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Research paper

Sodium chloride improves photosynthesis and water status in the succulent xerophyte *Zygophyllum xanthoxylum*

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Zygophyllum xanthoxylum, a C_3 woody species, is a succulent xerophyte that is well adapted to arid environments. Our previous investigations showed that Na^+ has a positive effect on the growth of *Z. xanthoxylum* under drought conditions, which was closely related to high Na^+ accumulation in leaves. To reveal the physiological mechanisms underlying how Na^+ accumulation improves the drought resistance of *Z. xanthoxylum*, 3-week-old seedlings were treated with a series of additional external NaCl concentrations (5–150 mM) in sand culture experiments. Seedlings were also subjected to water deficit (30% of field water capacity) in the presence or absence of additional NaCl (50 mM) in pot experiments. The results indicated that 50 mM NaCl could mitigate deleterious impacts of water deficit on the growth of *Z. xanthoxylum*, by improving the relative water content, inducing a significant drop in leaf water potential and, concomitantly, increasing leaf turgor pressure and chlorophyll concentrations resulting in an enhancement of overall plant photosynthetic activity (i.e., photosynthetic rate and water use efficiency). Furthermore, NaCl (50 mM) could alleviate the inhibitory effect of water deficit on the activity of photosystem II in *Z. xanthoxylum*. The contribution of Na^+ to the total osmotic potential varied from 8% in the control to 13% in plants subjected to water deficit and, surprisingly, to 28% in plants grown in the presence of 50 mM NaCl under water deficit; however, the contribution of K^+ significantly decreased from 13 to 8%. These findings suggest that, under arid environments, *Z. xanthoxylum* is able to accumulate a high concentration of Na^+ in its leaves and use it directly for osmotic adjustment, which was coupled with an improvement in leaf hydration and photosynthetic activity.

Keywords: photosynthesis, sodium, succulent xerophyte, water deficit, water status, *Zygophyllum xanthoxylum*.

Introduction

Drought is one of the major abiotic stresses constituting a serious threat to agricultural productivity throughout the world (Ben Hassine et al. 2010). This stress triggers desertification coupled with a progressive reduction of vegetation coverage and rapid soil erosion in arid and semi-arid regions (Martínez et al. 2005, Slama et al. 2007). Some plants, such as xerophytes, have, however, evolved multiple protective physiological mechanisms that allow them to survive and grow well in these harsh environments. To cope with the challenge of water deficiency for agriculture, there is increasing interest in studying the physiological behavior of xerophyte species to understand their drought-resistance mechanisms (Chaves and Oliveira 2004, Yamaguchi and Blumwald 2005, Sambatti and Caylor 2007, Ashraf 2010).

Drought resistance is a complex trait involving several interacting properties. One of the main physiological adaptations in plants is osmotic adjustment (OA), which is defined as the lowering of osmotic potential (Ψ_s) in plant tissues due to the net accumulation of cellular solutes in response to a fall in the water potential of the environment (Zhang et al. 1999, Ramanjulu and Sudhakar 2000). Besides organic solutes, such as proline (Raymond and Smirnoff 2002), glycine betaine (Rhodes and Hanson 1993), soluble sugars and polyols (Tattini et al. 1996, Sun et al. 1999), K^+ is thought to be involved in OA in both the vacuoles and cytosol of numerous glycophytic species under

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water deficit (De La Guardia and Benlloch 1980, Mengel and Arneke 1982). However, in some halophytes, Na$^+$, which used to be considered toxic to almost all glycophytes, was found to have a positive function in response to water deficit as evidenced by a specific increase in Na$^+$ absorption when plants were subjected to drought (Zhu 2003, Martínez et al. 2003, 2004). According to Glenn and Brown (1998) and Slama et al. (2007), drought resistance in *Atriplex canescens* and *Sesuvium portulacastrum* was linked through a common mechanism of Na$^+$ uptake, which was directly used for OA. But Martínez et al. (2005) estimated that despite a significant water deficit-induced stimulation of Na$^+$ absorption by *Atriplex halimus*, the direct contribution of this element to OA was negligible. Therefore, although Na$^+$ plays an important role in the growth of halophytes under drought conditions, the real contribution of Na$^+$ to OA is still a matter of debate. Furthermore, such data concerning xerophytes in arid and semi-arid regions are scarce.

Zygophyllum xanthoxylum, a C_3 perennial woody species native to the desert areas of northwest China, is a succulent xerophyte belonging to Zygophyllaceae with excellent adaptability to adverse arid environments (Liu et al. 1987, 1988, Pei et al. 2004). This species has a strong ability for sand-fixing, and for soil and water conservation in desert regions (Wang et al. 2004). Meanwhile, the high palatability and nutrient value make *Z. xanthoxylum* attractive as a forage crop in local regions, and its leaves, stems, roots and fruits have high medicinal value (Wu et al. 2004; Zhou et al. 2006). Wang et al. (2004) hypothesized that Na$^+$ may have positive effects on the growth of *Z. xanthoxylum* in arid environments, since it was found that the plant absorbed a great quantity of Na$^+$ from low-salinity soil that was transported to leaves under arid field conditions. Further studies confirmed that 50 mM NaCl could not only stimulate growth of *Z. xanthoxylum* under well-irrigated conditions, but also alleviate the deleterious impact of water deficit, which was closely related to the high Na$^+$ accumulation in leaves (L.J. Yue and S.M. Wang, unpublished data). However, the possible physiological mechanisms involved in these observations had not been explored.

