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NACL SALINITY-INDUCED CHANGES IN GROWTH, PHOTOSYNTHETIC PROPERTIES, WATER STATUS AND ENZYMATIC ANTIOXIDANT SYSTEM OF *NITRARIA ROBOROWSKII* KOM

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Abstract

In pot experiments, 1-year-old *Nitraria roborowskii* Kom plants were planted under control and four levels of NaCl. The plant growth, Na^+ and K^+ contents, maximal efficiency of photosystem II photochemistry (F_v/F_m), water potential, antioxidative enzyme activities and contents of photosynthetic pigments, relative water, soluble sugars, proline, hydrogen peroxide (H_2O_2), and malondialdehyde (MDA) in leaves were measured after 90 days of NaCl treatments. *N. roborowskii* showed tolerance to medium salinity (50 mM NaCl), whereas growth reduction in biomass was observed when ≥ 100 mM NaCl solutions were used for irrigation. Extreme salinity (400 mM NaCl) hardly impacted F_v/F_m, but a marked decreased in chlorophyll content was observed. A gradual decline of the leaf relative water content and water potential with increased NaCl feeding level was accompanied by increased Na^+ accumulation. The total proline and soluble sugar contents were not significantly affected by low salinity (50 mM NaCl); however, both of these values decreased gradually at high salinities (≥ 100 mM NaCl). MDA and H_2O_2 were not markedly affected at low salinity (50 mM NaCl), but both of these values remarkably accumulated at high salinities (≥ 100 mM NaCl). Superoxide dismutase (SOD), guaiacol peroxidase (POD) and catalase (CAT) presented the same variation trends, first increasing with concentrations of 100, 50 and 100 mM NaCl, respectively, and then decreasing compared to the control, whereas APX increased under all NaCl concentrations.

Key words: *Nitraria roborowskii* Kom, Chlorophyll content, Halophyte, LIPID peroxidation, Proline, Soluble sugar.

Introduction

Salinity is considered the main environmental extreme that detrimentally impacts plant growth and metabolism, particularly in arid and semi-arid areas (Munns & Tester, 2008). In China, 36 million areas are already saline (Yang, 2008). The semi-arid region of China is one of the regions most seriously affected by soil salinization (Wang & Jia, 2012). A requirement to exploit and utilize saline soils has borne a precondition to elucidate how plants respond and adapt to saline stress (Yıldıztugay *et al*., 2011).

Plants respond to saline stress through a series of changes in ecophysiological processes, including plant growth (Koyro *et al*., 2013; Rasool *et al*., 2013; Srinieng *et al*., 2015), photosynthesis properties (Koyro *et al*., 2013; Wankhade & Sanz, 2013) and lipid metabolism (Rasool *et al*., 2013; Wankhade & Sanz, 2013). Salinity can hurt plants in various way, with direct effects including ion toxicities and osmotic stress (Khan, 2000). Generally, osmotic adjustment can be achieved through the synthesis of organic solutes such as proline and sugars (Mišić *et al*., 2012; Parida & Jha, 2013) and the uptake of ions such as Na^+ and K^+ (Morant-Manceau *et al*., 2004; Vasquez *et al*., 2006). Moreover, salinity can cause oxidative stress through the excessive production of reactive oxygen species (ROS), such as superoxide radicals ($O_2^{\cdot-}$), hydroxyl radicals ($^{\cdot}OH$) and hydrogen peroxide (H_2O_2) (Koyro *et al*., 2013; Yıldıztugay *et al*., 2013). The accelerated accumulation of ROS can induce oxidative damage to multitudinous cellular constituents, such as proteins, membrane lipids and nucleic acids (Mittler, 2002). One of the most destructive oxidative impacts is the peroxidation of membrane lipids, which leads to the accompanying generation of malondialdehyde (MDA) (Sekmen *et al*., 2012, Koyro *et al*., 2013). Consequently, a high MDA level is a helpful biomarker of the lipid peroxidation and is therefore often used to emphasize the occurrence of oxidative stress situations as induced by salinity stress (Hernández & Almansa, 2002). To control the level of ROS, plants possess a well-developed intricate antioxidant defense system containing enzymatic and non-enzymatic antioxidative processes (Blokhina *et al*., 2003). The antioxidative enzymes contain superoxide dismutases (SOD), guaiacol peroxidase (POD), catalases (CAT) and ascorbate peroxidase (APX), which can remove, neutralize and/or eliminate oxidative species (Mittler, 2002, Blokhina *et al*., 2003). SOD is a crucial antioxidant enzyme, working as an $O_2^{\cdot-}$ scavenger in living organisms by promptly transforming $O_2^{\cdot-}$ into H_2O_2 and O_2 as a front-line defense against oxidative stress induced by ROS. H_2O_2 can destroy the cellular plasma membrane lipids and/or other biomolecules (Mittler, 2002). Accordingly, the effective detoxification of H_2O_2 is necessary to alleviate oxidative damage. CAT and some types of peroxidases can scavenge the resulting H_2O_2. CAT dismutates H_2O_2 into H_2O and O_2. APX catalyzes the decreased H_2O_2 using ascorbate as an electron donor in the first step of the ascorbate-glutathione cycle and is the most valuable plant peroxidase in H_2O_2 elimination (de Azevedo Neto *et al*., 2006). POD decomposes H_2O_2 through the oxidation of

co-substrates such as phenolic compounds and/or antioxidants (Blokhina *et al*., 2003).

