 g) was digested overnight with 25 ml of 0.1 M HNO_3 (Sibole *et al*. 2003). Concentrations of Na^+, K^+, Ca^{2+}, and Mg^{2+} in the acid extract were measured by an inductively coupled plasma-optical emission spectrometer (ICP-OES, *Agilent 735*, Santa Clara, CA, USA), and the Cl^- content was detected using the same extract by a chloride meter (*Jenway PC LM3*, London, UK).

Photosynthetic pigment and gas-exchange characteristics: Photosynthetic pigments were extracted by 80% acetone. The clear supernatant fraction obtained after centrifugation at 480 × *g* for 3 min was employed for determination, using the extinction coefficients and equations redetermined by Lichtenthaler (1987).

Net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were measured by a portable photosynthesis system (*LI-6400*, *LI-COR*, Lincoln, NE, USA) between 10:30 and 11:00 h. Four plants were chosen for each treatment under the following atmospheric conditions: PAR of 1,279 ± 57 μmol(photon) m^{-2} s^{-1}, CO_2 concentration of 384. 5 ± 4.7 μmol mol^{-1}, temperature of 29.6 ± 1.3°C, RH of 33.1 ± 1.2%. Water-use efficiency (WUE) was calculated as the ratio of P_N to E. Due to the irregular shape of *T. ramosissima* leaves, after photosynthetic measurement, the irregular leaves in the chamber were removed and carefully spread and scanned, the scanned images were processed, and the surface area of leaves calculated using the image analysis system *Delta-T Scan* (Cambridge, UK), and gas-exchange parameters were recalculated according to the actual effective photosynthetic area.

RWC and ψ_w: RWC was calculated according to Smart and Bingham (1974) and was obtained by the following formula: RWC = (FM – DM)/(TM – DM) × 100%. Turgid mass (TM) was measured after floating leaves in distilled water for 4 h at room temperature in darkness. Leaf ψ_w was detected on freshly cut leaves using a *WP4* dewpoint water potential meter (*Decagon Devices*, *Inc.*, Pullman, WA, USA).

Total soluble sugars and proline contents: The content of soluble sugars was determined by the anthrone method (Palma *et al*. 2009). The proline content was measured according to the method of Bates *et al*. (1973) using a spectrophotometer (*UV752 N*, *Shanghai Precision & Scientific Instrument Co.*, *Ltd.*, China).

MDA and H_2O_2 content: MDA content was measured based on the method of Kosugi and Kikugawa (1985). H_2O_2 concentration was determined according to the method of Sergiev *et al*. (1997) using a spectrophotometer (*UV752 N*, *Shanghai Precision & Scientific Instrument Co.*, *Ltd.*, China).

Enzyme extraction and assays: The supernatant fraction adopted for determining soluble protein content and SOD, POD, CAT, and APX activities was acquired by the method of Lu *et al*. (2010). Enzyme activities were measured using a spectrophotometer (*UV752 N*, *Shanghai Precision & Scientific Instrument Co.*, *Ltd.*, China). SOD (EC 1.15.1.1) activity was measured according to the method of Beauchamp and Fridovich (1971). CAT (EC 1.11.1.6) activity was assayed by detecting the consumption of H_2O_2 by the method of Aebi (1984). POD (EC 1.11.1.7) activity was obtained based on the method of Chance and Maehly (1955). APX (EC 1.11.1.11) activity was measured by the method of Nakano and Asada (1981).

Proteins were measured according to the method of Bradford (1976) taking bovine serum albumin as the standard protein.

Statistical analysis: Four independent experiments were carried out to evaluate the effects of NaCl treatments on selected physiological and biochemical indexes. Data presented here are means ± SD. Mean values were separated using *Tukey*'s tests at the 0.05 probability level. A linear regression analysis was employed to analyze the relationship between NaCl content and detected parameters at different salinity levels. The analysis was performed with *SPSS Inc.*, *version 13* (Chicago, USA). To acquire a multivariable view of tested parameters and the three species at all salinity concentrations, and also the interrelationships between tested parameters, the mean values of salt-tolerance coefficient of selected physiological and biochemical parameters for the three species and four salinity concentrations were exposed to principal component analysis (PCA). The salt-tolerance coefficient was calculated using the following formula: salt-tolerance coefficient = (mean value under different salinity concentrations/mean value under control) × 100%. The PCA was carried out according to correlation matrix data.

Results

Ion concentrations: In general, as the salinity concentration elevated, the content of toxic ions (Na^+ and Cl^-) also increased in the three species. Toxic ions in leaves of *H. ammodendron* and *P. euphratica* increased more dramatically compared to *T. ramosissima* (Fig. 1). Na^+ in leaves of *H. ammodendron* and *P. euphratica* was elevated by 56.2 and 183.9% at the highest NaCl treatment compared to control, however, it increased only 29.2% in leaves of *T. ramosissima* (Fig. 1*A–C*). Similarly, Cl^- in leaves of *H. ammodendron* and *P. euphratica* increased by 140.1 and 78.1% at the highest NaCl treatment compared to control, respectively, however, it was elevated only by 30.4% in leaves of *T. ramosissima* (Fig. 1*M–O*). Na^+ and Cl^- accumulated most in leaves of the three species, especially for *H. ammodendron*, in which Na^+ and Cl^- concentrations in leaves were 8 and 4 times higher than that in roots under 400 mM NaCl treatment. None remarkable effects of NaCl were observed for Ca^{2+} and Mg^{2+} concentrations (Fig. 1*G–L*), however, K^+ contents prominently increased in roots of *T. ramosissima* and significantly decreased in roots of *P. euphratica* (Fig. 1*E*,*F*).

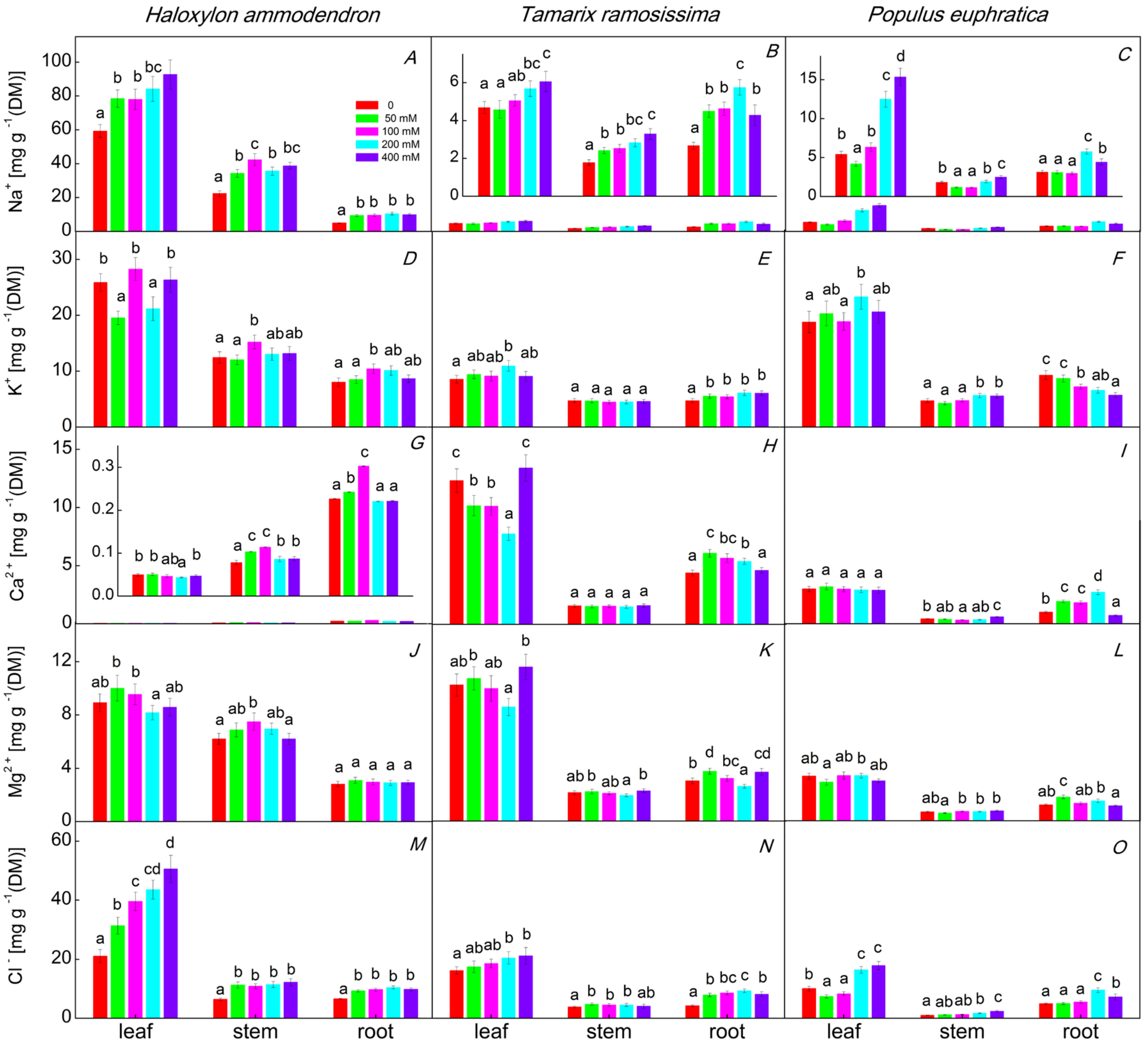

Fig. 1. Effects of NaCl exposure (0, 50, 100, 200, and 400 mM) on Na^+ (*A*–), K^+ (*D*–*F*), Ca^{2+} (*G*–*I*), Mg^{2+} (*J*–*L*), and Cl^- (*M*–*O*) contents in leaves, stems, and roots of *Haloxylon ammodendron*, *Tamarix ramosissima*, and *Populus euphratica*. Values are means ± SD ($n = 4$). *Different lowercase letters* indicate significant differences of ion contents in the same organ across the range of NaCl concentrations at $p<0.05$ according to *Fisher*'s LSD test.