The present work was initiated to determine whether NaCl can stimulate growth and enhance the drought resistance of *Z. xanthoxylum* seedlings through a role in OA and by modifying other physiological parameters. Plants were subjected to different NaCl treatments and water deficit in the presence or absence of additional NaCl. Parameters related to OA, photosynthesis and water status were assessed.

Materials and methods

Plant growth conditions and salt treatments in sand culture experiments

Seeds of *Z. xanthoxylum* were collected from wild plants in Alxa League (39°05′N, 105°34′E; elevation 1360 m) of Inner-Mongolia Autonomous Region, China. The mean annual rainfall and temperature were 60–150 mm and 8 °C, respectively, and the mean annual wind velocity was 3.4–4.7 m s^{-1}. The concentrations of available N, P and K in soil were 2.1, 0.08 and 4.7 μmol g^{-1} dry soil, respectively. After removal of the bracts, seeds were surface sterilized for 1 min in 75% ethanol (v/v) and rinsed three times with distilled water, soaked in distilled water for 1 day and then germinated at 25 °C in the dark for 2 days. Uniform seedlings were transplanted to a plugged hole in plastic containers (5 × 5 × 5 cm; one seedling/container) filled with sand irrigated with modified 1/2 strength Hoagland nutrient solution containing 2 mM KNO_3, 0.5 mM $NH_4H_2PO_4$, 0.25 mM $MgSO_4{\cdot}7H_2O$, 0.1 mM $Ca(NO_3)_2{\cdot}4H_2O$, 50 μM Fe-citrate, 92 μM H_3BO_3, 18 μM $MnCl_2{\cdot}4H_2O$, 1.6 μM $ZnSO_4{\cdot}7H_2O$, 0.6 μM $CuSO_4{\cdot}5H_2O$ and 0.7 μM $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$. Solutions were renewed every 3 days. Seedlings were grown in a greenhouse where the temperature was 28 °C/23 °C (day/night), the daily photoperiod was 16/8 h (light/dark; the flux density was ~800 μmol m^{-2} s^{-1}) and the relative humidity was about 65%.

Three-week-old plants were treated with modified 1/2 strength Hoagland nutrient solution supplemented with additional 0, 5, 50 or 150 mM NaCl. For the 150 mM NaCl treatment, plants were treated with 50 mM NaCl for 1 day, and were then transferred to the 150 mM NaCl treatment. Plants were harvested at 0, 1, 3, 5 and 7 days after treatments for physiological analysis. Treatment solutions were changed once every 2 days to maintain constant NaCl concentration.

Plant growth conditions and drought treatments in pot experiments

Seeds were germinated as described above and then seedlings were transplanted into plastic pots (18 cm high × 20 cm diameter) filled with 1 kg oven-dried vermiculite and irrigated with modified 1/2 strength Hoagland nutrient solution. The water content of the substrate was maintained at 70% of field water capacity (FWC) by weighing, and the rest of the growth conditions were the same as that in the sand culture experiments.

After 30 days, seedlings were divided into three groups: control (C), drought treatment (D) and drought with additional 50 mM NaCl treatment (D + S). The water content of the substrate in the C group was maintained at 70% of FWC by irrigating with modified 1/2 strength Hoagland nutrient solution during the experimental period. In the D and D + S groups, the water content of the substrate was maintained at 70% of FWC for 3 days by irrigating with modified 1/2 strength Hoagland nutrient solution without or with additional 50 mM NaCl; then water was withheld for 5 days to induce drought stress gradually. When the water content of the substrate had reduced to 30% of FWC, this value was maintained by irrigating with the corresponding solution. After 7 days, plants were harvested for further physiological analysis. Each treatment consisted of 6 pots containing 10 plants each. To minimize the effects of possible environmental gradients in the greenhouse, pots were randomly reassigned to new positions every day.

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Measurement of the relative growth rate

The relative growth rate (RGR) of whole plants was calculated using the formula RGR = $(\ln W_f - \ln W_i)/\Delta t$, where W_f and W_i are final and initial dry weights, respectively, and Δt is the time elapsed (days) between the two measurements; initial dry weight was determined before treatments (Martínez et al. 2005).

Measurement of photosynthesis-related parameters

Net photosynthesis rate (Pn) and stomatal conductance (Gs) were measured using an automatic photosynthetic measuring apparatus (LI-6400, LI-COR Biosciences, Lincoln, NE, USA) in the greenhouse. All the parameters were measured at a photosynthetic available radiation of 800 μmol m^{-2} s^{-1} (saturated light intensity), 65 ± 5% relative humidity, 28 ± 2 °C leaf temperature and 350 μmol mol^{-1} CO_2 concentration. The water use efficiency (WUE) was estimated using the following formula: WUE = Pn/Gs (Liu et al. 2005).

Chlorophyll fluorescence parameters were measured at room temperature using a portable fluorometer (PAM-2100, Walz, Germany) according to the experimental protocol of Lu et al. (2003). Fluorescence nomenclature was that of van Kooten and Snel (1990).

Leaf area was estimated by the Epson Perfection 4870 photo scanner (Epson America Inc., Long Beach, CA, USA).