Nitraria roborowskii Kom, a succulent and creeping growth shrub belonging to the *Nitraria* genus in Zygophyllaceae, is a typical desert halophyte and an ecologically and economically important species in arid and semiarid land with high-saline soils (Wang *et al*., 2007). In China, this species is mainly distributed in the provinces of Xinjiang, Gansu and Inner Mongolia; it often forms a dominant population and plays an important role in sand stabilization (Pan *et al*., 1999; Wang *et al*., 2007). Nevertheless, little information is available on its biophysiological mechanisms in a salt environment. Hence, it is necessary to research the relationship between *N. roborowskii* and salinity stress for arid and semiarid land conservation. In our research, we are interested in (i) evaluating the effect of salinity stress on the growth of *N. roborowskii*; (ii) determining the Na^+ and K^+ concentrations in the leaves, shoots and roots of *N. roborowskii;* and (iii) determining the activity levels of antioxidative enzymes (i.e., SOD, CAT, POD and APX), the contents of proline and soluble sugar in the leaves of *N. roborowskii,* and the water potential, relative water content, H_2O_2 content, lipid peroxidation, photosynthetic pigment content and chlorophyll fluorescence of *N. roborowskii* to observe the stress that is induced by salinity and the level of tolerance and the detoxification strategy used by *N. roborowskii*.

Materials and Methods

Plant material and experimental treatments: A pot experiment was carried out in the field 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.), from 10 April to 20 August 2011. During the experimental period, the temperature ranged from 10℃ to 40℃ by day and 5°C to 20°C by night, the relative humidity ranged from 20% to 60%, and the precipitation was approximately 5.6 mm. One-year-old *Nitraria roborowskii* Kom plants (n = 50) with an initial height of 15 cm were transplanted on 10 April into plastic pots (diameter 40 cm, height 32 cm) filled with 20 kg soils collected from a desert-oasis transitional zone (sampling depth of 0–30 cm) and previously passed through a sieve of 2 mm. Soils were Aeolian loose sediments, and the soil texture was highly homogeneous; the main fraction of the particle size was silt (> 88%), with sand (particle size 63–2000 μm) and clay (particle size < 2 μm) generally accounting for less than 5% of the soil texture (Thomas *et al*., 2006). The soil properties were as follows: pH 8.52 (soil water ratio 1:5), bulk density 1.494 g cm^{-3}, electrical conductivity 0.54 mS cm^{-1}, total salinity 1.749 g kg^{-1}, organic carbon 0.848 g kg^{-1}, organic matter 1.462 g kg^{-1}, total N 0.089 g kg^{-1}, total P 0.571 g kg^{-1}, total K 15.766 g kg^{-1}, available N 12.992 mg kg^{-1}, available P 2.362 mg kg^{-1}, and available K 224.751 mg kg^{-1}. To avoid plants from being burned by high temperature, the pots were embedded in the soil.

Irrigation with saline water began on 20 May, 40 days after transplanting. Saline growth conditions were simulated by applying NaCl to deionized water at five concentrations (0, 50, 100, 200 and 400 mM). To avoid salinity shock, the NaCl was added stepwise in aliquots of 50 mM daily until the given concentration was reached. Each treatment had 10 pots. After 90 days, the plants were harvested for analysis.

Measurements of growth: The plant shoot height, number of branches, east-west canopy diameter (D_{EW}) and north-south canopy diameter (D_{NS}) were recorded. The canopy area was calculated from the following equation: Canopy area = $[(1/4)\pi \times D_{EW} \times D_{NS}]$ (Jia *et al*., 2009). Whole plants were harvested to measure the biomass. After a rapid washing with abundant deionized fresh water and rinsing three times with deionized water, the plant leaves, branches and roots were separated and dried in an oven at 70°C until an invariable dry mass was acquired. Each treatment was replicated with six seedlings. Dry samples were used to determine ion contents on a dry-weight basis.

Na^+ and K^+ concentration analysis: Finely ground oven-dried tissues (0.1 g) were digested overnight with 25 ml of 0.1 M HNO_3 at room temperature (John *et al*., 2003). The concentrations of Na^+ and K^+ in the acid extract were measured by an inductively coupled plasma-optical emission spectrometer (ICP-OES, Agilent 735, Santa Clara, CA, USA).

Photosynthetic pigment contents and the maximal efficiency of photosystem II photochemistry (F_v/F_m): The photosynthetic pigment content was extracted with 80% acetone. The clear supernatant that was obtained after centrifugation at 480 × g for 3 min was used to estimate Chl using the extinction coefficients and the equations of Lichtenthaler (1987).

