



NaCl stimulates growth and alleviates drought stress in the salt-secreting xerophyte *Reaumuria soongorica*

Fang-Lan He^{a,b}, Ai-Ke Bao^{a,*}, Suo-Min Wang^{a,*}, Hong-Xi Jin^b

^a State Key Laboratory of Grassland Agro-ecosystems, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou, 730020, PR China

^b Gansu Desert Control Research Institute, Lanzhou, 733070, PR China



ARTICLE INFO

Keywords:

Reaumuria soongorica
Growth
Na⁺
Drought stress
Osmotic adjustment
Salt secretion

ABSTRACT

Reaumuria soongorica, a salt-secreting xerophytic shrub, is a dominant species in arid desert regions of northwest China. To investigate whether NaCl could stimulate the growth and alleviate drought stress in *R. soongorica*, the seedlings were treated with a range of different concentrations of 0–1.17 g kg⁻¹ NaCl, and also were withheld water for drought stress under without or supplementary 0.59 g kg⁻¹ NaCl. The results showed that 0.59 g kg⁻¹ NaCl (moderate NaCl) significantly stimulated the growth of *R. soongorica*, and also effectively alleviated deleterious impacts of drought stress on its growth and leaf morphological structure. Further analysis showed that moderate NaCl significantly improved photosynthetic capacity and mitigated light suppression of drought stress on photosystem II in *R. soongorica*, concomitantly, decreased osmotic potential, enlarged turgor pressure as well as increased the relative water content in leaves under drought stress. Furthermore, moderate NaCl induced an obvious increase of Na⁺ accumulation in tissues of *R. soongorica*, which resulted in a significant increase of the contribution of Na⁺ to leaf osmotic potential from 13 to 22% under drought stress. Additionally, moderate NaCl also promoted Na⁺ secretion via salt glands of leaf. These results suggest that the positive roles of NaCl on the growth and drought resistance of *R. soongorica* is due to the increase of Na⁺ accumulating in leaf tissues, facilitating plant to maintain higher photosynthetic activity and better water status in arid environment, simultaneously, salt secretion of salt glands protects plant from excess Na⁺ toxicity.

1. Introduction

Drought is one of major environment stress factors, inhibiting seriously plant growth and development by causing dehydration of plant tissues (Molnar et al., 2015; Wang et al., 2016a, 2016b; Daliakopoulos et al., 2017). Due to globe climate change, extreme drought event has been frequented in recent years, which is considered as a serious threat to agricultural productivity and ecological ecosystem stability in arid and semi-arid regions (Hua et al., 2017). Some xerophytes, growing in extreme drought environment over a long period of time, have evolved multiple drought-resistance mechanisms protecting them from drought damage and sustaining well growth in harsh environments (Kang et al., 2017; Moradi et al., 2017; Cui et al., 2019). Understanding their drought-resistance mechanisms would be important role for agriculture development and environment restoration in arid land (He et al., 2016; Kang et al., 2016; Jupa et al., 2017).

Plant drought resistance involves physiological (Jupa et al., 2017) and morphological modifications (Zhang et al., 2015; Shahidi et al., 2017). One of the most important physiological mechanisms allowing

for drought stress adaptation in plants is osmotic adjustment (Wu et al., 2015; Binks et al., 2016; Kang et al., 2017), which involves solutes accumulation in cell under low water potential conditions (Hatami et al., 2017). Osmotic regulators in plants are often composed of organic solutes and inorganic ions. Organic solutes, such as proline (Sperdoui and Moustakas, 2015; Zanella et al., 2016) and soluble sugars (Zhang et al., 2018), have been found to contribute to osmotic adjustment plants. Inorganic ions, especially K⁺, are thought to be involved in osmotic adjustment of plant under drought stress (Ahanger and Agarwal, 2017; Zahoor et al., 2017). Na⁺ is generally regarded as a toxic ion to almost all glycophytic species, but is determined to positively influence drought adaptation in some succulent or less succulent xerophytes (Kang et al., 2015; Cui et al., 2019). It had been found that the application of moderate NaCl showed a positive effect on stimulating growth and improving drought resistance of the xerophytes *Zygophyllum xanthoxylum*, *Haloxylon ammodendron*, and *Nitraria tangutorum*, which is consistent with the increase of Na⁺ accumulation in the leaves (Ma et al., 2012; Kang et al., 2015).

The salt-secreting shrub *Reaumuria soongorica* (Tamaricaceae) grows

* Corresponding authors.

E-mail addresses: baoaik@lzu.edu.cn (A.-K. Bao), smwang@lzu.edu.cn (S.-M. Wang).

in desert grasslands of northwest China as the constructive species (Xu et al., 2010), and faces frequently the challenges from various adverse environmental factors, such as extreme drought, severe salinization, extreme temperature fluctuations, and nutrient deficiency (Bai et al., 2008). This species has become one important species for vegetation restoration and reconstruction in arid desert regions of China, but is not yet widely used in artificial vegetation construction due to its low growth rate and weak drought resistance in the juvenile phase (He et al., 2017). Interestingly, its growth performance in the arid -salinization habitat is better than that in the corresponding arid habitat (He et al., 2017). Previous investigations showed that some salt-accumulating xerophytes possess a strong capability of accumulating Na^+ in their leaves under drought conditions, which results in a positive effect on growth and drought tolerance (Ma et al., 2012; Kang et al., 2015). Does Na^+ bring a positive effect on growth and drought resistance of salt-secreting xerophytes? However, there are few studies on effects of Na^+ on growth and drought tolerance of salt-secreting xerophytes under arid desert environment as yet.

To investigate whether NaCl can stimulate the growth and alleviate drought stress in *R. soongorica*, in this study, *R. soongorica* seedlings were treated with different concentrations of NaCl and drought stress by withholding water in the presence or absence of supplementary NaCl, and parameters related to growth, leaf cross section, photosynthesis activity, water status, ion accumulation and secretion, and osmotic adjustment were assayed.

2. Materials and methods

2.1. Plant growth and treatments

Seeds of *R. soongorica* were collected from vigorous plants growing wildly in a desert oasis transitional zone located in the Gansu Minqin National Studies Station for Desert Steppe Ecosystems (MSDSE), in November 2014. The station is located in Minqin County, Gansu Province, China (102°57'06"E, 38°31'22"N; elevation 1315–1375 m), where the average annual temperature, rainfall, and evaporation are 7.5 °C, 110.2 mm, and 2645 mm, respectively. In May 2015, seeds were sowed in plastic pots (40 cm height × 22 cm diameter with no drainage holes at the bottom) filled with 10 kg air-dried sandy soil and watered with underground water. The chemical properties of the sandy soil are described in Table 1. After emergence of seedlings, the soil water contents in pots were maintained at 65% field water capacity (FWC), determined gravimetrically. The seedlings of all pots were cultivated under field conditions in the experimental fields of the State Key Laboratory of Desertification and Aeolian Sand Disaster Combating (SLDASC), Wuwei, China (37°56'09.59"E and 102°35'51.64"N) with day/night temperatures of 17–32/6–21 °C, a relative humidity of 45–67 %, and a daily photon flux density of 1,700–2,700 $\mu\text{mol m}^{-2}\text{s}^{-1}$. Rainfall was restricted using a movable canopy during the experimental period. Pots were moved cyclically from east to west and north to south every three days. After 6 weeks, uniform seedlings were thinned to eight plants per pot and divided into five groups to which 0, 0.29, 0.59, 0.88, and 1.17 g kg^{-1} (dry soil) NaCl was supplied, respectively. Each group included 12 pots. The soil water contents of all the treatments were maintained at 60% of FWC for 45 days.

In May 2016, seeds were sowed and irrigated as described above. Pots were placed under the experimental fields of MSDSE (day/night

temperature, 16–39/13–25 °C; relative humidity, 41–57 %; and daily photon flux density, 1,900–2,800 $\mu\text{mol m}^{-2}\text{s}^{-1}$). After 6 weeks, uniform seedlings of 90 pots were thinned as described as above and divided averagely into two groups, which were supplied 0 (control) and 0.59 g kg^{-1} NaCl (NaCl), respectively, and cultivated at 60% of FWC for 75 days. After that, all seedlings from the control or NaCl treatment were watered for 70% of FWC and divided into well-watered (15 pots) and drought stress (30 pots) treatments, following the well-watered treatment was watered continuously for 70% of FWC, while the drought stress treatment was withheld water for 20 days. Rainfall was restricted by means of a temporary canopy during the experiment. The soil water contents of the pots after withholding water for 0, 5, 10, 15, and 20 days are provided in Table 2.

2.2. Determination of growth parameters and relative water content

After the height and crown diameter were measured, the plants were washed by distilled water and the shoots and roots were separated for determination of fresh weights and root lengths. The leaves were separated from branches and immediately used to determine the leaf area by a laser leaf area meter (ZH6118, Ghitest Instrument Co. Ltd, Beijing, China) or fresh weight, and then were soaked in distill in the dark for 16 h for weighing the turgid weight. The dry weights of samples were determined after keeping them in an oven at 80 ± 2 °C for 24 h. The relative growth rate (RGR) of plant was calculated by a formula as follows: $RGR = \ln W/t$, where W is the ration of the final and pre-treated plant fresh weight (g), t is the treated time (d) (Kang et al., 2014; Habibi, 2014). The leaf relative water content (RWC) was calculated with the expression: $RWC (\%) = 100 \times (\text{fresh weight} - \text{dry weight}) / (\text{turgid weight} - \text{dry weight})$ (Wu et al., 2015).

2.3. Measurement of leaf cross section

The leaf cross section was observed using the method of normal paraffin sectioning. Briefly, leaves were cut from branches, vacuumed by a syringe, and put in formalin-acetic acid-alcohol (FAA) solution to fix immediately. Fixed-samples were carried out washing, dehydration, transparent, wax penetration, and embedding. Sections (10 μ) were obtained using a rotary microtome (Huajun Co. Ltd, Shanghai, China), stained with safranin and light green, and mounted in Canada balsam. Sections were examined and photographed by a microimaging system (CX31-TR-K5, Olympus corporation, Tokyo, Japan). The length, width, and area of leaf cross section were measured using software Artios CAD 14.0 (Artios CAD, ESKO, Wisconsin, USA), and the length/width of leaf cross section was calculated.