Chlorophyll concentrations were estimated according to Porra et al. (1989). Fresh leaf samples were crushed thoroughly with 80% acetone in the dark and centrifuged at 9000 *g* for 10 min at 4 °C. Supernatant was taken as the source of chlorophyll and estimated by reading the absorbance at 645 and 663 nm with a UV spectrophotometer (UV-2102C, Unico Instrument Co., Ltd, Shanghai, China). Chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*) concentrations were estimated using the equations of Porra et al. (1989).

Measurement of the relative water content

The relative water content (RWC) was estimated using the following formula: RWC (%) = 100 × (fresh weight–dry weight)/(turgid weight–dry weight) (Slama et al. 2007).

Fresh weight was determined immediately after harvest. Turgid weight was obtained after the leaves were soaked in deionized water in test tubes at 4 °C overnight in the dark. Finally, leaves were dried in an oven at 80 °C for 48 h and weighed to obtain the dry weight.

Measurement of leaf water potential, osmotic potential and turgor pressure

Leaf water potential (Ψ_w) was measured using the PSYPRO water potential system (C-52 Sample Chamber, WESCOR Inc., Logan, UT, USA). For the osmotic potential (Ψ_s), leaf samples were frozen in liquid nitrogen after measuring Ψ_w. Cell sap was collected by thawing slowly and then Ψ_s was determined using a cryoscopic osmometer (Osmomat-030, Gonotec GmbH, Berlin, Germany) at 25 °C. The readings (mmol kg^{-1}) were used to calculate the solute potential (Ψ_s) in MPa with the formula $\Psi_s = -\text{moles of solute} \times R \times K$; here $R = 0.008314$ and $K = 298.8$. Leaf turgor pressure (Ψ_t) was estimated using the following formula (Ueda et al. 2003): leaf water potential (Ψ_w) = leaf osmotic potential (Ψ_s) + leaf turgor pressure (Ψ_t).

Measurement of leaf Na^+ and K^+ concentrations and the contributions of Na^+ and K^+ to leaf osmotic potential

Na^+ and K^+ concentrations were measured according to the method described by Wang et al. (2007). Briefly, Na^+ and K^+ were extracted from dried plant tissues in 100 mM acetic acid at 90 °C for 2 h. Then ion analysis was performed using an atomic absorption spectrophotometer (2655-00, Cole-Parmer Instrument Co., Vernon Hills, IL, USA). The Ψ_s values of Na^+ and K^+ (calculated osmotic potential; COP) were calculated by the Van't Hoff equation as described by Guerrier (1996): $COP = -nRT$; here n is the number of solute molecules, $R = 0.008314$ and $T = 298.8$. The contributions of Na^+ and K^+ to leaf osmotic potential were estimated by the formula $C = COP/\Psi_s \times 100\%$ (Guerrier 1996).

Measurement of available Na^+ and K^+ concentrations in the medium in pot experiments

Available Na^+ and K^+ concentrations in field soils from Yuzhong county (35°34′–36°32′N, 103°50–104°34′E, elevation 1520 m) and Lanzhou city (35°34′–37°07′N, 102°36′–104°34′E, elevation 1500 m), China, and in turfy soil, vermiculite and perlite were measured according to the method described by Wang et al. (2004). Briefly, available Na^+ and K^+ in the various media included the sum of soluble, exchangeable and available non-exchangeable Na^+ and K^+: soluble Na^+ and K^+ were extracted by soaking the soil in deionized water (water/soil, 5/1), and exchangeable and available non-exchangeable Na^+ and K^+ were extracted with 2 M cold HNO_3. Na^+ and K^+ concentrations were analyzed with an atomic absorption spectrophotometer (2655-00, Cole-Parmer Instrument Co.).

Data analysis

All the data were subjected to one-way analysis of variance (ANOVA) using SPSS 13.0 (SPSS Inc., Chicago, IL, USA). Duncan's multiple range tests were used to detect significant differences between means at a significance level of $P < 0.05$.

Results

Moderate concentrations of NaCl stimulate the growth of *Z. xanthoxylum* by enhancing photosynthesis and improving water status

Compared with the control (no additional NaCl), the addition of 5 and 50 mM NaCl significantly increased RGR by 30 and

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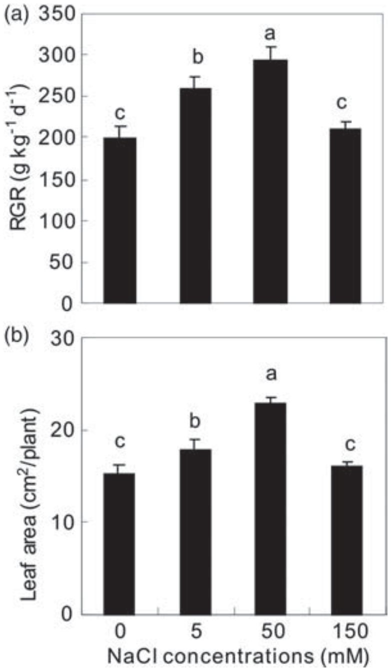

Figure 1. Relative growth rate (RGR) and leaf area of *Z. xanthoxylum* seedlings treated with 0, 5, 50 or 150 mM NaCl for 7 days. Values are means ± SD ($n = 6$) and bars indicate SD. Columns with different letters indicate significant difference at $P < 0.05$ (Duncan test).