F_v/F_m was measured using a portable fluorometer (OS-30, Opti-Sciences, USA) at 11:00 on a clear day. Chlorophyll fluorescence was measured in 3 leaves of the same plant, and four plants were chosen for each treatment.

Measurements of the leaf relative water content and water potential: The relative water content (RWC) was determined according to Smart and Bingham (1974) and calculated using the following formula:

$$RWC = (FW\text{-}DW)/(FSW\text{-}DW) \times 100\%.$$

Dry weight (DW) was obtained after oven-drying the plant leaves for 48 h at 70°C. The fully saturated weight (FSW) was measured after floating plant leaves on distilled water for 4 h at 20°C in the dark. The leaf water potential was measured on freshly cut leaves using a WP4 Dewpoint Water Potential Meter (Decagon Devices, Inc, Pullman, WA, USA).

Total soluble sugar and proline content determination: The concentration of soluble sugars was determined using the anthrone method (Palma *et al*., 2009). The proline concentration was determined by the method of Bates *et al.* (1973).

Lipid peroxidation and H_2O_2 content determination: Lipid peroxidation was determined as the amount of MDA and measured by the thiobarbituric acid reaction (Buege & Aust, 1978). H_2O_2 levels were determined according to the method of Sergiev *et al*. (1997).

Enzyme extraction and assays: The supernatant that was used to measure the SOD, POD, CAT and APX activities and soluble protein concentration was obtained according to the method of Lu *et al*. (2010). The SOD activity was detected by the method of Beauchamp & Fridovich (1971). The CAT activity was obtained according to the method of Aebi (1984). The POD activity was measured in accordance with the method of Chance & Maehly (1955). The APX activity was assayed according to the method of Nakano & Asada (1981). Proteins were detected by the method of Bradford (1976).

Statistical analysis: Six independent experiments were carried out to measure the influence of NaCl treatments on plant biomass and ion accumulation. For the selected biochemical parameters, four independent experiments with two parallel samples were performed. The data appearing here are the means ± SD. The mean values were separated by Fisher's LSD test at a 0.05 probability level. A linear regression analysis was used to evaluate the relationship between the NaCl concentration and the tested parameters at different salinities. The calculation was conducted by SPSS Ver. 13 Inc., Chicago, USA.

Results

Responses of growth parameters to NaCl treatment: The plant height of *N. roborowskii* still was not significantly influenced by NaCl exposure (Table 1). The crown area, number of branches and dry matter of the leaves, stems and roots peaked under the 50 mM NaCl treatment and decreased progressively with enhancing NaCl concentrations compared to the control. *N. roborowskii* required 50 mM NaCl to reach its maximum growth potential. The root-shoot ratio was negatively correlated with increasing NaCl concentration ($R = -0.967$, $p<0.01$).

Responses of the photosynthetic pigment content and F_v/F_m to NaCl treatment: The chlorophyll and carotenoid contents were maximal in leaves of *N. roborowskii* treated with 50 mM NaCl and decreased gradually with enhanced NaCl concentration (Table 2). The addition of NaCl at all concentrations had no noticeable negative influence on F_v/F_m. The Chl a/b ratio showed an irregular trend with increasing NaCl concentrations: it decreased remarkably by 23.3, 11.7 and 8.4% in 50, 100 and 200 mM NaCl-treated plants, respectively, and increased slightly by 3.4% in 400 mM NaCl-treated plants compared to the control. The Chl/Car ratio decreased with the increasing salinity ($R = -0.808$, $p<0.05$).

Responses of leaf relative water content and water potential to NaCl treatment: The relative water content and water potential in leaves of *N. roborowskii* were significantly affected by salinity; they decreased with the increasing NaCl concentration ($R_{RWC} = -0.892$, $p<0.05$; $R_{WP} = -0.915$, $p<0.05$) (Fig. 1). These values decreased by 4.7–15.7% and 12.1–55.6%, respectively, with increased NaCl concentration compared to the control.

Responses of total soluble sugar and proline content to NaCl treatment: The total soluble sugar and proline contents in the leaves of *N. roborowskii* were not markedly affected by low salinity (50 mM); however, these values decreased gradually at high salinities (≥ 100 mM NaCl) by 7.2–24.8% and 22.2–38.7%, respectively, compared to the control (Fig. 2).

Table 1. Effect of NaCl treatments on growth of *N. roborowskii*.