Na^+/Ca^{2+}, Na^+/K^+, and Na^+/Mg^{2+} ratios are often used to represent the degree of salt injury in plants. In general, the ratios in leaves, stems, and roots of the three species increased with increasing NaCl concentrations, and the ratios were substantially greater in *H. ammodendron* than that in *T. ramosissima* and *P. euphratica* (Fig. 1S, *supplement*).

Photosynthetic pigments: Chl *a*, *b*, (*a*+*b*), and carotenoid (Car) content reached the peak value in leaves of the three species under 50 mM NaCl treatment and gradually declined with the increasing NaCl concentration (Fig. 2). The significant decrease of Chl *a*, *b*, (*a*+*b*), and Car were detected at 200 mM NaCl in leaves of *H. ammodendron* (Fig. 2*A*,*D*,*G*,*J*), the significant reduction in Chl *b* and Car were measured at 200 mM NaCl in leaves of *T. ramosissima* (Fig. 2*E*,*K*); for Chl *a* and Chl (*a*+*b*), significant reductions were observed at 400 mM NaCl (Fig. 2*B*,*H*), however, the marked decrease of Chl *a*, *b*, (*a*+*b*), and Car was measured at 100 mM NaCl in leaves of *P. euphratica* (Fig. 2*C*,*F*,*I*,*L*). Chl (*a*+*b*) content in leaves of *H. ammodendron*, *T. ramosissima*, and *P. euphratica* was reduced by 27.4, 8.7, and 62.1% at the highest NaCl treatment, respectively, and the Car content decreased by 35.1, 18.3, and 74.9%, respectively. The Chl *a*/*b* ratio in leaves of *T. ramosissima* was not influenced by NaCl addition (Fig. 2*N*); it was reduced in leaves of *H. ammodendron* at higher NaCl treatment (Fig. 2*M*), however, it increased in leaves of *P. euphratica* at higher NaCl treatment (Fig. 2*O*). The Car/Chl ratio declined with elevated NaCl concentrations, a significant decrease in leaves of *H. ammodendron*, *T. ramosissima*, and *P. euphratica* was observed at 400, 200, and 100 mM NaCl, respectively (Fig. 2*P*–*R*).

Gas-exchange characteristics: The three species treated

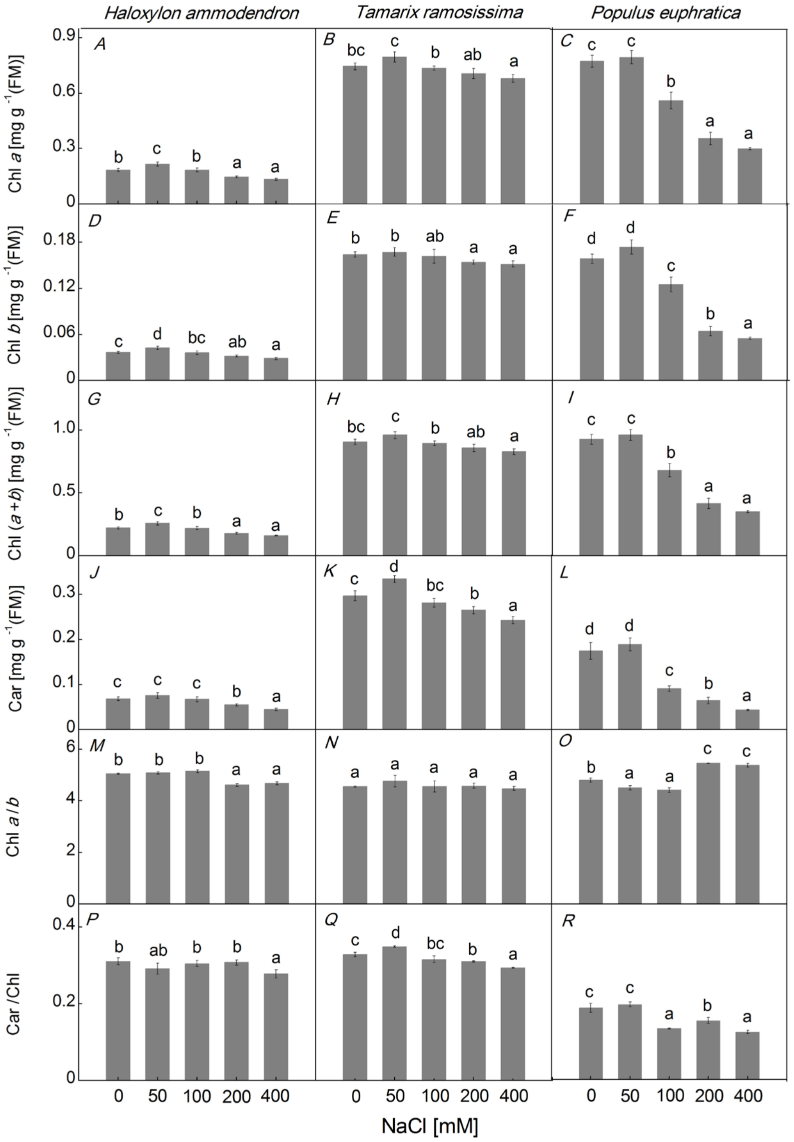

Fig. 2. Effects of NaCl exposure (0, 50, 100, 200, and 400 mM) on Chl *a* (*A–C*), Chl *b* (*D–F*), Chl (*a*+*b*) (*G–I*), Car (*J–L*), Chl *a*/*b* (*M–O*), and Chl/Car (*P–R*) in leaves of *Haloxylon ammodendron*, *Tamarix ramosissima*, and *Populus euphratica*. Values are means ± SD (*n* = 4). *Different lowercase letters* indicate significant differences at $p<0.05$ according to *Fisher*'s LSD test.

with 50 mM NaCl reached maximal values for P_N, E, and g_s (Fig. 3). However, increasing salinity stress (≥ 100 mM NaCl) resulted in a progressive decrease in P_N, E, and g_s compared with the control. The significant reduction in leaves of *H. ammodendron* and *T. ramosissima* was detected at 200 mM NaCl addition (Fig. 3*A*,*B*,*D*,*E*,*G*,*H*), while the significant reduction in leaves of *P. euphratica* was detected at 100 mM NaCl addition (Fig. 3*C*,*F*,*I*). C_i in leaves of *H. ammodendron* and *P. euphratica* increased with increasing NaCl concentration (Fig. 3*J*,*L*), however, it was not affected initially in leaves of *T. ramosissima*, while a significant increase was observed at 400 mM NaCl (Fig. 3*K*). Under higher NaCl treatment (≥ 100 mM NaCl), WUE in leaves of three species was promoted (Fig. 3*M*,*N*,*O*).

RWC and ψ_w: RWC in leaves of the three species was not affected under 50 mM NaCl concentration (Fig. 4*A–C*). Then it increased significantly in leaves of *H. ammodendron* with increasing NaCl concentration (≥ 100 mM NaCl) (Fig. 4*A*). In leaves of *T. ramosissima*, it decreased progressively with increasing NaCl exposure (Fig. 4*B*), and it initially decreased, and then it increased but still maintained at control level in leaves of *P. euphratica* (Fig. 4*C*).

Leaf ψ_w of the three species was remarkably affected by NaCl exposure, decreasing significantly with increasing NaCl concentration ($R_{H.\ ammodendron} = -0.992$; $R_{T.\ ramosissima} = -0.984$; $R_{P.\ euphratica} = -0.979$, $P<0.01$), it was reduced by 18.9–60.2%, 24.2–104.7%, and 14.2–97.3% in leaves of *H. ammodendron*, *T. ramosissima*, and *P. euphratica*, respectively, with elevated NaCl content compared to control (Fig. 4*D–F*).