47%, respectively, whereas 150 mM NaCl had no effects on RGR (Figure 1). Moreover, leaf area was significantly increased by 17 and 51% in the presence of additional 5 and 50 mM NaCl, respectively (Figure 1). The addition of 5 and especially 50 mM NaCl remarkably enhanced photosynthesis and increased water use efficiency (Figure 2). Pn, Gs and WUE increased with an increase of treatment time from 1 to 7 days in plants exposed to 50 mM NaCl and after 7 days of treatment were 128, 56 and 93% higher than those in control plants, respectively (Figure 2). However, photosynthetic characteristics and leaf area remained unaffected with time when plants were exposed to 150 mM NaCl (Figures 1 and 2).

Additional 5 and 50 mM NaCl significantly induced an increase in chlorophyll concentrations with the elongation of treatment time; an optimal effect was observed in the presence of 50 mM NaCl, where Chl *a* and Chl *b* concentrations were 155 and 200% higher, respectively, than those in control plants after 7 days (Figure 3). When plants were exposed to additional 150 mM NaCl, chlorophyll concentrations also increased but reached their highest values after 1 day, and then decreased gradually to the same level as the control after 7 days (Figure 3). By contrast, additional NaCl had no impact on chlorophyll fluorescence parameters (F_v/F_m, Φ_{PSII}, F_v'/F_m', *q*P, *q*N), regardless of the external concentrations of NaCl (5, 50, 150 mM) or the duration of exposure (1–7 days) (data not shown).

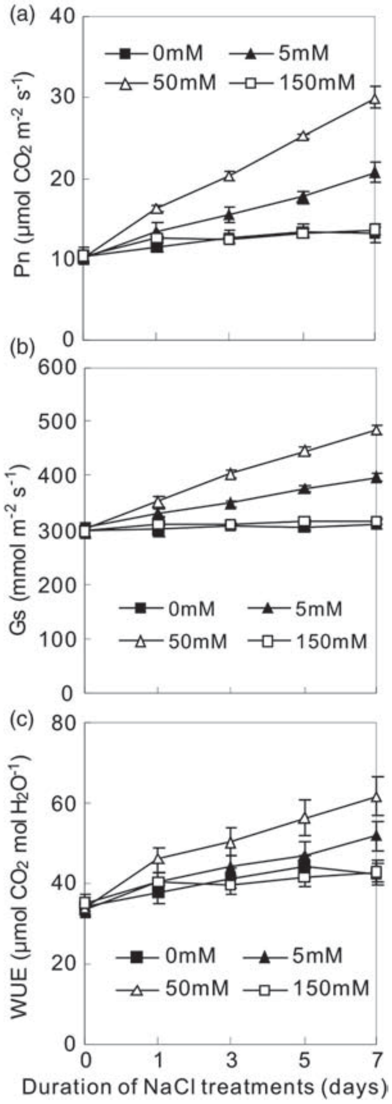

Figure 2. Net photosynthesis rate (Pn) (a), stomatal conductance (Gs) (b) and water use efficiency (WUE) (c) of leaves of *Z. xanthoxylum* seedlings treated with 0, 5, 50 or 150 mM NaCl for various periods (0, 1, 3, 5, 7 days). Values are means ± SD ($n = 6$) and bars indicate SD.

As shown in Table 1, Ψ_w and Ψ_s both significantly decreased with the increase in additional NaCl concentrations, whereas Ψ_t significantly increased—by 29%—when plants were treated with additional 50 mM NaCl compared with that of the control. There was no significant difference from the control when plants were exposed to 5 or 150 mM NaCl (Table 1). In addition, RWC increased with the increase of treatment time when plants were exposed to additional 5 and 50 mM NaCl, and again 50 mM NaCl had the optimal effect; RWC only increased for 1 day and then decreased gradually from 3 to 7 days under 150 mM NaCl (see Figure S1 available as Supplementary Data at *Tree Physiology* Online).

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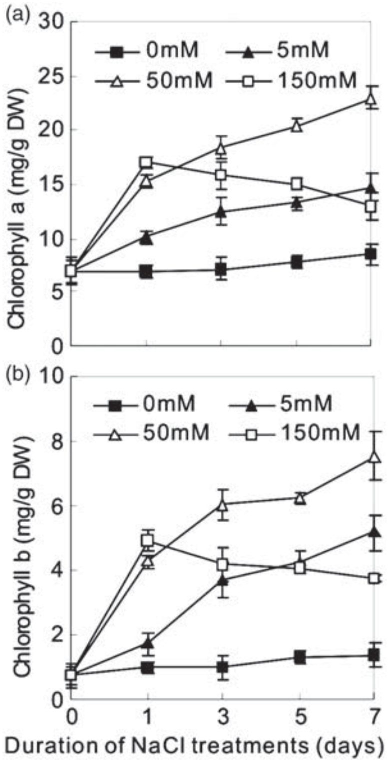

Figure 3. Chlorophyll *a* (a) and *b* (b) concentrations in leaves of *Z. xanthoxylum* seedlings treated with 0, 5, 50 or 150 mM NaCl for various periods (0, 1, 3, 5, 7 days). Values are means ± SD ($n = 6$) and bars indicate SD.

Table 1. Leaf water potential (Ψ_w), osmotic potential (Ψ_s) and turgor pressure (Ψ_t) of *Z. xanthoxylum* seedlings treated with 0, 5, 50 or 150 mM NaCl for 7 days. Values are means ± SD ($n = 6$). Columns with different letters indicate significant difference at $P < 0.05$ (Duncan test).