	NaCl treatment, mM				
	0	**50**	**100**	**200**	**400**
Shoot height (cm)	29.6 ± 2.45^{a}	30.1 ± 1.66^{a}	28.4 ± 1.88^{a}	27.7 ± 1.92^{a}	27.1 ± 2.42^{a}
Crown area (cm^2 $plant^{-1}$)	1744 ± 162.6^{c}	1948 ± 179.9^{c}	1549 ± 118.5^{b}	1044 ± 109.2^{a}	1028 ± 98.4^{a}
Number of branches per plant	9.50 ± 0.55^{b}	11.00 ± 1.26^{c}	9.17 ± 1.47^{ab}	8.50 ± 0.55^{ab}	8.00 ± 0.80^{a}
Leaf dry matter (g $plant^{-1}$)	3.30 ± 0.161^{d}	3.45 ± 0.216^{d}	3.01 ± 0.149^{c}	2.55 ± 0.187^{b}	1.86 ± 0.176^{a}
Stem dry matter (g $plant^{-1}$)	4.92 ± 0.281^{c}	5.45 ± 0.225^{d}	4.83 ± 0.211^{c}	4.35 ± 0.245^{b}	3.45 ± 0.215^{a}
Root dry matter (g $plant^{-1}$)	10.71 ± 1.013^{c}	11.02 ± 1.173^{c}	9.65 ± 0.631^{c}	7.32 ± 1.039^{b}	5.59 ± 0.697^{a}
Root shoot ratio	1.303 ± 0.160^{a}	1.236 ± 0.085^{a}	1.201 ± 0.077^{b}	1.057 ± 0.097^{b}	1.051 ± 0.087^{b}

Values are mean ± SD of six independent experiments. Different letters in the same column mean significant differences ($p < 0.05$) according to Fisher's LSD test

Table 2. Responses of photosynthetic pigment content and F_v/F_m in leaves of *N. roborowskii* to NaCl treatments.

NaCl treatment mM	Chl a (mg g^{-1} FW)	Chl b (mg g^{-1} FW)	Chl a + b (mg g^{-1} FW)	Car (mg g^{-1} FW)	Chl a/b	Chl/Car	F_v/F_m
0	0.422 ± 0.035^{b}	0.086 ± 0.009^{b}	0.507 ± 0.045^{b}	0.087 ± 0.005^{a}	4.93 ± 0.12^{c}	5.94 ± 0.33^{b}	0.839 ± 0.021^{a}
50	0.486 ± 0.026^{c}	0.129 ± 0.007^{c}	0.615 ± 0.033^{c}	0.112 ± 0.002^{b}	3.78 ± 0.07^{a}	5.49 ± 0.51^{ab}	0.852 ± 0.022^{a}
100	0.386 ± 0.032^{ab}	0.089 ± 0.005^{b}	0.474 ± 0.036^{ab}	0.085 ± 0.004^{a}	4.35 ± 0.19^{b}	5.57 ± 0.43^{ab}	0.837 ± 0.020^{a}
200	0.363 ± 0.017^{a}	0.080 ± 0.003^{ab}	0.444 ± 0.021^{ab}	0.083 ± 0.005^{a}	4.52 ± 0.06^{b}	5.33 ± 0.54^{ab}	0.823 ± 0.023^{a}
400	0.352 ± 0.032^{a}	$0.069 \pm 0.005a$	0.421 ± 0.038^{a}	0.080 ± 0.005^{a}	5.10 ± 0.12^{c}	5.27 ± 0.34^{a}	0.818 ± 0.026^{a}

Values are mean ± SD of four independent experiments. Different letters in the same column mean significant differences ($p<0.05$) according to Fisher's LSD test

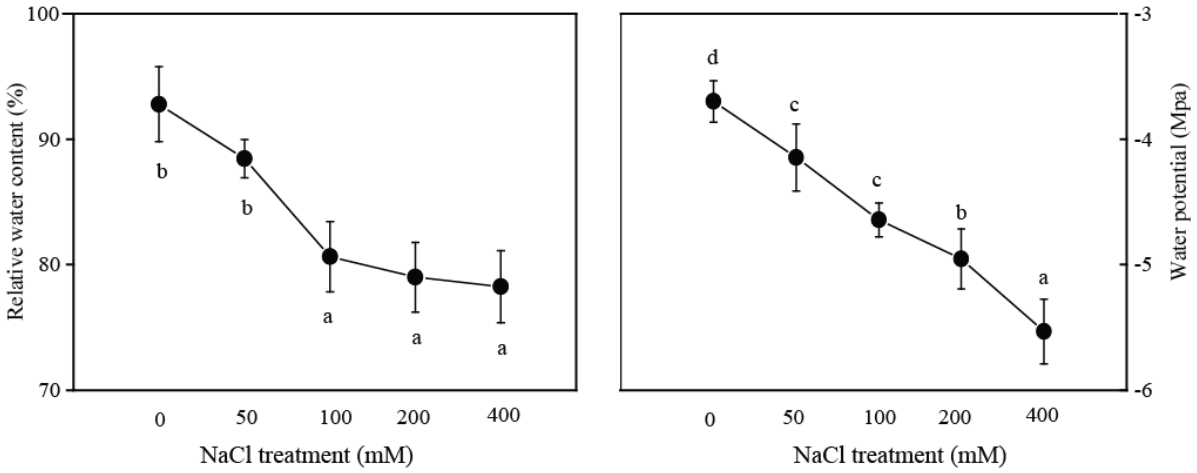

Fig. 1. Leaf relative water content and water potential under different NaCl concentrations in *N. roborowskii*. Each value represents mean ± SD of four independent experiments. The lines with different letters mean significant differences (p<0.05) according to Fisher's LSD test.