Total soluble sugars and proline content: Proline in leaves of *H. ammodendron* was increasing with increasing NaCl concentration (R = 0.981, $P<0.01$) (Fig. 5*A*), it initially increased and reached the peak value at 100

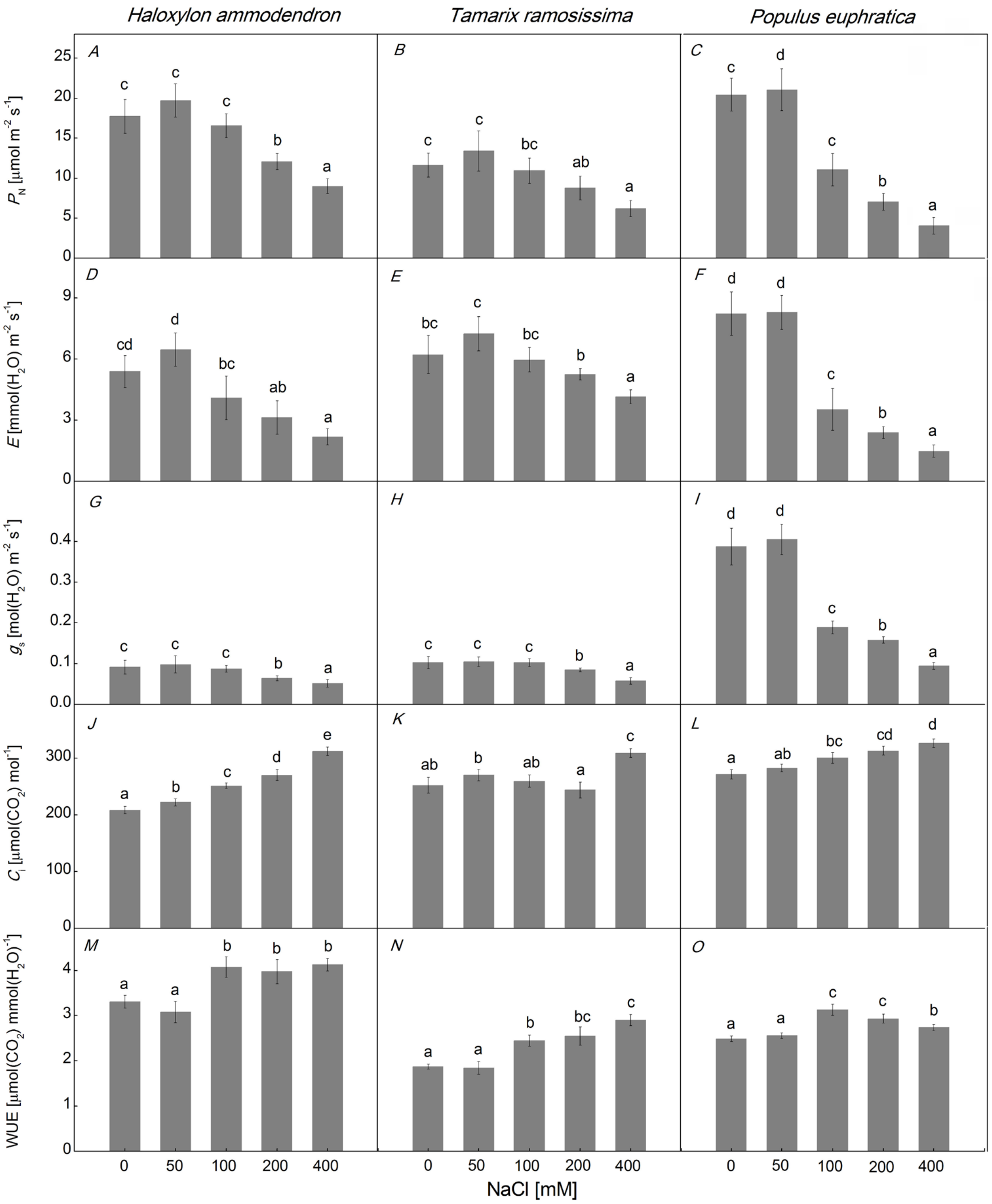

Fig. 3. Effects of NaCl exposure (0, 50, 100, 200, and 400 mM) on net photosynthetic rate (P_N) (*A–C*), transpiration rate (E) (*D–F*), stomatal conductance (g_s) (*G–I*), intercellular CO$_2$ concentration (C_i) (*J–L*), and water-use efficiency (WUE) (*M–O*) in leaves of *Haloxylon ammodendron*, *Tamarix ramosissima*, and *Populus euphratica*. Values are means ± SD (n = 4). *Different lowercase letters* indicate significant differences at $p<0.05$ according to *Fisher*'s LSD test.

and 50 mM NaCl in leaves of *T. ramosissima* and *P. euphratica*, respectively, and began to decrease but still maintained at control level even at the highest NaCl concentration (Fig. 5*B*,*C*).

Soluble sugar in leaves of *H. ammodendron* and *T. ramosissima* initially increased with increasing NaCl concentration and reached the peak value at 200 mM NaCl, however, it began declining significantly at 400 mM NaCl compared with control (Fig. 5*D*,*E*). It was significantly influenced by NaCl exposure in leaves of *P. euphratica*, decreasing with elevated NaCl concentrations ($R = -0.888$, $P<0.05$) (Fig. 5*F*).

MDA and H$_2$O$_2$ content: In general, H$_2$O$_2$ and MDA concentrations were not influenced by lower NaCl concentrations, however, both were remarkably accumulated at increasing NaCl concentrations (Fig. 6). H$_2$O$_2$ and MDA contents in leaves of *H. ammodendron* significantly

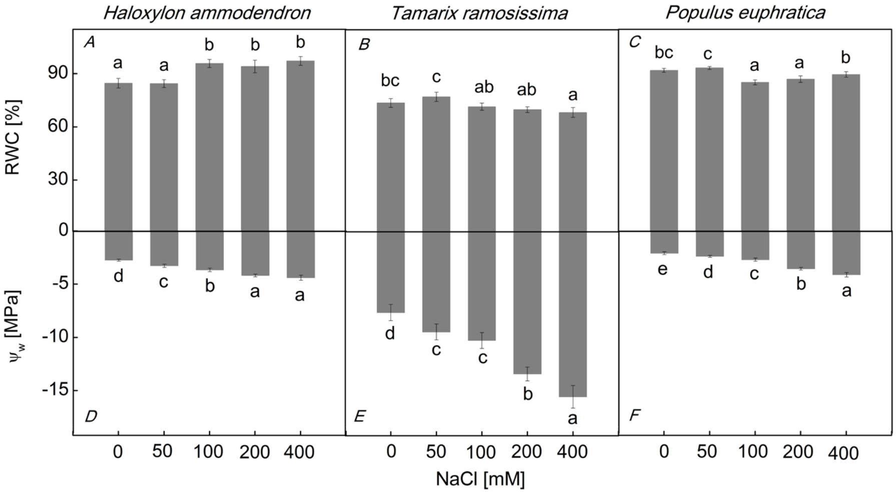

Fig. 4. Effects of NaCl exposure (0, 50, 100, 200, and 400 mM) on relative water content (RWC) (*A–C*) and water potential (ψ_w) (*D–F*) in leaves of *Haloxylon ammodendron*, *Tamarix ramosissima*, and *Populus euphratica*. Values are means ± SD ($n = 4$). *Different lowercase letters* indicate significant differences at $p<0.05$ according to *Fisher*'s LSD test.

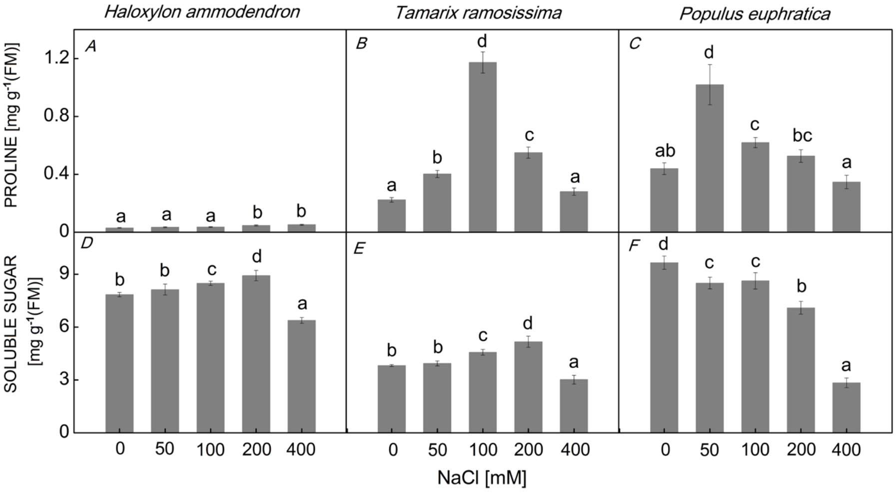

Fig. 5. Effects of NaCl exposure (0, 50, 100, 200, and 400 mM) on proline (*A–C*) and soluble sugar contents (*D–F*) in leaves of *Haloxylon ammodendron*, *Tamarix ramosissima*, and *Populus euphratica*. Values are means ± SD ($n = 4$). *Different lowercase letters* indicate significant differences at $p<0.05$ according to *Fisher*'s LSD test.

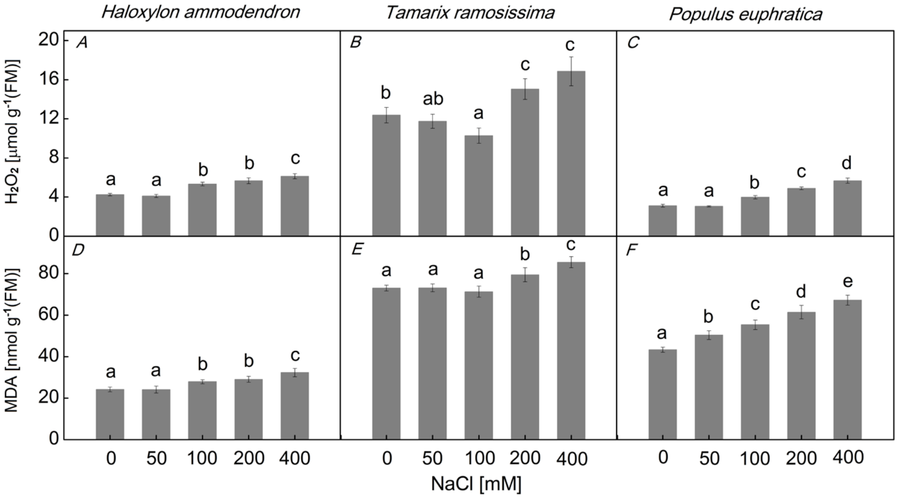

Fig. 6. Effects of NaCl exposure (0, 50, 100, 200, and 400 mM) on hydrogen peroxide (H_2O_2) (*A–C*) and malondialdehyde (MDA) (*D–F*) contents in leaves of *Haloxylon ammodendron*, *Tamarix ramosissima*, and *Populus euphratica*. Values are means ± SD ($n = 4$). *Different lowercase letters* indicate significant differences at $p<0.05$ according to *Fisher*'s LSD test.

increased under 100 mM NaCl (Fig. 6*A*,*D*), and both H_2O_2 and MDA significantly accumulated in leaves of *T. ramosissima* under 200 mM NaCl (Fig. 6*B*,*E*). H_2O_2 content in leaves of *P. euphratica* showed a similar tendency as in *H. ammodendron* (Fig. 6*C*). MDA content was increasing with elevated NaCl concentrations ($R = 0.999$, $P<0.001$) (Fig. 6*F*).