NaCl treatments (mM)	Ψ_w (MPa)	Ψ_s (MPa)	Ψ_t (MPa)
0	−0.47 ± 0.02 a	−0.99 ± 0.01 a	0.52 ± 0.05 bc
5	−0.59 ± 0.02 b	−1.15 ± 0.03 b	0.56 ± 0.03 b
50	−0.95 ± 0.03 c	−1.62 ± 0.08 c	0.67 ± 0.04 a
150	−1.92 ± 0.09 d	−2.34 ± 0.05 d	0.42 ± 0.07 c

The addition of NaCl alleviates the deleterious impact of water deficit on the photosynthesis and water status of Z. xanthoxylum

In comparison with the control (70% of FWC), RGR was significantly reduced when plants were subjected to water deficit (30% of FWC); however, a significant increase in RGR by 33% was observed in plants grown in the presence compared with the absence of additional 50 mM NaCl in the presence of water deficit (Table 2). Likewise, photosynthesis was inhibited by water deficit, while the presence of additional 50 mM NaCl significantly increased Pn, Gs and WUE by 73, 39 and 25%, respectively, compared with treatment without additional NaCl under water deficit (Table 2). The addition of 50 mM NaCl in the presence of water deficit resulted in a significant increase in leaf area (Table 2) and chlorophyll concentrations (Table 2). Furthermore, Φ_{PSII}, F_v'/F_m' and qP in *Z. xanthoxylum* treated with additional 50 mM NaCl were 38, 11 and 19% higher than in plants treated without additional NaCl, respectively, although no significant change was observed in F_v/F_m, whereas qN was lower by 39% (Table 3).

As shown in Table 4, water deficit significantly decreased Ψ_w and Ψ_s, and these two parameters showed an even greater reduction in the presence of additional 50 mM NaCl and water deficit, whereas the Ψ_t of plants that suffered water deficit was significantly reduced compared with the control; however, a significant increase (70%) in Ψ_t was induced by the addition of 50 mM NaCl under water deficit. In addition, water deficit triggered a significant reduction in RWC, and RWC was significantly increased by 22% by the addition of 50 mM NaCl in comparison with treatment without additional NaCl in the presence of water deficit (see Figure S2, available as Supplementary Data at *Tree Physiology* Online).

Na^+ and K^+ accumulation and their contribution to osmotic potential of Z. xanthoxylum under water deficit

To avoid the presence of excess Na^+ in the substrate during pot experiments (see 'Materials and methods' for details), we measured available Na^+ and K^+ concentrations in field soils

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Table 2. Relative growth rate (RGR), net photosynthesis rate (Pn), stomatal conductance (Gs), water use efficiency (WUE), leaf area, and chlorophyll *a* and *b* concentrations in leaves of *Z. xanthoxylum* seedlings. The treatments were: control (C) (70% FWC), drought stress (D) (30% of FWC) and drought stress together with salt (D + S) (30% of FWC with 50 mM NaCl) for 7 days. Values are means ± SD ($n = 6$). Columns with different letters indicate significant difference at $P < 0.05$ (Duncan test).

Treatments	RGR (g kg^{-1} day^{-1})	Pn (μmol CO$_2$ m^{-2} s^{-1})	Gs (mmol m^{-2} s^{-1})	WUE (μmol CO$_2$ mol H$_2$O^{-1})	Leaf area (cm^2 plant^{-1})	Chlorophyll *a* (mg g^{-1} DW)	Chlorophyll *b* (mg g^{-1} DW)
C	206 ± 6 a	16.1 ± 0.7 a	351.3 ± 5.6 a	45.9 ± 2.5 a	17.4 ± 1.0 a	12.5 ± 0.3 a	5.1 ± 0.3 a
D	139 ± 7 c	8.1 ± 0.4 c	228.8 ± 7.4 c	35.4 ± 3.0 b	11.0 ± 0.9 c	9.4 ± 0.4 b	3.4 ± 0.2 c
D + S	184 ± 12 b	14.0 ± 0.3 b	318.0 ± 12.5 b	44.0 ± 3.1 a	15.7 ± 0.5 b	12.3 ± 0.3 a	4.2 ± 0.4 b

Table 3. The maximal quantum yield of PSII photochemistry (F_v/F_m), the photochemical quenching coefficient (qP), the non-photochemical quenching coefficient (qN), the efficiency of excitation capture by the open PSII reaction center (F_v'/F_m') and the actual PSII efficiency (Φ_{PSII}) of *Z. xanthoxylum* seedlings. The treatments were: control (C) (70% FWC), drought stress (D) (30% of FWC) and drought stress together with salt (D + S) (30% of FWC with 50 mM NaCl) for 7 days. Values are means ± SD ($n = 6$). Columns with different letters indicate significant difference at $P < 0.05$ (Duncan test).