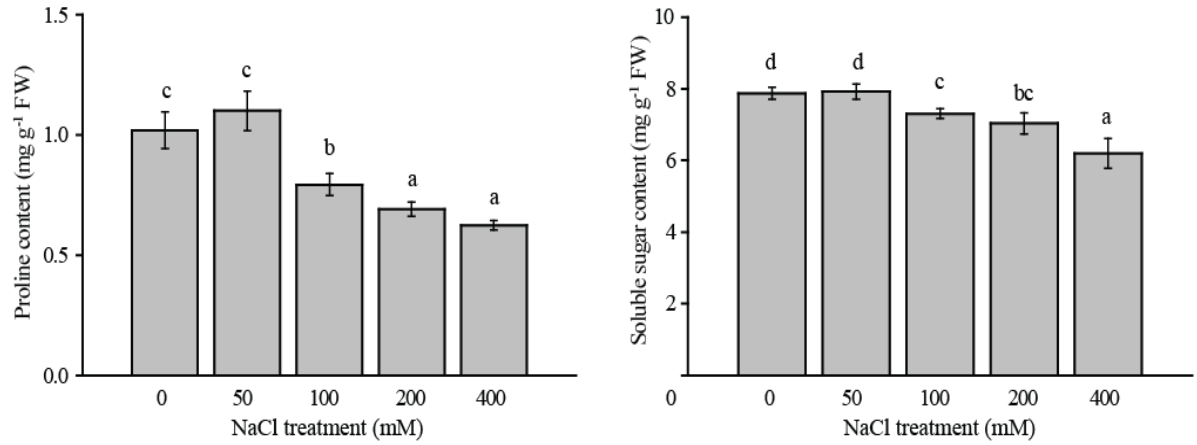

Fig. 2. Effect of different concentrations of NaCl on total soluble sugars and proline content in leaves of *N. roborowskii*. Each value represents mean ± SD of four independent experiments. The bars with different letters mean significant differences (p<0.05) according to Fisher's LSD test.

Responses of Na^+ and K^+ concentrations to NaCl treatment: The measured ions had different distributions in different tissues of *N. roborowskii* after the elevated NaCl treatments (Fig. 3). The content of Na^+ in leaves significantly accumulated with the increased NaCl concentration; however, in stems and roots, this content did not noticeably change with low concentrations of NaCl. In stems, this content increased drastically in the ≥ 100 mM salt solution, while in roots, it accumulated markedly in the ≥ 200 mM salt solution. The K^+ content increased in leaves in all of the NaCl treatments except for the 100 mM NaCl treatment, showing an insignificant change in stems under all of the NaCl treatments. In roots, this content accumulated only in the 400 mM NaCl treatment.

Responses of lipid peroxidation and H_2O_2 content to NaCl treatment: The H_2O_2 and MDA contents decreased slightly at low salinity (50 mM NaCl); however, these values significantly accumulated at elevated NaCl concentrations (≥ 100 mM) (Fig. 4). In the 400 mM NaCl treatment, the H_2O_2 and MDA contents were approximately 45.3% and 36.2% higher than the control, respectively.

Responses of antioxidative enzymes to NaCl treatment: In *N. roborowskii* grown under elevated NaCl concentrations, the activities of SOD, CAT, POD and APX changed differently (Fig. 5). The SOD and CAT activities increased and peaked in the 100 mM NaCl treatment, increasing by 39.8% and 46.5%, respectively, and then decreased gradually compared to the control (Fig. 5a, b). In the 400 mM NaCl treatment, the SOD and CAT activities were approximately 23.7% and 56.2% significantly lower than those of the control, respectively. The POD activity increased significantly in the low NaCl treatment (50 mM), peaked, increasing by 28.5%, and then significantly decreased by 27.6–72.2% with elevated NaCl concentrations compared to the control (Fig. 5c). In contrast to the SOD, POD and CAT activities, the APX activity increased with NaCl concentration, peaked at 200 mM, increasing by 76.2%, and was still markedly higher than the control level at the highest NaCl concentration (Fig. 5d).