Antioxidant enzymes: SOD, CAT, POD, and APX activities in leaves of the three species initially increased and then decreased gradually with elevated NaCl concentrations (Fig. 7). SOD, CAT, POD, and APX activities in

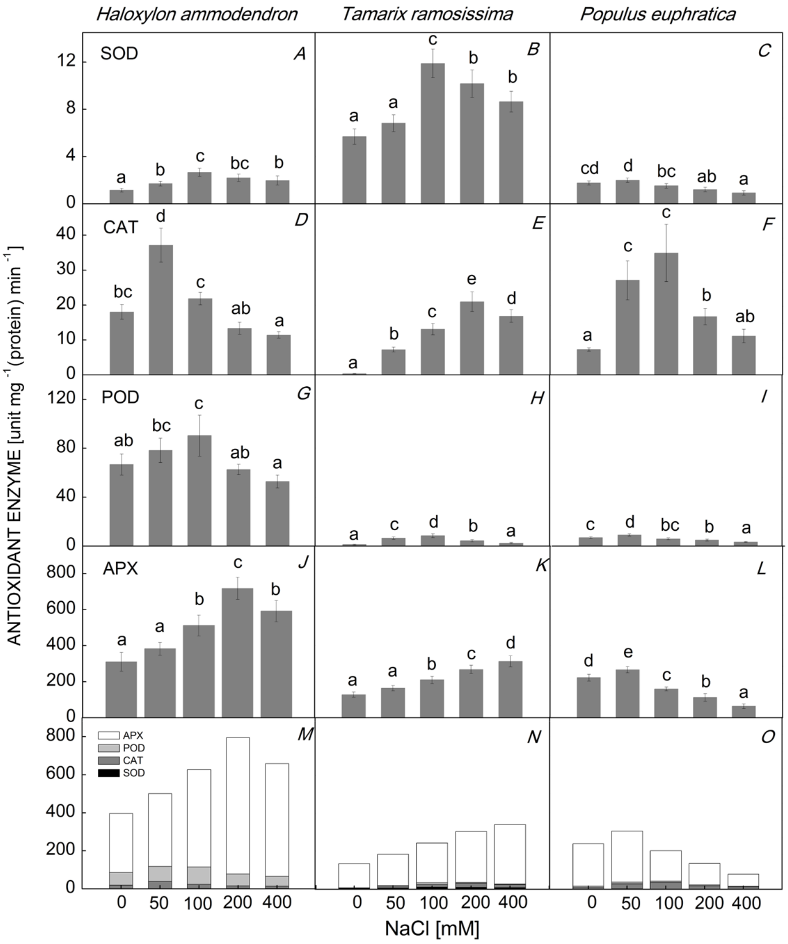

Fig. 7. Effects of NaCl exposure (0, 50, 100, 200, and 400 mM) on superoxide dismutases (SOD) (*A–C*), catalases (CAT) (*D–F*), guaiacol peroxidase (POD) (*G–I*), ascorbate peroxidase (APX) (*J–L*), and total enzyme activities (*M–O*) in leaves of *Tamarix ramosissima*, *Populus euphratica*, and *Haloxylon ammodendron*. Values are means ± SD (*n* = 4). *Different lowercase letters* indicate significant differences at $p<0.05$ according to *Fisher*'s LSD test.

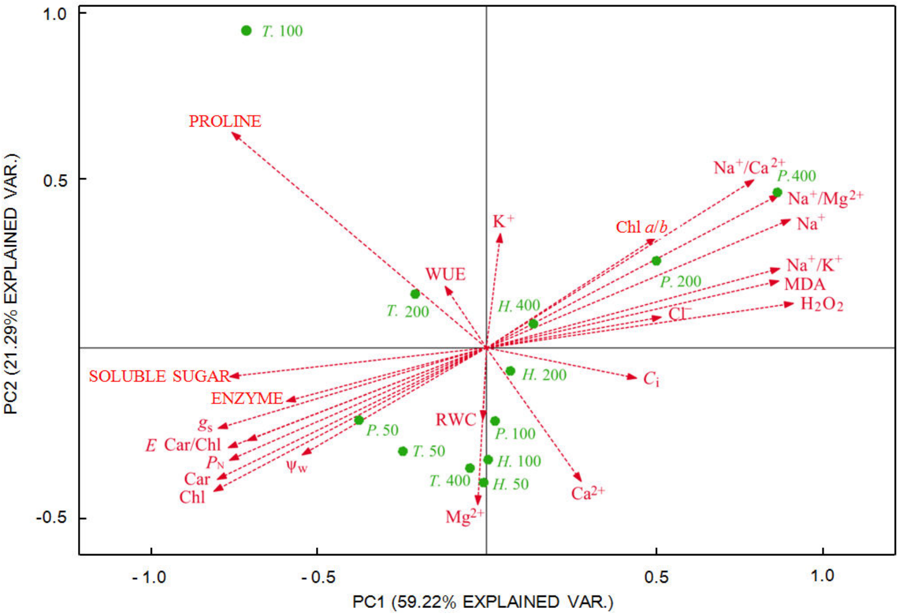

Fig. 8. Biplot of principal component analysis of the first two principal components of all tested parameters, species and salinity levels. Car – carotenoids; Chl – chlorophyll (*a*+*b*); Enzyme – total activities of all analyzed antioxidant enzymes; C_i – intercellular CO_2 concentration; *E* – transpiration rate; g_s – stomatal conductance; *H.* – *Haloxylon ammodendron*; H_2O_2 – hydrogen peroxide; MDA – malondialdehyde content; *P.* – *Populus euphratica*; P_N – net photosynthetic rate; RWC – relative water content; *T.* – *Tamarix ramosissima*; WUE – water-use efficiency; ψ_w – water potential; 50 – 50 mM NaCl treatment; 100 – 100 mM NaCl treatment; 200 – 200 mM NaCl treatment; 400 – 400 mM NaCl treatment.

leaves of *H. ammodendron* increased with increasing NaCl concentration and reached their maximum value at 100, 50, 100, and 200 mM NaCl concentrations, respectively, and began to decrease with increasing NaCl treatments. Among these enzymes only CAT decreased significantly lower than the control level under 400 mM NaCl concentration

(Fig. 7*A*,*D*,*G*,*J*). APX in leaves of *T. ramosissima* increased with increasing NaCl concentrations (Fig. 7*K*); SOD, CAT, and POD initially increased with elevated NaCl and reached their peak value at 100, 200, and 100 mM NaCl concentrations, and then began to decrease. POD was reduced to control level under 400 mM NaCl treatment, however, SOD and CAT were still higher than control level at 400 mM NaCl treatment (Fig. 7*B*,*E*,*H*). SOD, POD, and APX in leaves of *P. euphratica* peaked at 50 mM NaCl and then began decreasing under the control level (Fig. 7*C*,*I*,*L*); only CAT peaked at 100 mM NaCl and was still higher than control level (Fig. 7*F*). The total enzyme activities in leaves of *H. ammodendron* initially increased and showed the maximal value at 200 mM NaCl and then decreased at 400 mM NaCl being still higher than that of the control (Fig. 7*M*). The total enzyme activities in leaves of *T. ramosissima* increased with increasing NaCl concentrations ($R = 0.997$, $P<0.001$) (Fig. 7*N*), in leaves of *P. euphratica,* it reached the peak value at 50 mM NaCl concentration, and began to decrease significantly lower than control level with increasing NaCl concentration (Fig. 7*O*).

Principle component analysis (PCA): The results of the PCA obtained from data of all physiological and biochemical parameters of the three species subjected to different salinity levels are illustrated in Fig. 8. The first two components accounted for 80.5% of the total variation, and the first and second PCA accounted for 59.2 and 21.3% of the total variability, respectively. The leaf toxic ion (Na^+ and Cl^-) content, Na^+/Ca^{2+}, Na^+/K^+, and Ca^{2+}/Mg^{2+} ratios together with MDA and H_2O_2 concentrations were favored by *H. ammodendron* as well as *P. euphratica* under 200 and 400 mM NaCl treatment. Interestingly, photosynthetic pigments (Chl, Car, Car/Chl), gas-exchange parameters (P_N, E, g_s) were grouped with soluble sugar, antioxidant enzymes, RWC, ψ_w, and Mg^{2+}, and the angle among the vectors of these parameters was acute. All of the three species were identified by these parameters when treated with lower NaCl concentrations (50 mM), except for *T. ramosissima* under 400 mM NaCl.