Treatments	F_v/F_m	Φ_{PSII}	F_v'/F_m'	qP	qN
C	0.81 ± 0.02 a	0.60 ± 0.02 a	0.77 ± 0.03 a	0.78 ± 0.02 a	0.23 ± 0.02 b
D	0.74 ± 0.03 b	0.42 ± 0.04 b	0.67 ± 0.03 c	0.63 ± 0.05 c	0.36 ± 0.03 a
D + S	0.80 ± 0.04 ab	0.58 ± 0.02 a	0.73 ± 0.02 b	0.75 ± 0.02 b	0.22 ± 0.05 b

Table 4. Leaf water potential (Ψ_w), osmotic potential (Ψ_s), turgor pressure (Ψ_t), Na^+ and K^+ concentrations, and the contributions of Na^+ and K^+ to osmotic potential (Ψ_s) of *Z. xanthoxylum* seedlings. The treatments were: control (C) (70% FWC), drought stress (D) (30% of FWC) and drought stress together with salt (D + S) (30% of FWC with 50 mM NaCl) for 7 days. Values are means ± SD ($n = 6$). Columns with different letters indicate significant difference at $P < 0.05$ (Duncan test).

Treatments	Ψ_w (MPa)	Ψ_s (MPa)	Ψ_t (MPa)	Na^+ concentration (mmol g^{-1} DW)	K^+ concentration (mmol g^{-1} DW)	Contribution of Na^+ to Ψ_s (%)	Contribution of K^+ to Ψ_s (%)
C	−0.45 ± 0.02 a	−0.95 ± 0.03 a	0.50 ± 0.02 a	0.39 ± 0.06 c	0.59 ± 0.05 a	8	13
D	−1.09 ± 0.07 b	−1.33 ± 0.05 b	0.24 ± 0.07 c	0.64 ± 0.04 b	0.59 ± 0.07 a	13	13
D + S	−1.31 ± 0.03 c	−1.72 ± 0.05 c	0.41 ± 0.02 b	2.13 ± 0.09 a	0.58 ± 0.04 a	28	8

from Yuzhong county and Lanzhou city, China, and in turfy soil, vermiculite and perlite (see Table S1, available as Supplementary Data at *Tree Physiology* Online). Both lowest Na^+ and K^+ concentrations were found in vermiculite (4.8 ± 0.3 and 5.0 ± 0.2 μmol g^{-1} dry weight, respectively, see Table S1, available as Supplementary Data at *Tree Physiology* Online). Although Na^+ concentration in the soil from Alxa League (4.1 ± 0.1 μmol g^{-1} dry weight) where the seeds of Z. *xanthoxylum* were collected was lower than that in vermiculite (Wang et al. 2004), 5 kg of oven-dried soil was needed to fill the plastic pots (18 cm high × 20 cm diameter), whereas only 1 kg oven-dried vermiculite was required, which would have meant that four times more Na^+ would have been available when the pots were filled with the soil from Alxa League than with vermiculite. Thus, vermiculite with trace amounts of Na^+ was used in pot experiments.

Water deficit induced a significant increase in leaf Na^+ concentration by 1.6 times compared with the control (Table 4), although Na^+ levels in the substrate were the same between the control and drought treatments (see Table S1, available as Supplementary Data at *Tree Physiology* Online). As expected, leaf Na^+ concentration was 2.3 times higher in the presence than in the absence of additional 50 mM NaCl under water deficit (Table 4). However, leaf K^+ concentration remained unaffected by water deficit or the addition of 50 mM NaCl (Table 4). Na^+/K^+ ratio in the leaf was significantly increased by 57% in plants subjected to water deficit compared with the control, and a significant increase in leaf Na^+/K^+ ratio by 2.5 times was observed in plants grown in the presence compared with the absence of additional 50 mM NaCl and water deficit (data not shown).

The contribution of Na^+ to total osmotic potential of *Z. xanthoxylum* varied from 8% in the control to 13% in plants subjected to water deficit, and to 28% in plants grown in the presence of additional 50 mM NaCl under water deficit (Table 4). In contrast, no significant difference for the contribution of K^+ to total osmotic potential was found between the control and plants that suffered water deficit, and the contribution of K^+ decreased significantly from 13% in plants subjected to water deficit to 8% in plants grown in the presence of additional 50 mM NaCl and water deficit (Table 4).

Discussion

NaCl enhances photosynthesis and improves water status by involvement in OA

Zygophyllum xanthoxylum is well known as a succulent xerophyte of the desert regions of northwestern China, and high Na^+ accumulation in leaves might be one of its most important adaptations to aridity (Liu et al. 1987, 1988, Wang et al. 2004). In this study, we have shown that adding NaCl at a concentration of 50 mM was optimal for its growth (Figure 1) and photosynthetic capacity (Figure 2). Similar results have been reported for some other halophytes such as *Atriplex gmelini*, *A. halimus* and *Suaeda salsa* (Matoh et al. 1986, Bajji et al. 1998, Zhang and Zhao 1998; Wang et al. 2001, Martínez et al. 2005). Our research on *Z. xanthoxylum* has demonstrated that a moderate

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concentration of NaCl can improve its ability to cope with water deficit by enhancing the Na^+ concentration in leaves (Table 4) and photosynthesis, resulting in an increase in RGR (Table 2), indicating that this species has some properties of a salt-accumulating halophyte. In fact, although the available Na^+ concentration (4.1 μmol g^{-1} dry soil) in the soil of the natural habitat of *Z. xanthoxylum* was about 1/66th that (272.9 μmol g^{-1} dry soil) of the salt-accumulating halophyte *S. salsa*, leaf Na^+ concentration in *Z. xanthoxylum* was comparable to that in *S. salsa* (Wang et al. 2002, 2004). We propose that *Z. xanthoxylum* should be considered as a xero-halophyte species.