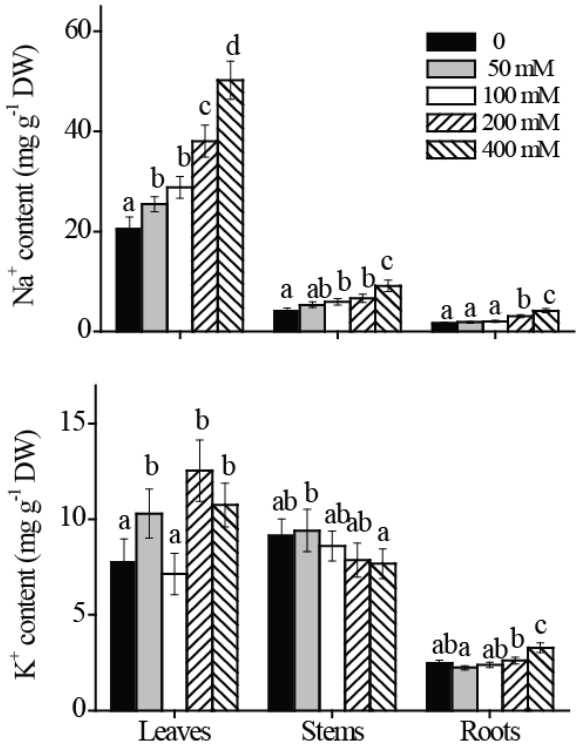

Fig. 3. Concentration of Na^+ and K^+ in leaves, stems and roots of *N. roborowskii* under different NaCl concentrations. Each value represents mean ± SD of four independent experiments. The bars with different letters in the same organ mean significant differences ($p < 0.05$) according to Fisher's LSD test.

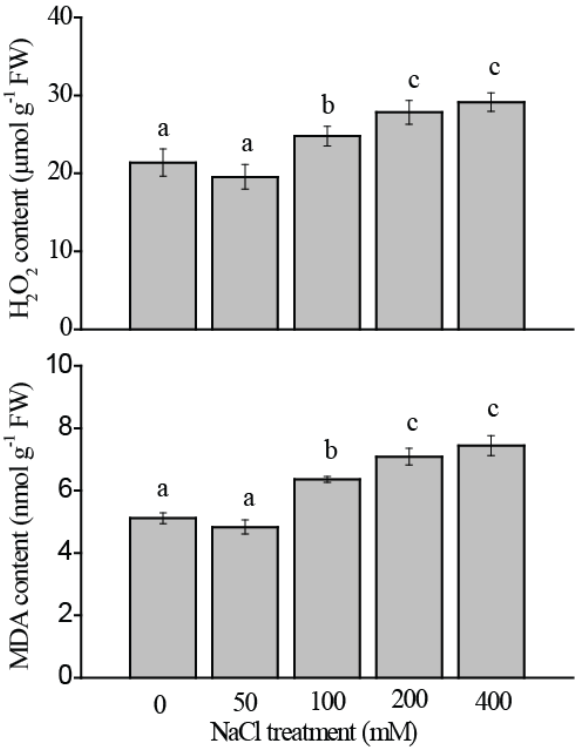

Fig. 4. Effect of different concentrations of NaCl on H_2O_2 content and lipid peroxidation (in terms of MDA content) in leaves of *N. roborowskii*. Each value represents mean ± SD of four independent experiments. The bars with different letters mean significant differences ($p<0.05$) according to Fisher's LSD test.