Discussion

Exposure of plants to stress conditions such as salinity not only reduces the total quantity of photosynthetic energy obtained by plants but also rearranges energy from growth to endurance processes such as ion homeostasis, decreasing oxidative damages, and osmotic regulation (Munns and Gilliham 2015).

Effects of salinity on ion concentrations: NaCl induced ion toxicity associated with the overaccumulation of Na^+ and Cl^-, and there were significant differences between species in the capacity for uptaking and utilizing of Na^+ and Cl^-; consequently, these three species displayed different salt-resistance mechanisms. *H. ammodendron* is a leaf succulent plant that can deposit a high amount of Na^+ and Cl^- in leaves. Succulence is considered to contribute to salt adjustment by enlarging the vacuolar volume convenient to ion accumulation (Greenway and Munns 1980, Ungar 1991). *Halogeton glomeratus*, a succulent halophyte, took up a large quantity of Na^+ in water-storage tissue in leaves, sustained the succulence of leaves, and also the water content of tissues in response to salt stresses (Wang *et al*. 2015). *Tamarix* species acquire ions by the root system and then transport the ions to salt glands in leaves using an apoplastic xylem pathway (Campbell *et al*. 1974). In this study, Na^+ in leaves of *T. ramosissima* accumulated significantly only under higher NaCl concentrations (≥ 200 mM NaCl); the similar results had been reported in recretohalophyte *Atriplex centralasiatica* (Li *et al*. 2006). *Tamarix* sp. uses excretion processes to remove salt ions and the glandular structures are not always specific to Na^+ and Cl^-, and other toxic ions such as Cd^{2+}, Li^+, and Pb^{2+} are also excreted by the salt glands on the surface of the leaves (Hagemeyer and Waisel 1988, Kadukova *et al*. 2008, Manousaki and Kalogerakis 2011). The capacity of *P. euphratica* to exclude Na^+ and Cl^- is associated with restricted root-to-shoot salt transport caused by the blocking of root apoplastic salt transport and sequestration of Cl^- in root cortical vacuoles (Chen *et al*. 2003, Wang *et al*. 2008). However, excessive accumulation of Na^+ in leaves of *P. euphratica* treated with higher NaCl concentrations indicates that the effective system for root exclusion, accumulation in shoots, and/or weakened Na^+ xylem transport were not working to restrain this toxic ion to reach the leaves (Munns *et al*. 2005).

K^+, Ca^{2+}, and Mg^{2+} take a great part in plant growth and development but also in the maintenance of cell turgor and osmotic regulation (Marschner 1995, Veatch-Blohm *et al*. 2012, Acosta-Motos *et al*. 2014). However, K^+ in roots of *P. euphratica* decreased significantly with an elevated level of salinity, as reported by Chen *et al*. (2001), owing to a competitive process between K^+ and Na^+ in the root medium. Since salinity increased Na^+ concentration in plants, it competed with K^+, Mg^{2+}, and Ca^{2+} which increased Na^+/K^+, Na^+/Mg^{2+}, and Na^+/Ca^{2+} ratios, inducing a disorder of K^+, Ca^{2+}, and Mg^{2+}-dependent metabolic processes in plants, causing cellular damage, decreases in net photosynthesis, and inhibition of growth (Carter *et al*. 2006).

Photosynthetic pigment and gas-exchange characteristics: Salinity severely impacts photosynthesis by the reduction in Chl and Car contents (Lu *et al*. 2017, Li *et al*. 2020), as was also seen in species in the present research at higher NaCl concentrations. In this study, *P. euphratica* showed a higher reduction in Chl, Car, and Car/Chl, and the significant reduction began at lower NaCl concentration than that of *H. ammodendron* and *T. ramosissima*. A decrease of Chl has often been linked to salinity stress (Borrelli *et al*. 2018, Hinojosa-Vidal *et al*. 2018). Reduction in Chl content might be attributed to direct salt stress effects on Chl degradation due to weakening bonds of the pigment–protein–lipid complex (Levitt 1980), Mg accumulation, and resulting in restrains of Chl synthesis; or salinity could promote the activity of the Chl-degrading enzyme chlorophyllase (Reddy and Vora 1986). The interconversion of Chl *a* and *b* effectively works on the establishment of desired Chl *a*/*b* ratio during the regulation of leaves to stress conditions (Ito *et al.* 1993). In our research, with the elevated NaCl concentration ($\geq$ 100 mM NaCl), the ratio of Chl *a*/*b* enhanced markedly in leaves of *P. euphratica*, suggesting a reduction of the light-harvesting complex of PSII (Pandey *et al.* 2009), or reduction in the LHC components because Chl *b* is mainly located in the complexes (Durnford *et al.* 2003). Cars are related to the photosynthetic complexes in thylakoid membranes. They harvest photons, keep the structure and function of the photosynthetic complexes, eliminate excess energy, and quench Chl triplet states (Demmig-Adams *et al.* 1996, Niyogi *et al.* 1999). In this study, Car concentration and Car/Chl ratio decreased under higher NaCl concentrations, especially in leaves of *P. euphratica*, suggesting that the energy dissipation mechanism failed to reduce the excess energy in PSI and PSII (Lu *et al.* 2003, Pandey *et al.* 2005).

The reduction in the Chl content caused the decrease of P_N, and changed g_s, inducing stomatal closure (Verslues *et al.* 2006). P_N of a plant normally decreases with elevated salinity stress (Garcia *et al.* 2017, Ahanger *et al.* 2020). In this study, the decrease of P_N reported in *P. euphratica* was more significant compared to *H. ammodendron* and *T. ramosissima*, and the variations in P_N were in accordance with Chl content changes. P_N exhibited a highly remarkable correlation with g_s, which suggested that stomatal closure drove, at least in part, P_N reduction under salt stress. It has been proven that long-term exposure to salt reduced P_N, which might be due to decreased g_s (Ouerghi *et al.* 2000). Leaf stomata closure attributed to toxic Na^+ and $Cl^–$ ions reduces g_s, which further reduces P_N and growth (Shahbaz and Zia 2011). The P_N and g_s were highly correlated with C_i values. A reduction in C_i should be accompanied by the reduction in P_N if stomatal limitations to CO_2 diffusion are a major limitation (Farquhar and Sharkey 1982). The subsequent increase in C_i could suggest nonstomatal limitations (Moya *et al.* 2002, Duan *et al.* 2018). Stomatal limitations are usually prominent in moderate salt environments, however, nonstomatal limitations are significant in high salt environments (Everard *et al.* 1994). In this study, the decreases in P_N and g_s in leaves of *H. ammodendron* and *P. euphratica* were accompanied by the increase in C_i with increasing NaCl concentrations, which indicated nonstomatal limitations. However, the decreases in P_N and g_s accompanied by higher C_i in leaves of *T. ramosissima* were present only under 400 mM NaCl, indicating that a nonstomatal factor was responsible for the reduction of photosynthesis at high salinity. Similar results were found in previous studies of *Malus halliana*, *M. robusta* (Zhu *et al.* 2019), and *Suaeda glauca* (Duan *et al.* 2018) under salinity stress conditions. It can be concluded therefore that the decrease in the P_N reported herein was due to biochemical limitations, such as a decline in photosynthetic pigments and salinity-induced oxidative stress (Huang *et al.* 2014, Panda *et al.* 2019). Furthermore, stomatal closure reduces the loss of water through transpiration, resulting in a decrease of *E*. It has been reported that the reduction in g_s under mild stress may be a protective mechanism against stress by allowing the plant to conserve water, consequently improving plant WUE (Chaves *et al.* 2009).

RWC, ψ_w, and compatible solute content: Salinity first weakens the plant's capacity of absorbing water, consequently, results in physiological water deficit. Water relations and the capacity of regulating the osmotic concentration take a great part in the development and overall growth of plants (Munns 2002). It has been reported that plants accumulate osmolytes or inorganic ions in saline environments to reduce their cellular water potential (Munns 2002, Shafeiee and Ehsanzadeh 2019). Sodium and chloride are regarded as energetically efficient osmolytes for osmotic regulation and are usually compartmentalized into the vacuole to minimize cytotoxicity (Blumwald *et al.* 2000). Moreover, soluble sugars and proline are principal compatible solutes involved in osmotic adjustment (Singh *et al.* 2015, Ahanger *et al.* 2020). In this study, the reduction of leaf water potential (ψ_w) in salt-treated plants indicates an osmotic effect and as a consequence difficulty for water uptake. High salinity exposure caused a significant increment in RWC in leaves of *H. ammodendron*. The capacity of *H. ammodendron* to hold relatively high RWC in leaves exposed to high salt treatments seems to play a protective role from the deleterious influences of excessive salt. In halophytes, enhanced leaf succulency could be a representative adaptive response and is accomplished by enlarging the size of mesophyll cells and the relative size of vacuoles (Shabala and Mackay 2011, Parida *et al.* 2016). Proline and soluble sugar in leaves of *H. ammodendron* increased slowly and soluble sugar even decreased significantly exposed to 400 mN NaCl compared with control. On the contrary, an enhancement of external NaCl from 50 to 400 mM led to a remarkable accumulation in internal Na^+ and $Cl^–$, indicating that the Na^+ and $Cl^–$ were the main contributor to osmotic regulation responding to low external water potential in *H. ammodendron*. It has been demonstrated that the accumulation of Na^+ can mitigate the detrimental effects of osmotic stress induced by salinity stress in other succulent plants (Ma *et al.* 2012, 2016); $Cl^–$ is beneficial for the growth of the xerophyte *Pugionium cornutum* by enhancing osmotic adjustment capacity under salt stresses (Cui *et al.* 2020). However, RWC in leaves of *T. ramosissima* was reduced progressively with increasing NaCl concentration. In general, plants can take water loss as a quick and economical way to osmotic regulation in response to osmotic stress (Lissner *et al.* 1999). Remarkable accumulations of proline and soluble sugars in leaves of *T. ramosissima*, except for 400 mM NaCl treatment, were detected under salt stress in comparison with control, suggested the accumulation of sucrose and proline could be an adaptive mechanism to restrain water loss. RWC in leaves of *P. euphratica* decreased initially, and the proline content increased compared to control ($\leq$ 200 mM NaCl), meanwhile, Na^+ and $Cl^–$ were only significantly accumulated at higher NaCl treatment compared with control (≥ 200 mM NaCl). In *P. euphratica*, proline might play an important role in osmotic adjustment under lower salinity stress, however, Na^+ and Cl^- might be the main contributor to alleviate the osmotic pressure in response to higher salinity stress.