Positive correlations were found between stomatal conductance and net photosynthesis rate in *Z. xanthoxylum* (see Figure S3a available as Supplementary Data at *Tree Physiology* Online) or with relative growth rate (see Figure S3b, available as Supplementary Data at *Tree Physiology* Online), suggesting that the significant stimulation of photosynthesis and growth in the presence of salt was strongly related to the enhancement in stomatal conductance, which was dependent on the opening of stomata (Franks et al. 2001). In addition, leaf development plays an important role in plant production since it affects the area available for photosynthesis, which is strongly linked to plant growth and biomass (Gifford and Evans 1981, Gutierrez-Boem and Thomas 1998). Franks (2006), Franks and Farquhar (2007)and Heidecker et al. (2003) found that the opening of stomata and leaf area development strongly depended on cell turgor, which in turn was determined by OA. In our study, Ψ_w and Ψ_s both decreased significantly with increase of NaCl concentration, whereas Ψ_t was significantly increased (by almost a third) when plants were treated with 50 mM NaCl compared with the control (Table 1). While water deficit alone significantly decreased Ψ_t, the presence of 50 mM NaCl induced a significant increase in Ψ_t (by 70% compared with treatment without additional NaCl under water deficit; Table 4). These results suggest that moderate concentrations of NaCl could increase Ψ_t by enhancing OA and, as a result, increase Pn, Gs and leaf area (Figures 1 and 2; Table 2).

The involvement of NaCl in OA also resulted in a significant improvement of water status: the decrease in Ψ_w enhanced water absorption (Blumwald et al. 2000), leading to an increase in RWC (see Figures S1 and S2, available as Supplementary Data at *Tree Physiology* Online). Similar results were also observed in the halophyte *S. portulacastrum* (Slama et al. 2007). It is noteworthy that RWC increased within 1 day and then decreased gradually from 3 to 7 days when plants were exposed to additional 150 mM NaCl (see Figure S1, available as Supplementary Data at *Tree Physiology* Online). The initial increase in RWC after 1 day was likely to have been induced by the way the treatment was imposed for 1 day in 50 mM NaCl before the concentration was raised to 150 mM for 7 days (see Materials and methods). This result further confirmed the positive effects of 50 mM NaCl on RWC in *Z. xanthoxylum*.

Na^+ plays an important role in OA in *Z. xanthoxylum* under water deficit

K^+ performs multiple physiological functions, including osmotic regulation, during drought (Clarkson and Hanson 1980, Schroeder et al. 1994, Gierth and Mäser 2007). When growing under water deficit, Na^+ accumulation has been assigned a positive function in some halophytes, but the importance of Na^+ to OA is controversial (Martínez et al. 2003, 2004, 2005, Slama et al. 2007). In our study, we comprehensively investigated the contribution of Na^+ and K^+ to OA of *Z. xanthoxylum* grown with a sufficient water supply (70% of FWC) and under water deficit (30% of FWC) in the presence or absence of additional NaCl (50 mM). When the water supply was adequate, K^+ accounted for 13% of the leaf osmotic potential of *Z. xanthoxylum*, which was 63% higher than Na^+. Water deficit induced a significant increase in leaf Na^+ concentration and its contribution to the leaf osmotic potential increased by 57% compared with that of the control plants, while the contribution of K^+ remained unchanged (Table 4). These results suggest that the drought resistance of *Z. xanthoxylum* might be improved by accumulating Na^+ rather than K^+ in leaves. Na^+ can be used as an osmoregulant contributing to OA in order to cope with water deficit. Indeed, it has been shown that *Z. xanthoxylum*, just like *S. salsa*, a salt-accumulating halophyte, has a strong selective capacity for Na^+ over K^+ (Wang et al. 2002, 2004). In spite of growing in low-salinity soils in desert regions, *Z. xanthoxylum* was able to accumulate large quantities of Na^+ from soil in aboveground tissues to meet the requirements of growth (Tobe et al. 2001, Wang et al. 2004). Martínez et al. (2005) reported that, for *A. halimus*, although leaf Na^+ concentration was increased under water deficit, the contribution of Na^+ to total osmotic potential was negligible. This apparent difference between *Z. xanthoxylum* and *A. halimus* might be explained by the fact that, in the latter, a considerable part of Na^+ accumulated in trichomes that cover the leaf surface (Mozafar and Goodin 1970), whereas in *Z. xanthoxylum*, Na^+ is sequestered in vacuoles in large cells mediated by the tonoplast Na^+/H^+ antiporter (ZxNHX, GenBank: EU103624) (Liu et al. 1987, Wang et al. 2004, Wu et al. 2011), allowing plants to use Na^+ as an osmoregulatory substance (Blumwald et al. 2000, Zhu 2003, Benlloch-González et al. 2005, Slama et al. 2007). Moreover, it is noteworthy that the addition of 50 mM NaCl significantly increased the leaf Na^+ concentration of *Z. xanthoxylum* by more than 2.3 times compared with the treatment without additional NaCl under water deficit, and the contribution of Na^+ to leaf osmotic potential increased from 13% in plants under water deficit to 28% in plants grown in the presence of additional 50 mM NaCl under water deficit; in contrast, the contribution of K^+ to leaf osmotic potential of *Z. xanthoxylum* reduced from 13 to 8% (Table 4). These results suggest that the enhancement of photosynthesis and tissue

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hydration induced by the addition of 50 mM NaCl in *Z. xanthoxylum* under water deficit (Table 2; see Figure S2, available as Supplementary Data at *Tree Physiology* Online) might partially be a consequence of the significant increase in leaf Na^+ concentration and the direct contribution of Na^+ to OA.