2.4. Measurement of photosynthesis and chlorophyll fluorescence parameters

Net photosynthesis rate (P_n), intracellular CO_2 concentration (C_i), stomatal conductance (G_s), and transpiration rate (T_r) were measured using an automatic photosynthetic system (IRGA, LI-6400XT, LI-COR Biosciences, Lincoln, NE, USA) between 9:30 and 11:30 am. All parameters were measured with a 1200 $\mu\text{mol m}^{-2}\text{s}^{-1}$ light intensity (saturated light intensity), 380 $\mu\text{mol mol}^{-1}$ CO_2 concentration, and 28 °C leaf temperature. Water use efficiency (WUE) was calculated with a formula as follows: $WUE = P_n/T_r$ (Habibi, 2014).

Table 1

Some chemical properties of sand soil filled into pots. Values are means from six soil samples \pm SE ($n = 6$).

Water soluble ($\mu\text{mol g}^{-1}$)		Changeable ($\mu\text{mol g}^{-1}$)		Available P ($\mu\text{mol g}^{-1}$)	Available N ($\mu\text{mol g}^{-1}$)	pH
Na^+	K^+	Na^+	K^+			
8.0 \pm 0.5	1.6 \pm 0.2	2.3 \pm 0.3	3.2 \pm 0.3	0.8 \pm 0.0	0.04 \pm 0.0	7.8 \pm 0.4

Table 2

The relative water contents of soil in 0–40 cm of pots with withholding water for 0, 5, 1015 and 20 days. Values are means from six pots \pm SE (n = 6). Different letters in lines indicate significant differences at $P < 0.05$ (Duncan's multiple range test).

Soil layers in pots	Relative water content of soil (%)				
	0 day	5 days	10 days	15 days	20 days
0-10cm	65 \pm 9.5 a	24 \pm 4.7 b	7.7 \pm 1.3 c	3.9 \pm 0.4 d	1.7 \pm 0.2 e
10-40cm	70 \pm 2.3 a	57 \pm 2.7 b	47 \pm 3.2 c	23 \pm 1.8 d	12 \pm 0.3 e

The minimum fluorescence (F_0) and maximum fluorescence (F_m) were measured using a chlorophyll fluorescence chamber (6400XT-40, LI-COR Biosciences, Lincoln NE, USA) connected to the photosynthetic measuring apparatus between 5:00 and 6:00 am after dark adaption for one night. The leaf temperature and CO_2 concentration were controlled at 25 °C and 380 $\mu\text{mol mol}^{-1}$, respectively. After activation under natural light for 2 h (from 8 a.m. to 10 a.m.), the constant fluorescence (F_s), minimum fluorescence (F'_0), and maximum fluorescence (F'_m) were measured, respectively, under the same conditions as above. The maximal quantum yield of photosystem II (PSII) photochemistry (F_v/F_m), the efficiency of excitation capture by the opening of the PSII reaction center (F_v'/F'_m), the actual PSII efficiency (Φ_{PSII}), the photochemical quenching coefficient (qP), and the non-photochemical quenching coefficient (qN), were calculated using the following formula: $F_v/F_m = (F_m - F_0)/F_m$, $F_v'/F'_m = (F'_m - F'_0)/F'_m$, $\Phi_{PSII} = (F_m - F_s)/F'_m$, $qP = (F'_m - F_s)/(F'_m - F'_0)$, and $qN = 1 - (F'_m - F'_0)/(F_m - F_0)$, respectively.

2.5. Determination of leaf water potential (Ψ_w), osmotic potential (Ψ_s), and turgor pressure (Ψ_t)

The leaves were separated from plants and their surfaces were cleaned up with a soft brush, and the leaf Ψ_w was measured using a PSYPRO water system (C-52 Sample Chamber, WESCOR Inc. Logan, UT, USA), then these leaves were placed in 5 ml pipette tips and stored at -80 °C for the measurement of Ψ_s . After the frozen leaves being thawed at 25 °C, the cell sap was extracted and centrifuged at 3000 \times g for 8 min. The Ψ_s of cell sap was calculated using the following formula: $\Psi_s = -n \times R \times T$, where n is the solute molar concentration measured using a cryoscopic osmometer (Osmomat-030, Gonotec GmbH, Berlin, Germany) at 25 °C, here $R = 0.008314$ and $T = 298.8$ (Wu et al., 2015). Ψ_t was calculated using the equation (Ma et al., 2012): $\Psi_w = \Psi_s + \Psi_t$.

2.6. Determination of Na^+ and K^+ accumulation in tissues and leaf Na^+ secretion

The concentrations of Na^+ and K^+ in the tissues of leaf and root were determined refer to the method described by Wu et al. (2015). Briefly, samples were washed with distilled water and dried in an oven, and then the dried samples were cut and grinded. The Na^+ and K^+ in the dried samples were extracted in 100 mM at 90 °C for 2 h and analysed using a flame photometer (2655-00, Cole-Parmer Instrument Co. Vernon Hills, IL, USA).

Na^+ secretion of salt glands was measured according to the methods as described by Wang et al. (2009). The shoots of plants were washed by distilled water before drought treatment, then the shoots with leaves were cut at different times of withholding water, rinsed in 50 ml distilled water for 2 min, and weighted orderly. Na^+ amount in the rinsed liquid was determined as above. Leaf Na^+ secretion was represented with the rinsed liquid Na^+ amount / sample fresh weight.

2.7. Determination of soluble sugar and proline concentrations

The secreta and dust on leaf surface were cleaned at different times of withholding water, then the leaves were separated from branches

and immediately frozen in liquid nitrogen. Soluble sugars and proline in leaves were measured in accordance with sulfuric acid-anthrone colorimetric method (Wu et al., 2015) and acid ninhydrin method (Bates et al., 1973; Wu et al., 2015), respectively, using a UV spectrophotometer (UV-2100, Unico Instrument Co. Ltd, Shanghai, China).

2.8. Calculation of osmotic potential of solutes and their contributions to leaf Ψ_s

The osmotic potential values of Na^+ , K^+ , soluble sugar, and proline were calculated using the following formula: $\Psi_s = -nRT$, here n is the molar concentration of solute molecules (mol/m^3), $R = 0.008314 \text{ m}^3 \text{ MPa mol}^{-1} \text{ K}^{-1}$, and $T = 298.8 \text{ K}$ (Guerrier, 1996). n was determined with the following formula: $n = C \times DW / (FW - DW)$, here C is the osmotic solute content of the dry material, FW and DW are the fresh weight and dry weight of leaf, respectively, and $(FW - DW)$ is the sap volume in a sample (Ming et al., 2012). The contributions of solutes to leaf Ψ_s were estimated using the following formula: Contribution rate (CR) = Ψ_{ss}/Ψ_{sl} , where Ψ_{ss} and Ψ_{sl} were solute calculated osmotic potential and leaf osmotic potential, respectively.

2.9. Statistical analysis

Data were presented as means with standard error and analyzed by one-way analysis of variance (ANOVA). All statistical tests were performed using the software SPSS 19.0 (SPSS Inc. Chicago, IL, USA). The significant differences between means were detected by Duncan's multiple range tests ($P < 0.05$).

3. Results

3.1. Growth performance of *R. soongorica* under different NaCl treatments

Compared to control, the supplementation of 0.29 and 0.59 g kg^{-1} NaCl significantly increased plant height, crown diameter, and root length, especially, supplementary 0.59 g kg^{-1} NaCl resulted in the highest increase rates on leaf area, shoot fresh weight, root fresh weight, and relative growth rate (RGR) by 21, 27, 27, and 14%, respectively (Fig. 1 and Table 3). However, the supplementation of 0.88 or 1.17 g kg^{-1} NaCl had no obvious effect on the growth of *R. soongorica* (Fig. 1 and Table 3).

3.2. Moderate NaCl stimulates the growth of *R. soongorica* under drought condition

As shown in Table 4, under well-watered condition, the supplementation of 0.59 g kg^{-1} NaCl (moderate NaCl) significantly stimulated the growth of *R. soongorica* compared to the corresponding control. Although withholding water for 20 days caused serious inhibition on the growth of *R. soongorica*, the supplementary 0.59 g kg^{-1} NaCl effectively alleviated the inhibitory influence of drought stress, in which the plant height, crown diameter, root length, leaf area, fresh weight, and RGR were 18, 20, 28, 14, and 17% higher than that in corresponding treatment without NaCl addition, respectively, even as big as those in the well-watered control (Table 4).



Fig. 1. The photos of 6-week-old *R. soongorica* seedlings grown in sandy soils supplementing with 0 (Control), 0.29, 0.59, 0.88, and 1.17 g kg⁻¹ NaCl at 60% field water capacity (FWC) for 45 days, respectively. Each groups included 10 plants.

3.3. Moderate NaCl alleviates the deformation of leaf morphological structure in *R. soongorica* exposed to drought stress

Under well-watered conditions, the leaf cross sections from both NaCl-supplied and control plants were consistently circle, of which the arrangement and distribution of all the tissues were very orderly; likewise, both the length and area of leaf cross section in NaCl-supplied group (NaCl) were obvious bigger than those in corresponding control, indicating that supplementary 0.59 g kg⁻¹ NaCl has an increased effect on leaf area of *R. soongorica* (Fig. 2a and b).

When subjected to drought stress (withholding water for 20 days), most cell shapes in leaf and their spatial distributions were serious changed (Fig. 2c and d), the length, width and area of all leaf cross sections from both NaCl-supplied and control were sharply reduced, and their length/width were significantly increased (Table 5). Furthermore, under drought condition, the length, width and area of leaf cross section in NaCl-supplied group were 15, 23, and 35% bigger, respectively, than those in treatment without NaCl addition, for instance, the length/width was 9.91% lower (Table 5).