MDA, H_2O_2 content, and enzyme activities: Oxidative stress is a consequence that has been considered as one of the main factors inducing cellular damage in plants subjected to diverse abiotic stresses, including salt stress (Singh *et al.* 2015, Chen *et al.* 2020). Salinity could induce oxidative stress by a generation of ROS in higher plants (Singh *et al.* 2015, Jiang *et al.* 2020). Protonation of $O_2^{\bullet-}$ generates the hydroperoxyl radical ($^{\bullet}HO$ and H_2O_2), which can change fatty acids into toxic lipid peroxides and damage biological membranes (Weckx and Clijsters 1996). MDA is a substance produced by lipid peroxidation while plants are subjected to oxidative stress and could be considered as a marker of the extent of oxidative stress (Li *et al.* 2017, El-Esawi *et al.* 2018). In the present study, high concentrations of NaCl remarkably enhanced the generation of H_2O_2, thereby, resulting in lipid peroxidation as evidence by the accumulation of MDA. However, oxidative damage was lesser in *H. ammodendron* and *T. ramosissima* than that in *P. euphratica*, as shown by the lower MDA concentration in leaves.

Salt-evoked oxidative damage can be relieved by the active response of the antioxidative system; antioxidative ability is generally regarded as the standard for selecting salt-tolerant species. Plants' pivotal ROS-scavenging mechanisms contain SOD, CAT, APX, and POD, and the balance between them is crucial (Mittler 2002, Asrar *et al.* 2020). SOD is the key antioxidant enzyme working as $O_2^{\bullet-}$ scavenger in living organisms through promptly changing $O_2^{\bullet-}$ into O_2 and H_2O_2, a first-line protector from oxidative stress induced by ROS. In the present study, induction of SOD was recorded in leaves of *H. ammodendron* and *T. ramosissima* but not in *P. euphratica* leaves exposed to higher salt concentrations. However, the substance generated by SOD activity is H_2O_2, and that remains toxic and should be removed by change into H_2O in the next process. In plants, CAT, APX, and POD are regarded as the most important in the degradation of H_2O_2 (Blokhina *et al.* 2003, Jiang *et al.* 2020). In this study, an enhancement in SOD activity in leaves of *T. ramosissima* subjected to all NaCl exposure concentrations occurred together with an enhancement in CAT, POD, and APX, which guaranteed the efficient removing of H_2O_2 by a reduced production of H_2O_2 at lower NaCl concentration (≤ 100 mM NaCl) and slower accumulation rate at higher NaCl concentration (≥ 200 mM NaCl). In *H. ammodendron*, H_2O_2 was eliminated by POD and CAT at lower and by APX at higher salinity stress. However, in *P. euphratica*, the significant accumulation of H_2O_2 measured at high NaCl treatments (≥ 100 mM) may be owed to a gradual reduction in SOD, CAT, POD, and APX activities. It has been reported that activities of antioxidative enzymes were significantly higher in the tolerant species in comparison to sensitive ones exposed to salinity stress (Kaya *et al.* 2015, Ibrahim *et al.* 2019). The ROS-scavenging system in *T. ramosissima* and *H. ammodendron* was more efficient in comparison to *P. euphratica*, as CAT, POD, and APX together with SOD activity in leaves of *T. ramosissima* and *H. ammodendron* reacted more efficiently in $O_2^{\bullet-}$ and H_2O_2 scavenging. Our results suggested that *T. ramosissima* and *H. ammodendron* may better defend against oxidative stress and can promptly modulate the antioxidant system.

Evaluation of salt-tolerance parameters by principal component analysis: Principal component analyses (PCAs) could present an overall explanation of the interrelationships between tested indexes. It screens which parameters could be considered as interchangeable, individual, or supplementary criteria for estimating salt tolerance when exposed to salt stress (El-Hendawy *et al.* 2017). The interrelationships between parameters can be explained by the angles between the parameter vectors. An acute angle suggests strong correlations, and the reverse is also true for the obtuse angle. A straight angle indicates a negative correlation between parameters, while a right angle illustrates no correlation between parameters (El-Hendawy *et al.* 2017). According to this theory, the leaf oxidative stress parameters (H_2O_2 and MDA) together with toxic ion concentrations and ion ratios (Na^+, Cl^-, Na^+/Ca^{2+}, Na^+/K^+, and Ca^{2+}/Mg^{2+}) were grouped. Moreover, the leaf photosynthetic parameters (photosynthetic pigments and gas-exchange parameters) were grouped with soluble sugar, antioxidant enzymes, and ψ_w. Therefore, these parameters, in these two groups respectively, represented a strong correlation between them and recommended to be taken as complementary screening criteria for salt tolerance evaluation. The straight angle between toxic ion, H_2O_2, MDA, and photosynthetic parameters, soluble sugar, enzymes, and ψ_w suggests that salinity can cause ion toxicity, osmotic and oxidative stress through the excessive accumulation of toxic ions (Na^+ and Cl^-), H_2O_2, and MDA, however, a plant can alleviate the stress by the accumulation of soluble sugars and promotion of antioxidative enzyme activities. Therefore, the toxic ion contents (Na^+ and Cl^-), ion ratios (Na^+/Ca^{2+}, Na^+/K^+, and Ca^{2+}/Mg^{2+}), H_2O_2, MDA, photosynthesis parameters, soluble sugars, enzymes, and ψ_w could be considered as interchangeable or individual screening criteria for salt tolerance evaluation.

Conclusion: All three woody plant species are well adapted to the mild salinity exposure (≤ 50 mM NaCl), indicating that those plant species possess moderate salt tolerance capacity and can grow well under the mild saline conditions of the groundwater in the southern rim of the Taklamakan Desert. Nevertheless, the plants showed different salt-tolerance mechanisms. While *H. ammodendron* acquired a large quantity of mineral salts and deposited salts in leaf, Na^+ in leaves of *T. ramosissima* accumulated significantly only under higher NaCl treatments (≥ 200 mM NaCl), *P. euphratica* restricted excessive Na^+ and Cl^- accumulation in leaves by root exclusion and transport pathway from roots to shoots. Furthermore, there were substantial differences in the response of antioxidant enzymes to salinity stresses and salinity evoked oxidative damage in the three species. The higher Na^+ and Cl^- concentrations, lower oxidative damage, and more efficient antioxidant enzyme activities suggested that *H. ammodendron* and *T. ramosissima* depended on the antioxidant protective mechanisms to defend against oxidative stress. Our results indicate the better tolerance of *H. ammodendron* and *T. ramosissima* to salinity stress compared to *P. euphratica*.

References

Acosta-Motos J.R., Álvarez S., Barba-Espín G. *et al.*: Salts and nutrients present in regenerated waters induce changes in water relations, antioxidative metabolism, ion accumulation and restricted ion uptake in *Myrtus communis* L. plants. – Plant Physiol. Bioch. **85**: 41-50, 2014.

Aebi H.: Catalase in vitro. – Method. Enzymol. **105**: 121-126, 1984.

Ahanger M.A., Mir R.A., Alyemeni M.N., Ahmad P.: Combined effects of brassinosteroid and kinetin mitigates salinity stress in tomato through the modulation of antioxidant and osmolyte metabolism. – Plant Physiol. Bioch. **147**: 31-42, 2020.

Allakhverdiev S.I., Kreslavski V.D., Klimov V.V. *et al.*: Heat stress: an overview of molecular responses in photosynthesis. – Photosynth. Res. **98**: 541-550, 2008.

Arndt S.K., Arampatsis C., Foetzki A. *et al.*: Contrasting patterns of leaf solute accumulation and salt adaptation in four phreatophytic desert plants in a hyperarid desert with saline groundwater. – J. Arid Environ. **59**: 259-270, 2004.

Asrar H., Hussain T., Qasim M. *et al.*: Salt induced modulations in antioxidative defense system of *Desmostachya bipinnata*. – Plant Physiol. Bioch. **147**: 113-124, 2020.

Bates L.S., Waldren R.P., Teare I.D.: Rapid determination of free proline for water stress studies. – Plant Soil **39**: 205-207, 1973.