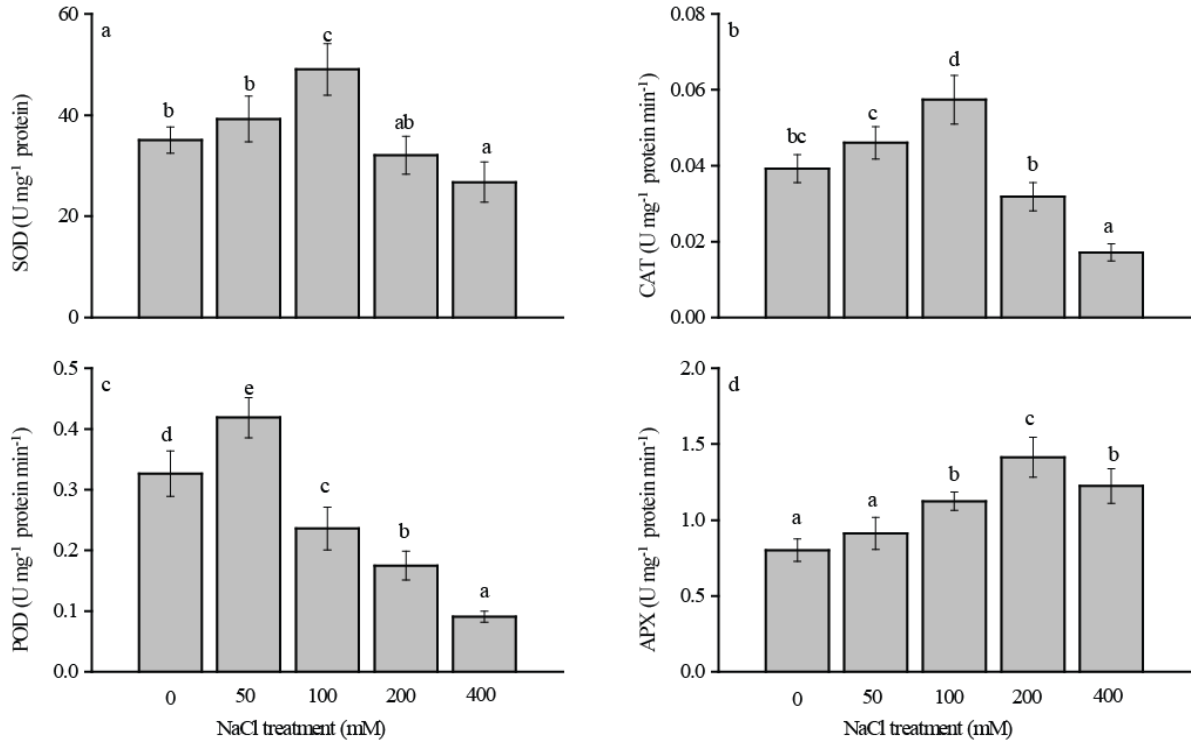

Fig. 5. Antioxidative enzyme (SOD, CAT, POD and APX) activities in leaves of *N. roborowskii* under different NaCl concentrations. a SOD activity, b CAT activity, c POD activity, d APX activity. Each value represents mean ± SD of four independent experiments. The bars with different letters mean significant differences ($p<0.05$) according to Fisher's LSD test.

Discussion

Suppressed plant growth is one of the most obvious results of salinity stress (Munns, 2002; Rasool *et al*., 2013). In this study, the growth of *N. roborowskii* was affected by 50 mM NaCl, thereby demonstrating the halophytic peculiarity of this genus, as previously reported by other authors (Chen *et al*., 2010; Hussin *et al*., 2013; Sharma & Ramawat, 2014). However, a reduction in growth of *N. roborowskii* under high saline conditions (≥ 100 mM) has been detected, and similar results have been reported in other halophytes (Gorai & Neffati, 2011; Khadhri *et al*., 2011). Salinity can decrease the capacity of plants to take up water, rapidly leading to reductions in growth rate, accompanied by a suite of metabolic changes (Munns, 2002).

The inhibition of photosynthesis may be associated with detected declines in plant growth (Agrawal *et al*., 2013; Koyro *et al*., 2013). Accordingly, the growth of *N. roborowskii* corresponds to chlorophyll contents. The chlorophyll content initially increased under the low NaCl concentration (50 mM). Similar results have been reported in salt-tolerant plants in which the chlorophyll content increased in plants that were cultivated in NaCl-containing soils (Parida *et al*., 2004; Yao *et al*., 2010); however, in other studies, the chlorophyll content decreased by NaCl addition (Sabra *et al*., 2012; Wankhade & Sanz, 2013). In our study, a decreased chlorophyll content was observed in the high NaCl treatments (≥ 100 mM). The decreased leaf chlorophyll content under NaCl stress could be attributed to damaged chlorophyll pigments, reduced chlorophyll synthesis and the instability of the pigment protein complex (Levitt, 1980). The Chl a/b ratio is often regarded as a parameter of the light-harvesting ability of chloroplasts and shows the shade or sun habits of plants (Anderson *et al*., 1988). A Chl a/b ratio value of 3.0 suggest the normal constitution of light-harvesting complexes, and variations in this ratio indicate adaptive changes (Das *et al*., 2002). In our study, the Chl a/b ratio fluctuated from 3.78 to 5.10 in *N. roborowskii*, which may reflect its normal adaptive features under salinity stress. The responses of the Chl/Car ratio to NaCl treatment were related to their salt tolerance: the lower the increase in the ratio, the greater was the plant tolerance to salinity (Rout & Shaw, 2001). Thus, maintaining a lower Chl/Car ratio compared to that of the control is one tolerance mechanism in *N. roborowskii* to adapt to salinity stress. The photosynthetic apparatus, especially PSII, is sensitive to various environmental stress conditions (Zivak *et al*., 2013; Hanachi *et al*., 2014; Li *et al*., 2014). Therefore, F_v/F_m may be regarded as an indicator to detect the tolerance capacity of plants to environmental factors (Ow *et al*., 2011; Schoedl *et al*., 2013), and an F_v/F_m ratio of 0.8 or above is characteristic of a healthy plant (Dan *et al*., 2000). In our study, F_v/F_m remained above 0.8 in *N. roborowskii*, suggesting the optimal functioning of PSII. Our study shows that the photosynthetic efficiency of PSII is well protected when exposed to salinity stress.