3.4. Moderate NaCl improves photosynthesis and water use efficiency (WUE) of *R. soongorica* exposed to drought stress

An obvious increase in net photosynthesis rate (Pn) and stomatal conductance (Gs) of the NaCl-supplied plants was observed before the withholding water (0 day, 70% of FWC, well-watered), respectively, in comparison to the plants without NaCl addition (Fig. 3a and b). Drought stress caused a sharp decrease in both Pn and Gs of *R. soongorica*, while the supplementary 0.59 g kg⁻¹ NaCl significantly increased Pn and Gs compared with those in the corresponding control during drought stress (Fig. 3a and b). Likewise, Ci in the all plants decreased gradually with the increase in water restriction from 5 to 15 days, and the extent of which in the NaCl-supplemented plants was less than the corresponding

control; at the time of withholding water for 20 days, the Ci in control group even increased rapidly compared with that of the plants for which water was withheld for 15 days, whereas the Ci in NaCl-supplied group still decreased (Fig. 3c). Additional, supplementary 0.59 g kg⁻¹ NaCl induced a sharp increase in WUE of *R. soongorica* exposed to severe drought stress (i.e. withholding water for 15 or 20 days).

Furthermore, drought stress triggered a significant reduction in the maximal quantum yield of photosystem II (PSII) photochemistry (F_v/F_m), the efficiency of excitation capture by the opening of the PSII reaction center (F_v'/F_m'), the actual PSII efficiency (Φ_{PSII}), and the photochemical quenching coefficient (qP) of *R. soongorica*, as well as an increase in the non-photochemical quenching coefficient (qN), respectively. However, supplementary 0.59 g kg⁻¹ NaCl effectively mitigated above influences of drought stress (Fig. 4).

3.5. Moderate NaCl improves leaf water status of *R. soongorica* under drought conditions

In comparison with the control, supplementary 0.59 g kg⁻¹ NaCl resulted a significant reduce in leaf water potential (Ψ_w) or osmotic potential (Ψ_s), and correspondingly, an obvious increase in leaf turgor pressure (Ψ_t) of *R. soongorica* under well-watered condition (Fig. 5a–c). Likewise, the Ψ_w , Ψ_s and Ψ_t in control or NaCl-supplied group were all decreased gradually with the prolonging of withholding water time, of which the Ψ_w or Ψ_s in NaCl-supplied group was always lower than that in corresponding control, while the Ψ_t was higher (Fig. 5a–c).

No obvious difference in leaf relative water content (RWC) between the control and NaCl-supplied groups was observed under well-watered or slight drought stress (withholding water for 5 days) (Fig. 5d). However, a conspicuous discrepancy in RWC between the two groups was shown when subjected to drought stress by withholding water for 10 days or even longer time, in which the RWC of the NaCl-supplied plants was higher by at least 12% than that of the corresponding control (Fig. 5d).

3.6. Na⁺ and K⁺ accumulations in tissues of *R. soongorica* and leaf Na⁺ secretion under drought conditions in the presence or absence of NaCl

Regardless of well-watered or drought stress conditions, supplementary 0.59 g kg⁻¹ NaCl increased the tissue Na⁺ concentrations in both leaf and root of *R. soongorica* by at least 52% and 63%, respectively, compared with those of the control plants (Fig. 6a and c). Likewise, a significant higher leaf Na⁺ secretion by at least 76% in the presence than the absence of supplementary 0.59 g kg⁻¹ NaCl during drought stress was observed (Fig. 6b). Severe drought stress significantly increased the Na⁺ concentration in leaves as well as decreased Na⁺ accumulation in roots of the plants either with or without NaCl supplement, than for well-watered conditions (Fig. 6a and c).

Additionally, both the tissue K⁺ concentrations in leaves and roots of *R. soongorica* exposed to NaCl-supplied or drought stress conditions always were maintained at a constant quantity (Fig. 7a and b), implying that the K⁺ concentration and distribution in plant tissues were unaffected by supplying NaCl or drought stress.

Table 3

Plant height, crown diameter, root length, leaf area, shoot fresh weight, root fresh weight, and relative growth rate (RGR) of 6-week-old *R. soongorica* seedlings grown in sandy soils were supplied with 0, 0.29, 0.59, 0.88 or 1.17 g kg⁻¹ NaCl at 60% field water capacity (FWC) for 45 days. Values are means from twelve samples \pm SE (n = 12). Different letters in columns indicate significant differences at $P < 0.05$ (Duncan's multiple range test).

Supplementary NaCl (g kg ⁻¹)	Plant height (cm)	Crown diameter (cm ²)	Root length (cm)	Leaf area (cm ²)	Shoot fresh weight (g)	Root fresh weight (g)	RGR $\times 10^3$ (g kg ⁻¹ d ⁻¹)
0	6.54 \pm 0.15 c	4.13 \pm 0.14 c	11.27 \pm 0.15 c	6.32 \pm 0.23 b	0.45 \pm 0.02 b	0.30 \pm 0.02 b	16.33 \pm 0.85 b
0.29	7.85 \pm 0.27 b	4.73 \pm 0.09 b	14.04 \pm 0.67 b	6.93 \pm 0.45 ab	0.51 \pm 0.04 ab	0.34 \pm 0.03 ab	17.27 \pm 0.45 ab
0.59	8.76 \pm 0.33 a	5.23 \pm 0.32 a	16.75 \pm 0.57 a	7.63 \pm 0.32 a	0.57 \pm 0.02 a	0.38 \pm 0.03 a	18.65 \pm 0.34 a
0.88	6.56 \pm 0.24 c	4.27 \pm 0.12 c	12.13 \pm 0.65 c	6.56 \pm 0.14 b	0.48 \pm 0.03 b	0.32 \pm 0.02 b	16.41 \pm 0.21 b
1.17	6.53 \pm 0.12 c	4.25 \pm 0.14 c	12.03 \pm 0.45 c	6.45 \pm 0.21 b	0.47 \pm 0.02 b	0.31 \pm 0.01 b	16.38 \pm 0.15 b

Table 4

Plant height, crown diameter, root length, leaf area, shoot fresh weight, root fresh weight, and relative growth rate (RGR) of 6-week-old *R. soongorica* seedlings grown in sandy soil were supplied with 0 (Control) and 0.59 g kg⁻¹ NaCl (NaCl) at 60% of FWC for 75 days and then under well-watered (70% FWC) or drought stress (withholding water) for 20 days. Values are means from twelve samples \pm SE (n = 12). Different letters in columns indicate significant differences at $P < 0.05$ (Duncan's multiple range test).

Treatments		Plant height (cm)	Crown diameter (cm ²)	Root length (cm)	Leaf area (m ²)	Shoot fresh weight (g)	Root fresh weight (g)	RGR $\times 10^3$ (g kg ⁻¹ d ⁻¹)
Well-watered	Control	14.42 \pm 0.46 b	14.23 \pm 0.42 b	26.38 \pm 1.52 b	0.95 \pm 0.03 b	0.68 \pm 0.02 b	0.95 \pm 0.03 b	15.48 \pm 0.87 b
	NaCl	16.18 \pm 0.57 a	16.45 \pm 0.76 a	31.23 \pm 1.23 a	1.05 \pm 0.03 a	0.77 \pm 0.03 a	1.05 \pm 0.03 a	17.65 \pm 0.74 a
Drought stress	Control	12.53 \pm 0.42 c	12.27 \pm 0.63 c	21.22 \pm 1.13 c	0.85 \pm 0.03 c	0.58 \pm 0.01 c	0.85 \pm 0.03 c	14.21 \pm 0.54 c
	NaCl	14.75 \pm 0.52 b	14.78 \pm 0.58 b	27.25 \pm 1.32 b	0.98 \pm 0.02 a	0.70 \pm 0.02 b	0.98 \pm 0.02 a	16.17 \pm 0.48 b

3.7. Soluble sugar and proline concentrations in leaves of *R. soongorica* under drought conditions in the presence or absence of NaCl

Severe drought stress, i.e. 20 days of drought, resulted in an evident increment in soluble sugar and proline concentrations in the leaves of the all plants compared with the well-watered, of which the leaf soluble sugar and proline concentrations in NaCl-supplied plants were 12% and 20% lower than the control, respectively (Fig. 8a and b).

3.8. Contributions of main solutes to Ψ_s of *R. soongorica* under drought conditions in the presence or absence of NaCl

As shown in Table 6, supplementary 0.59 g kg⁻¹ NaCl sharply increased the contribution of Na⁺ to leaf Ψ_s of *R. soongorica*, whereas significantly decreased in the contribution of corresponding K⁺, proline or soluble sugar. Furthermore, the contribution of Na⁺ to leaf Ψ_s in the NaCl-supplied plants with withholding water for 20 days reached at about 22%, which was about 6 or 14 times as big as the contribution of corresponding K⁺ or sum of sugar and proline (Table 6).