Beauchamp C., Fridovich I.: Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. – Anal. Biochem. **44**: 276-287, 1971.

Blokhina O., Virolainen E., Fagerstedt K.V.: Antioxidants, oxidative damage and oxygen deprivation stress: a review. – Ann. Bot.-London **91**: 179-194, 2003.

Blumwald E., Aharon G.S., Apse M.P.: Sodium transport in plant cells. – BBA-Biomembranes **1465**: 140-151, 2000.

Borrelli G.M., Fragasso M., Nigro F. *et al.*: Analysis of metabolic and mineral changes in responses to salt stress in durum wheat (*Triticum turgidum* ssp. durum) genotypes, which differ in salinity tolerance. – Plant Physiol. Bioch. **133**: 57-70, 2018.

Bradford M.M.: A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. – Anal. Biochem. **72**: 248-254, 1976.

Campbell N., Thomson W.W., Platt K.: The apoplastic pathway of transport to salt glands. – J. Exp. Bot. **25**: 61-69, 1974.

Carter J.L., Colmer T.D., Veneklaas E.J.: Variable tolerance of wetland tree species to combined salinity and waterlogging is related to regulation of ion uptake and production of organic solutes. – New Phytol. **169**: 123-134, 2006.

Chance B., Maehly A.C.: Assay of catalases and peroxidases. – In: Colowick S.P., Kaplan N.O. (ed.): Methods in Enzymology. Pp. 764-775. Academic Press, New York 1955.

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.

Chen J., Zhang H., Zhang X., Tang M.: Arbuscular mycorrhizal symbiosis mitigates oxidative injury in black locust under salt stress through modulating antioxidant defense of the plant. – Environ. Exp. Bot. **175**: 104034, 2020.

Chen S., Li J., Wang S. *et al.*: Salt, nutrient uptake and transport, and ABA of *Populus euphratica*: a hybrid in response to increasing soil NaCl. – Trees **15**: 186-194, 2001.

Chen S., Li J., Wang S. *et al.*: Effects of NaCl on shoot growth, transpiration, ion compartmentation and transport in regenerated plants of *Populus euphratica* and *Populus tomentosa*. – Can. J. For. Res. **33**: 967-975, 2003.

Cui Y.N., Li X.T., Yuan J.Z. *et al.*: Chloride is beneficial for growth of the xerophyte *Pugionium cornutum* by enhancing osmotic adjustment capacity under salt and drought stresses. – J. Exp. Bot. **71**: 4215-4231, 2020.

Cui Y.Q., Ma J.Y., Sun W.: Application of stable isotope techniques to the study of soil salinization. – J. Arid Land **3**: 285-291, 2011. doi: 10.3724/SP.J.1227.2011.00285.

Demmig-Adams B., Gilmore A.M., Adams III W.W.: In vivo functions of carotenoids in higher plants. – FASEB J. **10**: 403-412, 1996.

Duan H., Ma Y., Liu R. *et al.*: Effect of combined waterlogging and salinity stresses on euhalophyte *Suaeda glauca*. – Plant Physiol. Bioch. **127**: 231-237, 2018.

Durnford D.G., Price J.A., McKim S.M., Sarchfield M.L.: Light-harvesting complex gene expression is controlled by both transcriptional and posttranscriptional mechanisms during photoacclimation in *Chlamydomonas reinhardtii*. – Physiol. Plantarum **118**: 193-205, 2003.

El-Esawi M.A., Alaraidh I.A., Alsahli A.A. *et al.*: *Bacillus firmus* (SW5) augments salt tolerance in soybean (*Glycine max* L.) by modulating root system architecture, antioxidant defense systems and stress-responsive genes expression. – Plant Physiol. Bioch. **132**: 375-384, 2018.

El-Hendawy S.E., Hassan W.M., Al-Suhaibani N.A. *et al.*: Comparative performance of multivariable agro-physiological parameters for detecting salt tolerance of wheat cultivars under simulated saline field growing conditions. – Front. Plant Sci. **8**: 435, 2017.

Everard J.D., Gucci R., Kann S.C. *et al.*: Gas exchange and carbon partitioning in the leaves of celery (*Apium graveolens* L.) at various levels of root zone salinity. – Plant Physiol. **106**: 281-292, 1994.

Fan L., Wang G.N., Hu W. *et al.*: Transcriptomic view of survival during early seedling growth of the extremeophyte *Haloxylon ammodendron*. – Plant Physiol. Bioch. **132**: 475-489, 2018.

FAO, ITPS: Status of the World's Soil Resources (SWSR). Main Report. Pp. 650. Food and Agriculture Organization of the United Nations and Intergovernmental Technical Panel on Soils, Rome 2015.

Farquhar G.D., Sharkey T.D.: Stomatal conductance and photosynthesis. – Ann. Rev. Plant Physio. **33**: 317-345, 1982.

Garcia J.D., Dalmolin Â.C., França M.G.C., Mangabeira P.A.O.: Different salt concentrations induce alterations both in photosynthetic parameters and salt gland activity in leaves of the mangrove *Avicennia schaueriana*. – Ecotox. Environ. Safe. **141**: 70-74, 2017.

Gechev T., Petrov V.: Reactive oxygen species and abiotic stress in plants. – Int. J. Mol. Sci. **21**: 7433, 2020.

Gibert E., Gentelle P., Liang K.Y.: Chemical and isotopic evolution of surficial waters in the Taklamakan desert (Southern Xinjiang, China). – In: Isotopes in Water Resources Management: Proceedings of a Symposium on Isotopes in Water Resources Management, 20–24 March 1995. Pp. 211-212. IAEA, Vienna 1996.

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

Hagemeyer J., Waisel Y.: Excretion of ions (Cd^{2+}, Li^+, Na^+ and

Cl^-) by *Tamarix aphylla*. – Physiol. Plantarum **73**: 541-546, 1988.

Hinojosa-Vidal E., Marco F., Martínez-Alberola F. *et al.*: Characterization of the responses to saline stress in the symbiotic green microalga *Trebouxia* sp. TR9. – Planta **248**: 1473-1486, 2018.

Huang C.J., Wei G., Jie Y.C. *et al.*: Effects of concentrations of sodium chloride on photosynthesis, antioxidative enzymes, growth and fiber yield of hybrid ramie. – Plant Physiol. Bioch. **76**: 86-93, 2014.

Ibrahim W., Qiu C.W., Zhang C. *et al.*: Comparative physiological analysis in the tolerance to salinity and drought individual and combination in two cotton genotypes with contrasting salt tolerance. – Physiol. Plantarum **165**: 155-168, 2019.

Ito H., Tanaka Y., Tsuji H., Tanaka A.: Conversion of chlorophyll *b* to chlorophyll *a* by isolated cucumber etioplasts. – Arch. Biochem. Biophys. **306**: 148-151, 1993.

Jiang D.X., Chu X., Li M. *et al.*: Exogenous spermidine enhances salt-stressed rice photosynthetic performance by stabilizing structure and function of chloroplast and thylakoid membranes. – Photosynthetica **58**: 61-71, 2020.

Kadukova J., Manousaki E., Kalogerakis N.: Pb and Cd accumulation and phyto-excretion by salt cedar (*Tamarix smyrnensis* Bunge). – Int. J. Phytoremediat. **10**: 31-46, 2008.

Kaya C., Ashraf M., Sonmez O. *et al.*: Exogenous application of thiamin promotes growth and antioxidative defense system at initial phases of development in salt-stressed plants of two maize cultivars differing in salinity tolerance. – Acta Physiol. Plant. **37**: 1741, 2015.

Kosugi H., Kikugawa K.: Thiobarbituric acid reaction of aldehydes and oxidized lipids in glacial acetic acid. – Lipids **20**: 915-920, 1985.

Levitt J.: Responses of Plants to Environmental Stresses: Water, Radiation, Salt and Other Stresses. Pp. 607. Academic Press, New York 1980.

Li N.N., Wang X., Ma B.J. *et al.*: Expression of a Na^+/H^+ antiporter RtNHX1 from a recretohalophyte *Reaumuria trigyna* improved salt tolerance of transgenic *Arabidopsis thaliana*. – J. Plant Physiol. **218**: 109-120, 2017.

Li P., Zhu Y., Song X., Song F.: Negative effects of long-term moderate salinity and short-term drought stress on the photosynthetic performance of Hybrid *Pennisetum*. – Plant Physiol. Bioch. **155**: 93-104, 2020.

Li W.Q., Liu X.J., Zhao K.F., Liu H.L.: [Growth, development and ions distribution of three halophytes under salt stress.] – Chin. J. Eco.-Agr. **14**: 49-52, 2006. [In Chinese]

Lichtenthaler H.K.: Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. – Method. Enzymol. **148**: 350-382, 1987.

Lissner J., Schierup H.H., Comín F.A., Astorga V.: Effect of climate on the salt tolerance of two *Phragmites australis* populations. I. Growth, inorganic solutes, nitrogen relations and osmoregulation. – Aquat. Bot. **64**: 317-333, 1999.

López-Serrano L., Canet-Sanchis G., Selak G.V. *et al.*: Physiological characterization of a pepper hybrid rootstock designed to cope with salinity stress. – Plant Physiol. Bioch. **148**: 207-219, 2020.

Lu C., Jiang G., Wang B., Kuang T.: Photosystem II photochemistry and photosynthetic pigment composition in salt-adapted halophyte *Artimisia anethifolia* grown under outdoor conditions. – J. Plant Physiol. **160**: 403-408, 2003.