It has been frequently reported that some organic solutes, including proline, glycine betaine and soluble sugars, act as the main stress-induced substances involved in OA (Sun et al. 1999, Raymond and Smirnoff 2002). However, for *Z. xanthoxylum* and other xerophytes, such as *Nitraria sibirica* (which also belongs to Zygophyllaceae), proline and soluble sugars are less important than Na^+ for their adaptation to arid environments (Wang et al. 2004, Li et al. 2005), and proline and soluble sugars contribute even less to OA than K^+ (Li et al. 2005). Moreover, the addition of 50 mM NaCl significantly decreased the concentrations of proline and soluble sugars in *Z. xanthoxylum* under different osmotic stresses (Cai et al. 2011). Whether other compatible solutes such as glycine betaine play important roles in drought resistance needs to be further analyzed.

NaCl could increase chlorophyll concentrations in Z. xanthoxylum

Chlorophyll concentration is an important physiological index directly related to photosynthesis in plants. Some research has demonstrated that NaCl could stimulate chlorophyll biosynthesis by activating key enzymes (Brownell and Bielig 1996, Subbarao et al. 1999). Moreover, Na^+ could play an important role in the proliferation of chloroplasts instead of K^+ (Marschner 1971, Subbarao et al. 1999). Such changes may have occurred in *Z. xanthoxylum*, leading to the increase in chlorophyll concentrations (Figure 3; Table 2). Furthermore, the degradation of chlorophyll under abiotic stress could possibly be attributed, at least partially, to the sensitivity of membranes to oxidative stress (Louda et al. 1998, Barry 2009). Water deficit is known to increase the level of reactive oxygen species, which are well recognized for lipid peroxidation of chloroplast membranes, disintegrating thylakoids and leading to reduced chlorophyll concentrations under water deficit (Kato and Shimizu 1985, Yamauchi and Minamide 1985, Yamauchi and Watada 1994). Our previous research demonstrated that water deficit caused a significant increase in leaf malondialdehyde (MDA) concentration, which represents the degree of cell membrane damage under water deficit (Li et al. 1998); however, the addition of 50 mM NaCl significantly decreased leaf MDA concentration by 28% in *Z. xanthoxylum* grown under water deficit (L. J. Yue and S. M. Wang, unpublished data), indicating that an appropriate concentration of NaCl might alleviate the impact of water deficit on the chloroplast membrane. This may be another reason why the addition of 50 mM NaCl increased the chlorophyll concentrations of *Z. xanthoxylum* under water deficit (Table 2).

NaCl could mitigate deleterious impacts of water deficit on photosystem II in Z. xanthoxylum

Photosystem II (PSII) is believed to play a key role in the response of photosynthesis to environmental perturbations (Baker 1991). However, there are disparate results in the literature on the effects of NaCl on PSII photochemistry. Some studies showed that 100 mM NaCl inhibited PSII activity in salt-sensitive *Sorghum vulgare* (Masojidek and Hall 1992) and *Apium graveolens* (Everard et al. 1994), whereas other studies indicated that 100 mM NaCl had no effect on PSII activity in salt-tolerant *Gossypium hirsutum* (Brugnoli and Björkman 1992) and *Hordeum vulgare* (Morales et al. 1992, Abadía et al. 1999), and the halophyte *S. salsa* (Lu et al. 2003). In our study of *Z. xanthoxylum*, NaCl had no effects on PSII photochemistry either in a dark-adapted or in a light-adapted state, since F_v/F_m, F_v'/F_m', Φ_{PSII}, qP and qN were unaffected by different salt treatments (data not shown), suggesting that the response of PSII to NaCl was similar to other salt-tolerant plants. However, when PSII photochemistry was inhibited by water deficit, the presence of NaCl (50 mM) could enhance the resistance of PSII photochemistry to drought: Φ_{PSII}, F_v'/F_m' and qP in *Z. xanthoxylum* treated with additional 50 mM NaCl were 38, 11 and 19% higher, respectively, than in plants treated without additional NaCl under water deficit whereas qN was lower by 39% (Table 3). Similar results were observed by Lu et al. (2003): 100–400 mM NaCl had no effects on PSII photochemistry, but increased the resistance of PSII to heat stress in the halophyte *S. salsa*, which was shown to be a consequence of the involvement of Na^+ in the maintenance of grana stacking, providing a suitable environment for energy transfer between PSII and PSI (Brownell and Bielig 1996, Qiu et al. 2003), or associated with the increased resistance of O_2-evolving complex and reaction centers of PSII (Lu et al. 2003).

In conclusion, our results suggest that Z. *xanthoxylum* is able to accumulate a high concentration of Na^+ in leaves under arid environments and use it directly as an osmoregulatory substance, which is coupled with an improvement in leaf hydration and photosynthetic activity. In addition, the ability of NaCl to improve plant performance under water deficit may be due to the induction of significant increases in chlorophyll concentrations and the resistance of photosystem II to water deficit.

Supplementary data

Supplementary data for this article are available at *Tree Physiology* online.

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