4. Discussion

4.1. Moderate NaCl stimulates the growth of *R. soongorica* by improving photosynthetic capacity and mitigating light suppression of drought stress on PSII

Na⁺ distributing in soil or culture medium reaches the leaves of salt-secreting species via transpiration stream and then is divided into two parts, one part of which is quickly secreted via salt glands while another part is temporarily storage in leaves (Ramadan, 2001). It had been proved that some succulent xerophytes are able to absorb a large amount of Na⁺ from barren soil and accumulate them in tissues, which had a positive effect on their growth, especially exposed to drought condition (Ma et al., 2012; Kang et al., 2015). In the present study, the supplementation of 0.59 g kg⁻¹ NaCl stimulated effectively the growth of *R. soongorica*, where the growth-related parameters, including plant height, root length, leaf area, fresh weight, and RGR were all significantly increased compared to the treatment without additional NaCl (Table 3). Moreover, all these parameters in the NaCl-supplied group withheld water for 20 days were also significantly increased, compared with the corresponding control (Table 4). These results indicate that

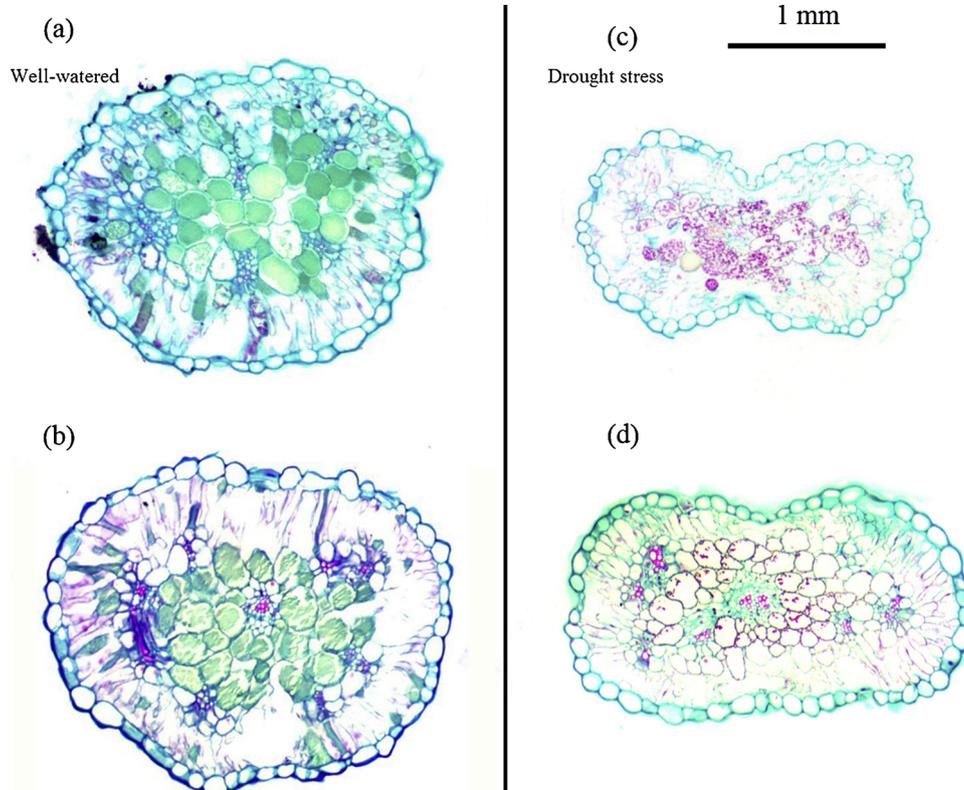


Fig. 2. Leaf cross section of 6-week-old *R. soongorica* seedlings grown in sandy soils supplementing with 0 (Control) (a, c) and 0.59 g kg⁻¹ NaCl (NaCl) (b, d) for 75 days and then under well-watered (70% FWC) or drought stress (withholding water) for 20 days.

Table 5

The length, width, area, and length/width of leaf cross section of 6-week-old *R. soongorica* seedlings grown in sandy soil were supplied with 0 (Control) and 0.59 g kg⁻¹ NaCl (NaCl) at 60% of FWC for 75 days and then under well-watered (70% FWC) or drought stress (withholding water) for 20 days. Values are means from six samples \pm SE (n = 6). Different letters in columns indicate significant differences at $P < 0.05$ (Duncan's multiple range test).

Treatments		Leaf cross section			
		Length (mm)	Width (mm)	Area (mm ²)	Length/width
Well-watered	Control	2.61 \pm 0.02 b	2.02 \pm 0.02 a	3.82 \pm 0.02 b	1.29 \pm 0.02 c
	NaCl	2.65 \pm 0.01 a	2.04 \pm 0.01 a	3.92 \pm 0.03 a	1.30 \pm 0.02 c
Drought stress	Control	2.27 \pm 0.02 c	1.07 \pm 0.02 c	2.24 \pm 0.02 d	2.12 \pm 0.03 a
	NaCl	2.62 \pm 0.02 b	1.32 \pm 0.02 b	3.02 \pm 0.01 c	1.91 \pm 0.02 b

moderate NaCl effectively stimulates the growth of *R. soongorica* under well-watered or drought stress conditions.

Photosynthesis is one of essential factors influencing plant growth, and strongly dependent on the opening of the stomata (Hu et al., 2010; Ma et al., 2012). Stomatal opening degree has a strong dependence on cell turgor, which is determined by tissue osmotic potential (Heidecher et al., 2003; Franks and Farquhar, 2007). Our results showed that, regardless of treated with drought stress or not, the supplementation of 0.59 g kg⁻¹ NaCl can help *R. soongorica* seedlings to maintain the higher level of Pn and Gs than those plants without additional NaCl, especially under severe drought stress (Fig. 3a and b); meanwhile, the addition of 0.59 g kg⁻¹ NaCl also resulted in an obvious increase in the Ψ_t of *R. soongorica* (Fig. 5c). These results suggest that 0.59 g kg⁻¹ NaCl might enhance Gs by aggrandizing Ψ_t , and as a result, improve Pn.

It has been reported that photosynthetic non-stomatal limitation in plants suffering from severe drought stress mainly results from the changes in chloroplast structure, damage to membrane systems, serious degradation to photosynthetic pigments, damage to the electron transfer system, and so forth (Xu et al., 2009; Fleck et al., 2012; Campos et al., 2014). Farquhar and Sharkey (1982) proposed that the change in Ci can be used as a parameter to assess whether the plant photosynthesis is reduced by stomatal limitation or non-stomatal limitation under stress conditions, with identical declining trends of Ci and Pn mainly representing stomatal limitation, and with the opposite trend indicating non-stomatal limitation. In this study, the Ci and Pn of *R. soongorica* in the absence of NaCl treatment decreased synchronously from 0 to 15 days of drought stress, but Ci exhibited a converse trend from 15–20 days of drought stress (Fig. 3a and c). Similarly, both Ci and

Pn in the presence of NaCl treatment decreased continuously from 0 to 20 days after withholding water (Fig. 3a and c). These results suggest that the plants without NaCl addition suffered mainly from non-stomatal limitation during 15–20 days of drought stress, while the supplementation of 0.59 g kg⁻¹ NaCl resulted in the plants mainly undergoing stomatal limitation. Therefore, we suppose that moderate NaCl could mitigate the harmful effects of drought stress on the mesophyll cells.

Chlorophyll fluorescence analysis has been feasibly applied in previous studies to detect and quantify the effects of stress environments on photosynthesis, particularly photosystem II (PSII) (Baker, 2008). Under well-watered conditions, the F_v/F_m , F_v/F_m' , Φ_{PSII} , qP , and qN in the NaCl-supplemented *R. soongorica* plants did not differ from those of the control (Fig. 4), implying that the supplementation of 0.59 g kg⁻¹ NaCl had no effect on the PSII photochemistry of *R. soongorica* grown in well-watered conditions. However, when exposed to moderate or severe drought stress (10 or 15 days after withhold water, less than 47% FWC), the supplementary 0.59 g kg⁻¹ NaCl significantly increased the F_v/F_m , F_v/F_m' , Φ_{PSII} , and qP of *R. soongorica* compared with that without additional NaCl, while the qN was markedly decreased, suggesting that the addition of a moderate amount of NaCl could relieve the light suppression on PSII in *R. soongorica* under drought stress. Our data are consistent with previous studies from the halophyte *S. salsa* (Lu et al., 2003), as well as in the xerophyte *Z. xanthoxylum* (Ma et al., 2012). It was proposed that Na⁺ plays a significant role in photophosphorylation (Aftab et al., 2011; Gattward et al., 2012) and electron transport between PSI and PSII (Lu et al., 2003; Qiu et al., 2010).

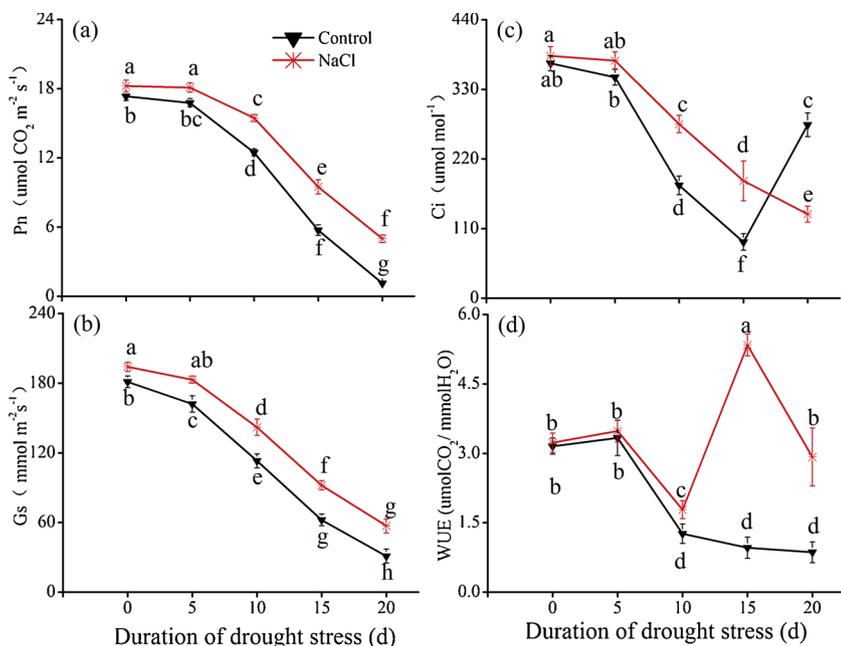


Fig. 3. Net photosynthesis rate (Pn) (a), stomatal conductance (Gs) (b), intracellular CO₂ concentration (Ci), and water use efficiency (WUE) (d) of 6-week-old *R. soongorica* seedlings grown in sandy soils supplementing with 0 (Control) and 0.59 g kg⁻¹ NaCl (NaCl) at 60% of FWC for 75 days and then withholding water for 0, 5, 10, 15, and 20 days. Values are means from nine samples \pm SE (n = 9) and bars indicate SE. Different letters in curves indicate significant differences at $P < 0.05$ (Duncan's test).