Lu Y., Lei J.Q., Zeng F.J. *et al.*: Effect of NaCl-induced changes in growth, photosynthetic parameters, water status and enzymatic antioxidant system of *Calligonum caput-medusae* Schrenk seedlings. – Photosynthetica **55**: 96-106, 2017.

Lu Y., Li X.R., He M.Z. *et al.*: Seedling growth and antioxidative enzymes activities in leaves under metal stress differ between two desert plants: a perennial (*Peganum harmala*) and an annual (*Halogeton glomeratus*) grass. – Acta Physiol. Plant. **32**: 583-590, 2010.

Ma Q., Bao A.K., Chai W.W. *et al.*: Transcriptomic analysis of succulent xerophyte *Zygophyllum xanthoxylum* in response to salt treatment and osmotic stress. – Plant Soil **402**: 343-361, 2016.

Ma Q., Yue L.J., Zhang J.L. *et al.*: Sodium chloride improves photosynthesis and water status in the succulent xerophyte *Zygophyllum xanthoxylum*. – Tree Physiol. **32**: 4-13, 2012.

Manousaki E., Kalogerakis N.: Halophytes – An emerging trend in phytoremediation. – Int. J. Phytoremediat. **13**: 959-969, 2011.

Marschner H.: Mineral Nutrition of Higher Plants. 2nd Edition. Pp. 889. Academic Press, London 1995.

Min X.J., Zang Y.X., Sun W., Ma J.Y.: Contrasting water sources and water-use efficiency in coexisting desert plants in two saline-sodic soils in northwest China. – Plant Biol. **21**: 1150-1158, 2019.

Mittler R.: Oxidative stress, antioxidants and stress tolerance. – Trends Plant Sci. **7**: 405-410, 2002.

Moya J.L., Tadeo F.R., Gómez-Cadenas A. *et al.*: Transmissible salt tolerance traits identified through reciprocal grafts between sensitive Carrizo and tolerant Cleopatra citrus genotypes. – J. Plant Physiol. **159**: 991-998, 2002.

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

Munns R.: Genes and salt tolerance: bringing them together. – New Phytol. **167**: 645-663, 2005.

Munns R., Gilliham M.: Salinity tolerance of crops − what is the cost? – New Phytol. **208**: 668-673, 2015.

Munns R., James R.A., Läuchli A.: Approaches to increasing the salt tolerance of wheat and other cereals. – J. Exp. Bot. **57**: 1025-1043, 2005.

Nakano Y., Asada K.: Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. – Plant Cell Physiol. **22**: 867-880, 1981.

Niyogi K.K.: Photoprotection revisited: genetic and molecular approaches. – Annu. Rev. Plant Phys. **50**: 333-359, 1999.

Ouerghi Z., Cornic G., Roudani M. *et al.*: Effect of NaCl on photosynthesis of two wheat species (*Triticum durum* and *T. aestivum*) differing in their sensitivity to salt stress. – J. Plant Physiol. **156**: 335-340, 2000.

Palma F., Lluch C., Iribarne C. *et al.*: Combined effect of salicylic acid and salinity on some antioxidant activities, oxidative stress and metabolite accumulation in *Phaseolus vulgaris*. – Plant Growth Regul. **58**: 307-316, 2009.

Pan C.C., Liu C.A., Zhao H.L., Wang Y.: Changes of soil physico-chemical properties and enzyme activities in relation to grassland salinization. – Eur. J. Soil Biol. **55**: 13-19, 2013.

Panda A., Rangani J., Parida A.K.: Cross talk between ROS homeostasis and antioxidative machinery contributes to salt tolerance of the xero-halophyte *Haloxylon salicornicum*. – Environ. Exp. Bot. **166**: 103799, 2019.

Pandey D.M., Choi I., Yeo U.-D.: Photosystem 2-activity and thylakoid membrane polypeptides of *in vitro* cultured chrysanthemum as affected by NaCl. – Biol. Plantarum **53**: 329-333, 2009.

Pandey D.M., Kang K.-H., Yeo U.-D.: Effects of excessive photon on the photosynthetic pigments and violaxanthin de-epoxidase activity in the xanthophyll cycle of spinach leaf. – Plant Sci. **168**: 161-166, 2005.

Parida A.K., Veerabathini S.K., Kumari A., Agarwal P.K.: Physiological, anatomical and metabolic implications of salt tolerance in the halophyte *Salvadora persica* under hydroponic culture condition. – Front. Plant Sci. **7**: 351, 2016.

Rajhi I., Ben Moussa S., Neji I. *et al*.: Photosynthetic and physiological responses of small seeded faba bean genotypes (*Vicia faba* L.) to salinity stress: identification of a contrasting pair towards salinity. – Photosynthetica **58**: 174-185, 2020.

Rattan A., Kapoor D., Kapoor N. *et al*.: Brassinosteroids regulate functional components of antioxidative defense system in salt stressed maize seedlings. – J. Plant Growth Regul. **39**: 1465-1475, 2020.

Reddy M.P., Vora A.B.: Changes in pigment composition, Hill reaction activity and saccharides metabolism in bajra (*Pennisetum typhoides* S & H) leaves under NaCl salinity. – Photosynthetica **20**: 50-55, 1986.

Sehar Z., Masood A., Khan N.A.: Nitric oxide reverses glucose-mediated photosynthetic repression in wheat (*Triticum aestivum* L.) under salt stress. – Environ. Exp. Bot. **161**: 277-289, 2019.

Sergiev I., Alexieva V., Karanov E.: Effect of spermine, atrazine and combination between them on some endogenous protective systems and stress markers in plants. – Compt. Rend. Acad. Bulg. Sci. **51**: 121-124, 1997.

Shabala S., Mackay A.: Ion transport in halophytes. – Adv. Bot. Res. **57**: 151-199, 2011.

Shafeiee M., Ehsanzadeh P.: Physiological and biochemical mechanisms of salinity tolerance in several fennel genotypes: Existence of clearly-expressed genotypic variations. – Ind. Crop. Prod. **132**: 311-318, 2019.

Shahbaz M., Zia B.: Does exogenous application of glycinebetaine through rooting medium alter rice (*Oryza sativa* L.) mineral nutrient status under saline conditions? – J. Appl. Bot. Food Qual. **84**: 54-60, 2011.

Shi Y., Wang Z., Lui C., Yang Z.: [Glacial resources of the arid regions in Northwest China and their utilization.] – In: Zhao S. (ed.): [Utilization and Development of Natural Resources in Arid and Semi-Arid Lands.] Pp. 26-32. Science Press, Beijing 1989. [In Chinese]

Sibole J.V., Cabot C., Poschenrieder C., Barceló J.: Efficient leaf ion partitioning, an overriding condition for abscisic acid-controlled stomatal and leaf growth responses to NaCl salinization in two legumes. – J. Exp. Bot. **54**: 2111-2119, 2003.

Singh M., Kumar J., Singh S. *et al*.: Roles of osmoprotectants in improving salinity and drought tolerance in plants: a review. – Rev. Environ. Sci. Biotechnol. **14**: 407-426, 2015.

Singh M., Kumar J., Singh V.P., Prasad S.M.: Plant tolerance mechanism against salt stress: the nutrient management approach. – Biochem. Pharmacol. **3**: 165, 2014.

Smart R.E., Bingham G.E.: Rapid estimates of relative water content. – Plant Physiol. **53**: 258-260, 1974.

Sofy M.R., Elhawat N., Alshaal T.: Glycine betaine counters salinity stress by maintaining high K^+/Na^+ ratio and antioxidant defense via limiting Na^+ uptake in common bean (*Phaseolus vulgaris* L.). – Ecotox. Environ. Safe. **200**: 110732, 2020.

Ungar I.A.: Ecophysiology of Vascular Halophytes. Pp. 221. CRC Press, Boca Raton 1991.

Veatch-Blohm M.E., Malinowski M., Keefer D.: Leaf water status, osmotic adjustment and carbon assimilation in colored calla lilies in response to saline irrigation. – Sci. Hortic.-Amsterdam **144**: 65-73, 2012.

Verslues P.E., Agarwal M., Katiyar-Agarwal S. *et al.*: Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stress that affect plant water status. – Plant J. **45**: 523-539, 2006.

Wang J.C., Meng Y.X., Li B.C. *et al.*: Physiological and proteomic analyses of salt stress response in the halophyte *Halogeton glomeratus*. – Plant Cell Environ. **38**: 655-669, 2015.

Wang R., Chen S., Zhou X. *et al*.: Ionic homeostasis and reactive oxygen species control in leaves and xylem sap of two poplars subjected to NaCl stress. – Tree Physiol. **28**: 947-957, 2008.

Weckx J.E.J., Clijsters H.M.M.: Oxidative damage and defense mechanisms in primary leaves of *Phaseolus vulgaris* as a result of root assimilation of toxic amounts of copper. – Physiol. Plantarum **96**: 506-512, 1996.

Zeng F.J., Bleby T.M., Landman P.A. *et al*.: Water and nutrient dynamics in surface roots and soils are not modified by short-term flooding of phreatophytic plants in a hyperarid desert. – Plant Soil **279**: 129-139, 2006.

Zhu Y.F., Wu Y.X., Hu Y. *et al*.: Tolerance of two apple rootstocks to short-term stress: focus on chlorophyll degradation, photosynthesis, hormone and leaf ultrastructures. – Acta Physiol. Plant. **41**: 87, 2019.