Water rations in plants are influenced by salinity (Gorai & Neffati, 2011; Nabati *et al*., 2011; Sai Kachout *et al*., 2011). In *N. roborowskii*, the leaf water potential peaked (down to –5.53 MPa) under the highest salinity treatment (400 mM NaCl), and similar responses have been found in other halophytes such as *Reaumuria vermiculata* (Gorai & Neffati, 2011) and *Atriplex halimus* (Hassine & Lutts, 2010) in which the leaf water potential decreased with increasing salinity level. In plants, this reduction was due to decreased RWC and leaf turgor potential (Gimeno *et al*., 2012). Given that plants accumulate non-toxic compatible solutes that maintain the turgor and water content (Hare *et al*., 1988), the accumulation of proline and sucrose may be an adaptive mechanism to prevent water loss. Proline helps plants to maintain cell turgor, protect membrane integrity, and prevent protein denaturation under various environmental stresses (Hong *et al*., 2000), and sucrose is often proposed as one of the osmolytes that can maintain turgor and sufficient hydration during water loss (Sánchez *et al*., 2004). An increase in proline, sucrose and soluble sugars has been widely reported under various stresses, including salinity stress (Gorai & Neffati, 2011; Lokhande *et al*., 2013; Sajid & Aftab *et al*., 2014). However, a significant accumulation of proline and soluble sugar was not detected in *N. roborowskii* under any NaCl concentration. In *N. roborowskii,* the Na^+ content rather than the soluble sugar and proline contents significantly increased under salinity stress, indicating that Na^+ was the pivotal contributor to osmotic adjustment in response to low external water potential in succulent plants (Cai *et al*., 2011; Zhang *et al*., 2004). At the cellular level, the use of organic solutes for osmotic adjustment could be an energy-consuming process (Flowers & Colmer, 2008), and the synthesis of organic solutes, such as proline, costs more energy compared to the uptake of Na^+.

Oxidative stress is one of the main factors causing cellular damage in plants when subjected to diverse stress conditions, including salinity stress (Koyro *et al*., 2013; Rasool *et al*., 2013). In higher plants, salinity induces oxidative stress by the production of ROS (Sekmen *et al*., 2012, Guzmán-Murillo *et al*., 2013). The protonation of $O_2^{\cdot-}$ can generate hydroperoxy radicals (H_2O_2 and $^{\cdot}OH$), which can transform fatty acids into harmful lipid peroxides, destroying biological membranes (Grant & Loake, 2000). MDA is a product of lipid peroxidation in plants under oxidative stress and is a biomarker of the degree of oxidative stress (Castelli *et al*., 2010). In *N. roborowskii,* the increased NaCl concentration at a high level markedly increased the production of H_2O_2 and correspondingly caused lipid peroxidation, as suggested by the accumulated MDA content, which is equivalent to the results of salinity stress in other higher plants (Sekmen *et al*., 2012; Yıldıztugay *et al*., 2013; Achakzai *et al*., 2014).

$O_2^{\cdot-}$ and H_2O_2 are the central constituents of signal transduction and can initiate the defense genes that encode antioxidative enzymes containing SOD, consequently enhancing the capacity for the elimination of $O_2^{\cdot-}$ (Mittler *et al*., 2004). Upon exposure to stress, SOD upregulates the activities of several antioxidative enzymes in plants, as presented in this research, and appears to be responsible, at least in part, for the comprehensive oxidative tolerance (Taha *et al*., 2000). *N. roborowskii* has shown a certain ability to upregulate SOD activity under lower salinity stress. Moreover, the

decreased SOD activity may be associated with non-enzymatic antioxidative processes promoted by antioxidant compounds (i.e., phenolics), which can be scavengers under high stress conditions (Koca *et al*., 2007). Furthermore, very high SOD activities may be harmful for plants due to high H_2O_2 production, which inhibits other enzymes such as APX (Asada, 1994). The product of SOD activity is H_2O_2, which is toxic and must be eliminated by conversion to H_2O in subsequent reactions. In plants, CAT, POD and APX play important roles in the degradation of H_2O_2 (Mittler, 2002; Blokhina *et al*., 2003). In the present study, a significant increase in POD and CAT activities was noticed only in the 50 and 100 mM concentrations compared to the control, respectively, suggesting that the POD and CAT activities function in the H_2O_2 detoxifying process only under a certain level of H_2O_2 accumulation. In response to higher saline environments, POD and CAT were restrained, possibly as a result of ROS-triggered inhibition. The significant upregulation of APX activity was observed under high concentrations of NaCl. In the plant cells, ascorbate is the most important reducing substrate for H_2O_2 detoxification and is used by APX to reduce H_2O_2 to water (Noctor & Foyer, 1998). The increase in APX activity is a significant indicator of high salinity tolerance (Sergio *et al*., 2012), and induced APX activity has been found in many other salt-tolerant plants (Amor *et al*., 2006, Yıldıztugay *et al*., 2011, Radić *et al*., 2013). In *N. roborowskii*, H_2O_2 was scavenged by POD and CAT at lower and by APX at higher saline situations. Those results suggest that *N. roborowskii* is equipped with a responsive and balanced antioxidant system, making this species moderately inducible under hyperosmotic and/or hyperionic situations triggered by NaCl.

Conclusion

The data presented here suggest that (i) similar to other halophytes, the growth of *N. roborowskii* is not affected at low salinity, yet decreases at higher salinities; (ii) despite the synthesis of Chl content being inhibited under high salinity stress, the photosynthetic efficiency of PSII is only slightly affected; (iii) *N. roborowskii* can take up a high content of Na^+ in a saline environment and use it directly as an osmoregulatory substance; (iv) H_2O_2 accumulation and lipid peroxidation indicate the appearance of oxidative stress at high salinities; and (v) the SOD, CAT, POD and APX antioxidant system of *N. roborowskii* is moderately inducible under salt stress.

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