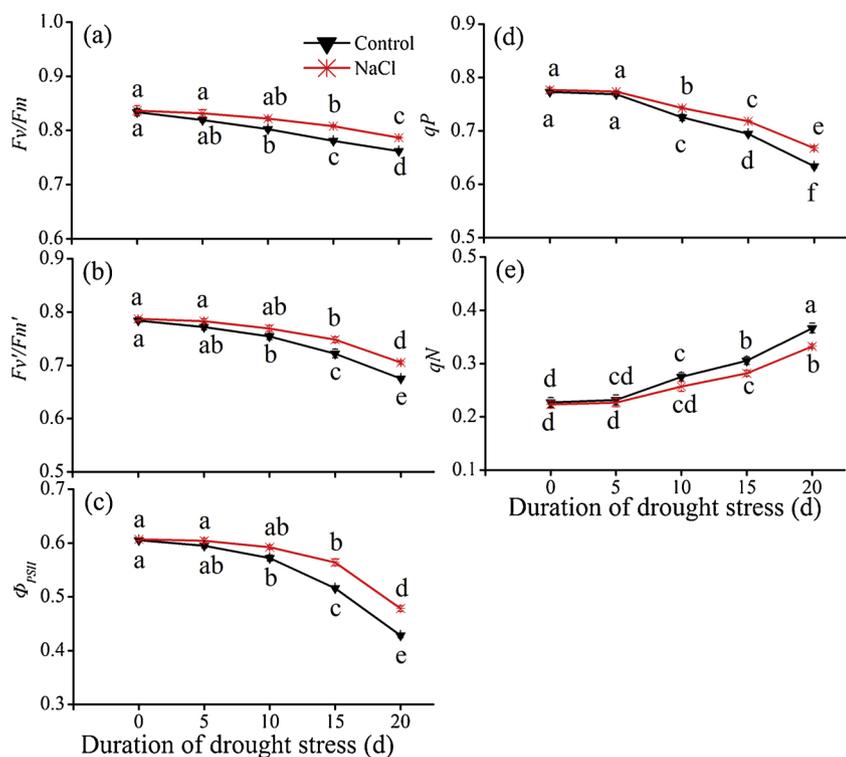


Fig. 4. The maximal quantum yield of photosystem II (PSII) photochemistry (F_v/F_m) (a), the efficiency of excitation capture by the opening of the PSII reaction center (F_v/F_m') (b), the actual PSII efficiency (Φ_{PSII}) (c), the photochemical quenching coefficient (qP) (d), and the non-photochemical quenching coefficient (qN) (e) of 6-week-old *R. soongorica* seedlings grown in sandy soils supplementing with 0 (Control) and 0.59 g kg⁻¹ NaCl (NaCl) at 60% of FWC for 75 days and then withholding water for 0, 5, 10, 15, and 20 days. Values are means from nine samples \pm SE (n = 9) and bars indicate SE. Different letters in curves indicate significant differences at $P < 0.05$ (Duncan's test).

4.2. Moderate NaCl maintains the leaf morphology structures of *R. soongorica* by improving osmotic adjustment ability

Leaf is the main site of photosynthesis and respiration of a plant, and also sensitive to changes in external ecological factors. Drought stress often results in a series of changes in leaf morphology or structure of high plants (Silva et al., 2010), which may reflect the degree of deleterious impact of drought stress on leaves of plant to a certain degree (Zhang et al., 2015). In well-watered condition, *R. soongorica* possesses rot-shaped leaf, and the cross section of which is composed of cellular-layer, epidermal cell, palisade cell, vascular bundle and sponge tissue

from outside to inside, arranging orderly (Liu et al., 2007). We also observed similar leaf morphology structures from *R. soongorica* under well-watered conditions in this study (Fig. 2a and b); when subjected to drought stress (withholding water for 20 days), however, most cell shapes of leaf tissues and their spatial distributions were seriously changed, while the supplementation of 0.59 g kg⁻¹ NaCl obviously shrunk these changes (Fig. 2c and d). Moreover, all the decreases of length, width, and area of leaf cross section caused by drought stress were effectively lessened by supplying 0.59 g·kg⁻¹ NaCl (Table 5). These results indicate that moderate NaCl effectively alleviated deleterious impact of drought stress on cell shape and their spatial

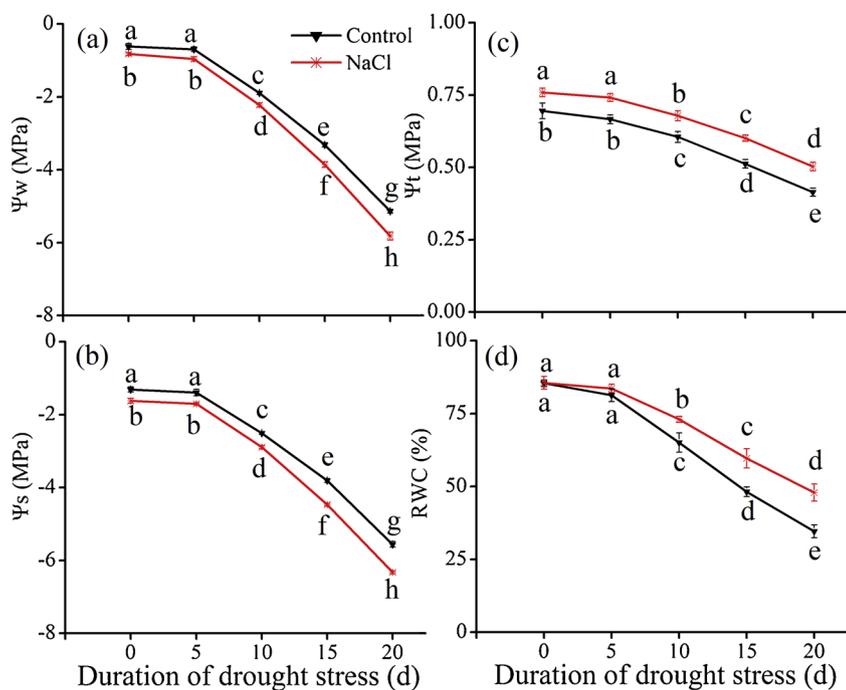


Fig. 5. Leaf water potential (Ψ_w) (a), osmotic potential (Ψ_s) (b), turgor pressure (Ψ_t) (c), and relative water content (RWC) (d) of 6-week-old *R. soongorica* seedlings grown in sandy soils supplementing with 0 (Control) and 0.59 g kg⁻¹ NaCl (NaCl) at 60% of FWC for 75 days and then withholding water for 0, 5, 10, 15, and 20 days. Values are means from six samples \pm SE (n = 6) and bars indicate SE. Different letters in curves indicate significant differences at $P < 0.05$ (Duncan's test).

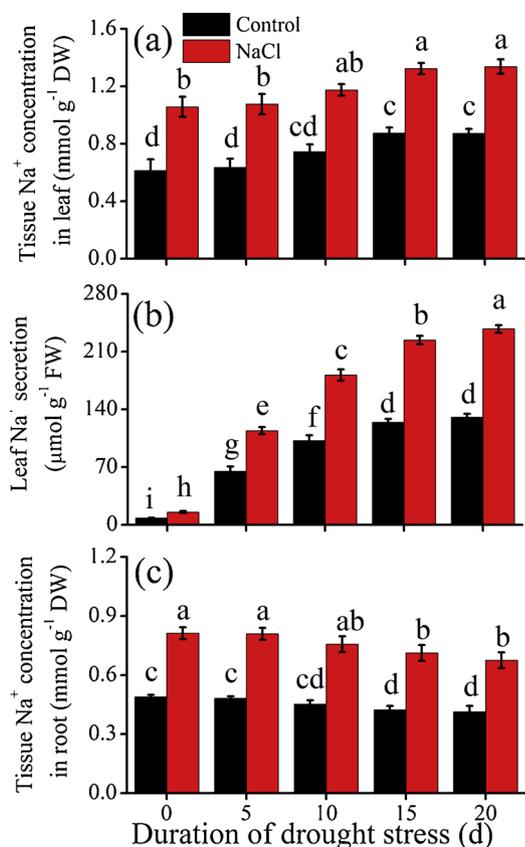


Fig. 6. Tissue Na^+ accumulations in leaf (a) and root (c), and leaf Na^+ secretion (b) of 6-week-old *R. soongorica* seedlings grown in sandy soils supplementing with 0 (Control) and 0.59 g kg^{-1} NaCl (NaCl) at 60% of FWC for 75 days and then withholding water for 0, 5, 10, 15, and 20 days. Values are means from six samples \pm SE ($n = 6$) and bars indicate SE. Different letters in columns indicate significant differences at $P < 0.05$ (Duncan's test).

distribution in leaves of *R. soongorica*.

Usually, drought stress results in a serious water deficit in plant organs, especially leaves due to baring in dry space and strong transpiration (Amoroso et al., 2015). Cell turgor pressure has a strong dependence on water status of a plant, and leaf water deficit can lead to changes of the shapes, arranges, and spatial distributions of cells in leaf tissues since there is a decrease of turgor pressure (Bosabalidis and Kofidis, 2002). Our results showed that drought stress triggered a greatly diminishing in both leaf Ψ_t and RWC of *R. soongorica*, but moderate NaCl increased the Ψ_t and RWC were by 22 and 54%, respectively, compared with the corresponding control (Fig. 5c and d), indicating that moderate NaCl enlarge Ψ_t and enhance in RWC of *R. soongorica* subjected to drought stress. Therefore, we suggest that moderate NaCl could effectively prevent from deformation of leaf morphology in *R. soongorica* exposed to drought stress by raising RWC and augmenting leaf Ψ_t .

Osmotic adjustment is an important strategy for plants to deal with drought stress, from which plants often sustains better growth performance by accumulating a large amount of solutes in cells to decrease osmotic potential (Ψ_s) and maintain higher turgor (Ψ_t) under drought stress (Chaves et al., 2003; Farooq et al., 2009). There is increasing experimental evidence that a decrease in Ψ_s can enhance water absorption ability of plants (Wu et al., 2015; Jupa et al., 2017). In our study, the leaf Ψ_s of *R. soongorica* was sharply reduced with strengthening of drought stress, and the reduction extent of Ψ_s was accelerated by supplying moderate NaCl (Fig. 5b), suggesting that *R. soongorica* possesses a strongly capability of osmotic adjustment in arid environment, which can be strengthened by supplying moderate NaCl. Moreover,

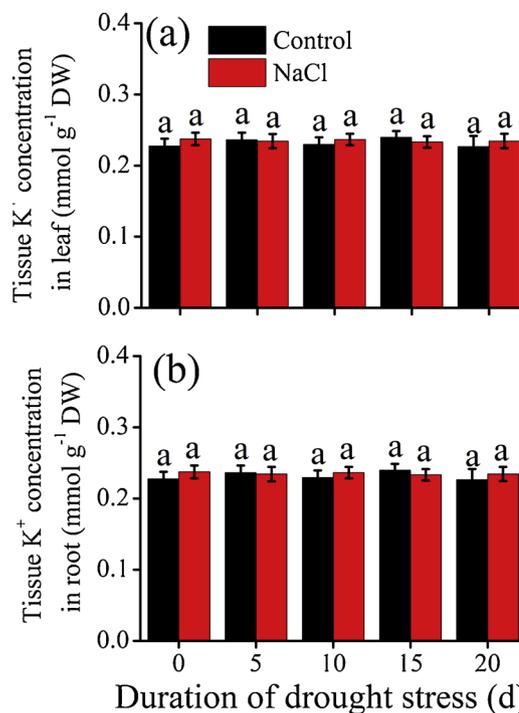


Fig. 7. Tissue K^+ accumulations in leaf (a) and root (b) of 6-week-old *R. soongorica* seedlings grown in sandy soils supplementing with 0 (Control) and 0.59 g kg^{-1} NaCl (NaCl) at 60% of FWC for 75 days and then withholding water for 0, 5, 10, 15, and 20 days. Values are means from six samples \pm SE ($n = 6$) and bars indicate SE. Different letters in columns indicate significant differences at $P < 0.05$ (Duncan's test).

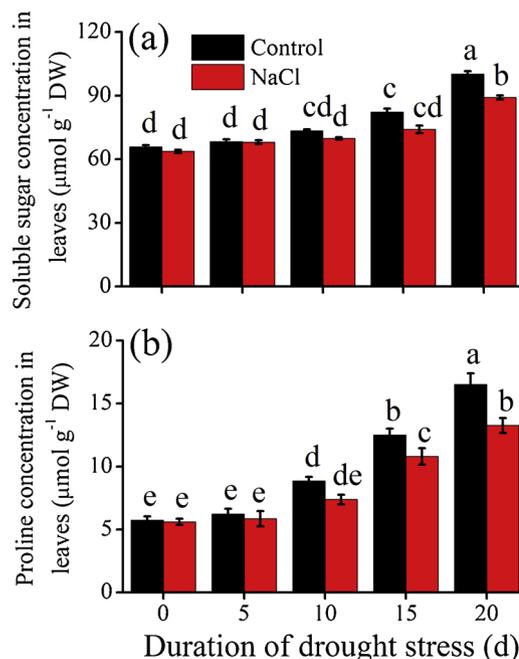


Fig. 8. Leaf soluble sugar (a) and proline (b) concentrations of 6-week-old *R. soongorica* seedlings grown in sandy soils supplementing with 0 (Control) and 0.59 g kg^{-1} NaCl (NaCl) at 60% of FWC for 75 days and then withholding water for 0, 5, 10, 15, and 20 days. Values are means from six samples \pm SE ($n = 6$) and bars indicate SE. Different letters in columns indicate significant differences at $P < 0.05$ (Duncan's test).

the supplementation of 0.59 g kg^{-1} NaCl helped the plant to maintain a higher RWC when subjected to drought stress (Fig. 5d), indicating that the positive effect of NaCl on improving RWC in the leaves of *R.*

Table 6

The contributions of Na^+ , K^+ , soluble sugar and proline to leaf osmotic potential (Ψ_s) of 6-week-old *R. soongorica* seedlings grown were supplied with 0 (Control) and 0.59 g kg^{-1} NaCl (NaCl) at 60% of FWC for 75 days and then with withholding for 20 days. Values are means from six samples \pm SE ($n = 6$). Different letters in columns indicate significant differences at $P < 0.05$ (Duncan's multiple range test).

Time of withholding water (d)	Treatments	Contributions of Na^+ , K^+ , soluble sugar and proline to leaf Ψ_s (%)			
		Na^+	K^+	Soluble sugar	Proline
20	Control	$13 \pm 0.37 \text{ b}$	$4.3 \pm 0.12 \text{ a}$	$1.7 \pm 0.02 \text{ a}$	$0.3 \pm 0.02 \text{ a}$
	NaCl	$22 \pm 1.03 \text{ a}$	$3.9 \pm 0.10 \text{ b}$	$1.4 \pm 0.03 \text{ b}$	$0.2 \pm 0.01 \text{ b}$

soongorica might be due to more Na^+ accumulation in plants resulted in a lower osmotic potential and higher water absorption capacity in plant.

It is well known that K^+ can fulfill multiple physiological functions for plant, especially osmotic adjustment under low water potential conditions (Kanai et al., 2011; Hosseini et al., 2016), and Na^+ can replace K^+ , at least to a certain degree, particularly in its osmotic function (Wakeel et al., 2011; Ran et al., 2014). A positive effect of Na^+ accumulation in xerophyte has been proved under drought stress, since Na^+ can play an important role in decreasing the leaf osmotic potential of those species (Ma et al., 2012). In this study, we examined the dynamic changes of main solutes in *R. soongorica* with or without NaCl-supplemented during drought stress by withholding water. Notably, both tissue Na^+ concentrations in leaves of the NaCl-supplemented and control plants were at least 4.7 and 2.9 times higher than those of the corresponding K^+ during this period, respectively, even 11 times as big as the sum of the soluble sugar and proline concentrations (Figs. 6–8), indicating that *R. soongorica* possesses a special capacity to absorb Na^+ over K^+ from soil and transport it from roots into leaves under saline and drought conditions; at the same time, Na^+ concentration was enhanced in leaf tissues, whilst reduced in roots of plants when exposed to severe drought stresses (Fig. 6a and c), implying *R. soongorica* could regulate the amount of Na^+ accumulations in leaf and root tissues under different drought conditions. This might not only avoid excess Na^+ toxicity to root system, but also decrease leaf osmotic to enhance water absorption capacity, which should be an important physiological mechanism of adaption to arid desert habitat. Additionally, the supplementation of 0.59 g kg^{-1} NaCl resulted a sharp increase in the Na^+ contribution to leaf Ψ_s , whereas a significant reduction in the contributions of K^+ , soluble sugar or proline; moreover, in NaCl-supplied group, the contribution Na^+ to leaf Ψ_s exceeded 22%, while the contribution of K^+ , soluble sugar, or proline was only 3.9%, 1.4% or 0.2% (Table 6). These results further confirm that Na^+ plays an important role in osmotic adjustment of *R. soongorica*.

4.3. Salt secretion of salt glands in *R. soongorica* plays an important role in adapting to high salinity and arid environments

Salt glands are prevalent on the surface of stem or leaf of salt-secreting plants, which act an important role in regulating ion balance, keeping osmotic pressure stability, and enhancing salinity tolerance (Ding et al., 2010). The structural characteristics of salt glands in different species are diverse, which in the dicotyledonous salt-secreting species are multi-cellular and sunken into the epidermis (Feng et al., 2014; Yuan et al., 2015). Some species in Tamaricaceae possess high salt secretion ability (Yuan et al., 2015), such as *Tamarix aphylla*, whose salt glands are identified composed of six secreting cells and two collecting cells (Thomson and Platt-Aloia, 1985). Salt secretion of salt glands in recretohalphytes was believed to be an active physiological process, and need large amounts of energy (Yuan et al., 2016). Ions secreted via salt glands include many kinds of cationic and anionic ions, depending strongly on the environment (Feng et al., 2014), and Na^+ secretion is higher than other cationic (Ma et al., 2011). In our study, a significant higher Na^+ secretion of leaf salt glands by at least 76% in the presence than the absence supplementary 0.59 g kg^{-1} NaCl was

observed in *R. soongorica* during water deficit (Fig. 6b). Zhou et al. (2012) observed similar result that, Na^+ secreted via salt glands of shoot of *R. soongorica* seedlings exposed to the additional 150 mM NaCl for 7 days was increased by 22 times compared with the corresponding control. These results suggest that indicating that *R. soongorica* possesses a strong capability of secreting Na^+ in higher NaCl environment. Recretohalphytes often excess ions to external environment through salt glands once growth and ion accumulation is balanced, which also is one of the possible avoidance mechanisms when exposed to high salt environments (Dassanayake and Larkin, 2017). In this study, no significant inhibition on the growth of *R. soongorica* grown in sandy soil supplied with 0.88 or 1.17 g kg^{-1} NaCl for 45 days was observed, compared to the corresponding control (Table 3). This result suggests that the better growth performance of *R. soongorica* in high salinization environments is due to a strongly capability of secreting ions via salt glands, which secreted excess Na^+ out plant and avoided ion toxicity. Therefore, we suppose that salt secretion of salt gland of *R. soongorica* should play an important role in adapting to salinity and arid environments.

4.4. Conclusions

Our results suggest that 0.59 g kg^{-1} NaCl effectively stimulates the growth of *R. soongorica* by improving photosynthetic capacity and alleviating light suppression of drought stress on PSII. Moreover, the addition of moderate NaCl can facilitate *R. soongorica* to maintain its leaf morphology structures under drought conditions, since Na^+ participates the osmotic adjustment to maintain higher Ψ_t and RWC in leaf. Additionally, the salt secretion via salt glands plays an important role in *R. soongorica* adapting to high salinity and drought conditions. These findings laid a basis for understanding the physiological mechanisms of salt-secreting xerophytes in response to adverse environments.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

CRediT authorship contribution statement

Fang-Lan He: Funding acquisition, Formal analysis, Writing - original draft. **Ai-Ke Bao:** Supervision, Writing - review & editing. **Suo-Min Wang:** Supervision, Writing - review & editing. **Hong-Xi Jin:** Funding acquisition.

Acknowledgements

This work was granted by the National Natural Science Foundation of China (318601753, 31670405, and 31372360), Innovative Research Groups of Foundation of Gansu Province, China (145RJIA335 and 18JR3RA018), and the Fundamental Research Funds for the Central Universities (lzujbky-2018-k01).

References

- Aftab, T., Khan, M.M.A., Idrees, M., Naeem, M., Hashmi, N., Varshney, L., 2011. Enhancing the growth, photosynthetic capacity and artemisinin content in *Artemisia annua* L. By irradiated sodium alginate. *Radiat. Phys. Chem.* 80, 833–836. <https://doi.org/10.1016/j.radphyschem.2011.03.004>.
- Ahanger, M.A., Agarwal, R.M., 2017. Potassium up-regulates antioxidant metabolism and alleviates growth inhibition under water and osmotic stress in wheat (*Triticum aestivum* L.). *Protoplasma* 254, 1471–1486. <https://doi.org/10.1007/s00709.016.1037.0>.
- Amoroso, M.M., Daniels, L.D., Villalba, R., Cherubini, P., 2015. Does drought incite tree decline and death in Austrocedrus chilensis forests? *J. Veg. Sci.* 26, 1171–1183. <https://doi.org/10.1111/jvs.12320>.
- Bai, J., Xu, D.H., Kang, H.M., Chen, K., Wang, G., 2008. Photoprotective function of photorespiration in *Reaumuria soongorica* during different levels of drought stress in natural high irradiance. *Photosynthetica* 46, 232–237. <https://doi.org/10.1007/s11099-008-0037-5>.
- Baker, N.R., 2008. Chlorophyll fluorescence: a probe of photosynthesis in vivo. *Annu. Rev. Plant Biol.* 59, 89–113. <https://doi.org/10.1146/annurev.arplant.59.032607.092759>.
- Bates, L.S., Waldren, R.P., Teare, I.D., 1973. Rapid determination of free proline for water-stress studies. *Plant Soil* 39, 205–207. <https://doi.org/10.1007/BF00018060>.
- Binks, O., Meir, P., Rowland, L., Da, C.A., Vasconcelos, S.S., 2016. Plasticity in leaf-level water relations of tropical rainforest trees in response to experimental drought. *New Phytol.* 211, 477–488. <https://doi.org/10.1111/nph.13927>.
- Bosabalidis, A.M., Kofidis, G., 2002. Comparative effects of drought stress on leaf anatomy of two olive cultivars. *Plant Sci.* 163, 375–379. [https://doi.org/10.1016/S0168-9452\(02\)00135-8](https://doi.org/10.1016/S0168-9452(02)00135-8).
- Campos, H., Trejo, C., Peña-Valdivia, C.B., García-Nava, R., Conde-Martínez, F.V., Cruz-Ortega, M.R., 2014. Stomatal and non-stomatal limitations of bell pepper (*Capsicum annuum* L.) plants under water stress and re-watering: delayed restoration of photosynthesis during recovery. *Environ. Exp. Bot.* 98, 56–64. <https://doi.org/10.1016/j.envexpbot.2013.10.015>.
- Chaves, M.M., Maroco, J.P., Pereira, J.S., 2003. Understanding plant responses to drought—from genes to the whole plant. *Funct. Plant Biol.* 30, 239–264. <https://doi.org/10.1111/pbr.12004>.
- Cui, Y.N., Xia, Z.R., Ma, Q., Wang, W.Y., Chai, W.W., Wang, S.M., 2019. The synergistic effects of sodium and potassium on the xerophyte *Apocynum venetum* in response to drought stress. *Plant Physiol. Biochem.* 135, 489–498. <https://doi.org/10.1016/j.plaphy.2018.11.011>.
- Daliakopoulos, I.N., Panagea, I.S., Tsanis, I.K., Grillakis, M.G., Koutroulis, A.G., Hessel, R., Mayor, A.G., Ritsema, C.J., 2017. Yield responses of Mediterranean rangelands under a changing climate. *Land Degrad. Develop.* 28, 1962–1972. <https://doi.org/10.1002/ldr.2717>.
- Dassanayake, M., Larkin, J.C., 2017. Making plants break a sweat: the structure, function, and evolution of plant salt glands. *Front. Plant Sci.* 8, 406. <https://doi.org/10.3389/fpls.2017.00406>.
- Ding, F., Yang, J.C., Yuan, F., Wang, B.S., 2010. Progress in mechanism of salt excretion in recretohalophytes. *Front. Biol.* 5, 164–170. <https://doi.org/10.1007/s11515-010-0032-7>.
- Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra, S., 2009. Plant drought stress: effects, mechanisms and management. *Agron. Sustain. Dev.* 29, 185–212. <https://doi.org/10.1051/agro:2008021>.
- Farquhar, G.D., Sharkey, T.D., 1982. Stomatal conductance and photosynthesis. *Ann. Rev. Plant Physiol.* 33, 317–345. <https://doi.org/10.1146/annurev.pp.33.060182.001533>.
- Feng, Z., Sun, Q., Deng, Y., Sun, S., Zhang, J., Wang, B., 2014. Study on pathway and characteristics of ion secretion of salt glands of *Limonium bicolor*. *Acta Physiol. Plant.* 36, 2729–2741. <https://doi.org/10.1007/s11738-014-1644-3>.
- Fleck, I., Peña-Rojas, K., Aranda, X., 2012. Mesophyll conductance to CO₂ and leaf morphological characteristics under drought stress during *Quercus ilex* L. Resprouting. *Ann. Forest Sci.* 67. <https://doi.org/10.1051/forest/2009114>. 308–308.
- Franks, P.J., Farquhar, G.D., 2007. The mechanical diversity of stomata and its significance in gas-exchange control. *Plant Physiol.* 143, 78–87. <https://doi.org/10.1104/pp.106.089367>.
- Gattward, J.N., Almeida, A.A., Souza, J.Q., Gomes, F.P., Kronzucker, H.J., 2012. Sodium–potassium synergism in *Theobroma cacao*: stimulation of photosynthesis, water-use efficiency and mineral nutrition. *Physiol. Plant.* 146, 350–362. <https://doi.org/10.1111/j.1399-3054.2012.01621.x>.
- Guerrier, G., 1996. Fluxes of Na⁺, K⁺ and Cl⁻, and osmotic adjustment in *Lycopersicon pimpinellifolium* and *L. esculentum* during short-and long-term exposures to NaCl. *Physiol. Plantarum.* 97, 583–591. <https://doi.org/10.1016/j.scienta.2012.02.002>.
- Habibi, G., 2014. Silicon supplementation improves drought tolerance in canola plants. *Russ. J. Plant Physiol.* 61, 784–791. <https://doi.org/10.1134/S.1021443714060077>.
- Hatami, M., Hadian, J., Ghorbanpour, M., 2017. Mechanisms underlying toxicity and stimulatory role of single-walled carbon nanotubes in *Hyoscyamus niger* during drought stress stimulated by polyethylene glycol. *J. Hazard. Mater.* 324, 306–320. <https://doi.org/10.1007/s11738-009-0410-4>.
- He, C.Y., Zhang, G.Y., Zhang, J.G., Duan, A.G., Luo, H.M., 2016. Physiological, biochemical, and proteomic profiling reveals key pathways underlying the drought stress response of Hippophae rhamnoides. *Proteomics* 16, 2688–2697. <https://doi.org/10.1002/pmic.201600160>.
- He, F.L., Jin, H.X., Guo, C.X., Ma, J.M., Wu, H., 2017. Vegetation composition and community similarity of *Haloxylon ammodendron* plantation at different degree of degradation on the edge of Minqin Oasis. *J. Desert Res.* 37, 1–7 (in Chinese).
- Hosseini, S.A., Hajirezaei, M.R., Christiane, S., Nese, S., Nicolaus, V.W., 2016. A potential role of flag leaf potassium in conferring tolerance to drought-induced leaf senescence in barley. *Front. Plant Sci.* 7, 206. <https://doi.org/10.3389/fpls.2016.00206>.
- Hu, L.X., Wang, Z.L., Huang, B.R., 2010. Diffusion limitation and metabolic factors associated with inhibition and recovery of photosynthesis from drought stress in a C₃ perennial grass species. *Physiol. Plantarum* 139, 93–106. <https://doi.org/10.1111/j.1399-3054.2010.01350.x>.
- Hua, T., Wang, X.M., Zhang, C.X., Lang, L.L., Lim, H., 2017. Responses of vegetation activity to drought in northern China. *Land Degrad. Dev.* 28, 1913–1921. <https://doi.org/10.1002/ldr.2709>.
- Jupa, R., Plichta, R., Paschoá, Z., Nadezhkina, N., Gebauer, R., 2017. Mechanisms underlying the long-term survival of the monocot *Dracaena marginata* under drought condition. *Tree Physiol.* 37, 1182–1197. <https://doi.org/10.1093/treephys/tpx072>.
- Kanai, S., Moghaieb, R.E., Elshemy, H.A., Panigrahi, R., Mohapatra, P.K., 2011. Potassium deficiency affects water status and photosynthetic rate of the vegetative sink in green house tomato prior to its effects on source activity. *Plant Sci.* 180, 368–374. <https://doi.org/10.1016/j.plantsci.2010.10.011>.
- Kang, J.J., Zhao, W.Z., Su, P.X., Zhao, M., Yang, Z.H., 2014. Sodium (Na⁺) and silicon (Si) coexistence promotes growth and enhances drought resistance of the succulent xerophyte *Haloxylon ammodendron*. *Soil Sci. Plant Nutr.* 60, 659–669. <https://doi.org/10.1080/00380768.2014.935695>.
- Kang, J.J., Zhao, W.Z., Zhao, M., Zheng, Y., Yang, F., 2015. NaCl and Na₂SiO₃ coexistence strengthens growth of the succulent xerophyte *Nitraria tangutorum* under drought. *Plant Growth Regul.* 77, 223–232. <https://doi.org/10.1007/s10725-015-0055-9>.
- Kang, P., Bao, A.K., Kumar, T., Pan, Y.Q., Bao, Z.L.T., Wang, F., Wang, S.M., 2016. Quality of stress tolerance, productivity, and forage quality in T₁ transgenic Alfalfa co-overexpressing *ZxNHX* and *ZxVP1-1* from *Zygophyllum xanthoxylum*. *Front. Plant Sci.* 7, 1598. <https://doi.org/10.3389/fpls.2016.01598>.
- Kang, J.J., Zhao, W.Z., Zheng, Y., Zhang, D.M., Zhou, H., Sun, P.C., 2017. Calcium chloride improves photosynthesis and water status in the C₄ succulent xerophytes *Haloxylon ammodendron* under water deficit. *Plant Growth Regul.* 82, 467–478. <https://doi.org/10.1007/s10725-017-0273-4>.
- Liu, Y.B., Wang, G., Liu, J., Zhao, X., 2007. Anatomical, morphological and metabolic acclimation in the resurrection plant *Reaumuria soongorica* during dehydration and rehydration. *J. Arid Environ.* 70, 183–194. <https://doi.org/10.1016/j.jaridenv.2006.12.020>.
- Lu, C.M., Qiu, N.W., Wang, B.S., Zhang, J.H., 2003. Salinity treatment shows no effects on photosystem II photochemistry, but increases the resistance of photosystem II to heat stress in halophyte *Suaeda salsa*. *J. Exp. Bot.* 54, 851–860. <https://doi.org/10.1093/jxb/erg080>.
- Ma, H., Tian, C., Feng, G., Yuan, J., 2011. Ability of multicellular salt glands in *Tamarix* species to secrete Na⁺ and K⁺ selectively. *Sci. China Life Sci.* 54, 282–289. <https://doi.org/10.1007/s11427-011-4145-2>.
- Ma, Q., Yue, L.J., Zhang, J.L., Wu, G.Q., Bao, A.K., Wang, S.M., 2012. Sodium chloride improves photosynthesis and water status in the succulent xerophyte *Zygophyllum xanthoxylum*. *Tree Physiol.* 32, 4–13. <https://doi.org/10.1093/treephys/tpr098>.
- Ming, D.F., Pei, Z.F., Naeem, M.S., Gong, H.J., Zhou, W.J., 2012. Silicon alleviates PEG-induced water-deficit stress in upland rice seedlings by enhancing osmotic adjustment. *J. Agron. Crop Sci.* 198, 14–26. <https://doi.org/10.1111/j.1439-037X.2011.00486.x>.
- Molnar, I., Gaspar, L., Sarvari, E., Dulai, S., Hoffmann, B., Molnar-Lang, M., Galiba, G., 2015. Physiological and morphological responses to water stress in *Aegilops biuncialis* and *Triticum aestivum* genotypes with differing tolerance to drought. *Funct. Plant Biol.* 31, 1149–1159. <https://doi.org/10.1071/FP03143>.
- Moradi, P., Ford-Lloyd, B., Pritchard, J., 2017. Metabolic approach reveals the biochemical mechanisms underlying drought stress tolerance in thyme. *Anal. Biochem.* 527, 49–62. <https://doi.org/10.1016/j.ab.2017.02.006>.
- Qiu, N.W., Lu, Q.T., Lu, C.M., 2010. Photosynthesis, photosystem II efficiency and the xanthophyll cycle in the salt-adapted halophyte *Atriplex centralasiatica*. *New Phytol.* 159, 479–486. <https://doi.org/10.1046/j.1469-8137.2003.00825.x>.
- Ramadan, T., 2001. Dynamics of salt secretion by *Sporobolus spicatus* (Vahl) Kunth from sites of differing salinity. *Ann. Bot.* 87, 259–266. <https://doi.org/10.1006/anbo.2000.1326>.
- Ran, E., Bengal, A., Dag, A., Schwartz, A., Yermiyahu, U., 2014. Sodium replacement of potassium in physiological processes of olive trees (var. Barnea) as affected by drought. *Tree Physiol.* 34, 1102–1117. <https://doi.org/10.1093/treephys/tpu081>.
- Shahidi, R., Yoshida, J., Coughon, M., Reheul, D., Labeke, M.C.V., 2017. Morpho-physiological responses to dehydration stress of perennial ryegrass and tall fescue genotypes. *Funct. Plant Biol.* 44, 612–623. <https://doi.org/10.1071/FP16365>.
- Silva, E.N., Ribeiro, R.V., Ferreira, S.L., 2010. Comparative effects of salinity and water stress on photosynthesis, water relations and growth of *Jatropha curcas* plants. *J. Arid Environ.* 74, 1130–1137. <https://doi.org/10.1016/j.jaridenv.2010.05.036>.
- Sperdoui, I., Moustakas, M., 2015. Differential blockage of photosynthetic electron flow in young and mature leaves of *Arabidopsis thaliana* by exogenous proline. *Photosynthetica* 53, 471–477. <https://doi.org/10.1007/s11099-015-0116-3>.
- Thomson, W., Platt-Aloia, K., 1985. The ultrastructure of the plasmodesmata of the salt glands of *Tamarix* as revealed by transmission and freeze-fracture electron microscopy. *Protoplasma* 125, 13–23. <https://doi.org/10.1007/BF01297346>.
- Wakeel, A., Farooq, M., Qadir, M., Schubert, S., 2011. Potassium substitution by sodium in plants. *Crit. Rev. Plant Sci.* 30, 401–413. <https://doi.org/10.1080/07352689.2011.587728>.
- Wang, C.M., Zhang, J.L., Liu, X.S., Li, Z., Wu, G.Q., Cai, J.Y., Flowers, T.J., Wang, S.M., 2009. Puccinellia tenuiflora maintain a low Na⁺ level under salinity by limiting unidirectional Na⁺ influx resulting in a high selectivity for K⁺ over Na⁺. *Plant Cell Environ.* 32, 486–496. <https://doi.org/10.1111/j.1365-3040.2009.01942.x>.
- Wang, F., Tong, W.J., Zhu, H., Kong, W.L., Peng, R.H., Liu, Q.C., Yao, Q.H., 2016a. A

- novel Cys₂/His₂ zinc finger protein gene from sweet potato, lbZFP1, is involved in salt and drought tolerance in transgenic Arabidopsis. *Planta* 243, 783–797. <https://doi.org/10.1007/s00425-015-2443-9>.
- Wang, X., Xiao, H., Cheng, Y., Ren, J., 2016b. Leaf epidermal water-absorbing scales and their absorption of unsaturated atmospheric water in *Reaumuria soongorica*, a desert plant from the northwest arid region of China. *J. Arid Environ.* 128, 17–29. <https://doi.org/10.1016/j.jaridenv.2016.01.005>.
- Wu, G.Q., Feng, R.J., Liang, N., Yuan, H.J., Sun, W.B., 2015. Sodium chloride stimulates growth and alleviates sorbitol-induced osmotic stress in sugar beet seedlings. *Plant Growth Regul.* 75, 307–316. <https://doi.org/10.1007/s10725-014-9954-4>.
- Xu, Z.C., Zhou, G.S., Shimizu, H., 2009. Effect of soil drought with nocturnal warming on leaf stomatal traits and mesophyll cell ultrastructure of a perennial grass. *Crop Sci.* 49, 1843–1851. <https://doi.org/10.2135/cropsci2008.12.0725>.
- Xu, D.H., Su, P.X., Zhang, R.Y., Li, H.L., Zhao, L., Wang, G., 2010. Photosynthetic parameters and carbon reserves of a resurrection plant *Reaumuria soongorica* during dehydration and rehydration. *Plant Growth Regul.* 60, 183–190. <https://doi.org/10.1007/s10725-009-9440-6>.
- Yuan, F., Lyu, M.J.A., Leng, B.Y., Zheng, G.Y., Feng, Z.T., Li, P.H., Zhu, X.G., Wang, B.S., 2015. Comparative transcriptome analysis of developmental stages of the *Limonium bicolor* leaf generates insights into salt gland differentiation. *Plant Cell Environ.* 38, 1637–1657. <https://doi.org/10.1111/pce.12514>.
- Yuan, F., Leng, B.Y., Wang, B.S., 2016. Progress in Studying Salt Secretion from the Salt Glands in Recretholophytes: How Do Plants Secrete Salt? *Front. Plant Sci.* 7, 977. <https://doi.org/10.3389/fpls.2016.00977>.
- Zahoor, R., Zhao, W., Abid, M., Dong, H., Zhou, Z., 2017. Potassium application regulates nitrogen metabolism and osmotic adjustment in cotton (*Gossypium hirsutum* L.) functional leaf under drought stress. *J. Plant Physiol.* 215, 30–38. <https://doi.org/10.1016/j.jplph.2017.05.001>.
- Zanella, M., Borghi, G.L., Pirone, C., Thalmann, M., Pazmino, D., Costa, A., Santelia, D., Trost, P., Sparla, F., 2016. β -amylase 1 (*BAMI*) degrades transitory starch to sustain proline biosynthesis during drought stress. *J. Exp. Bot.* 67, 1819–1826. <https://doi.org/10.1093/jxb/erv572>.
- Zhang, F.J., Zhang, K.K., Du, C.Z., Li, J., Xing, Y.X., Yang, L.T., Li, Y.R., 2015. Effect of drought stress on anatomical structure and chloroplast ultrastructure in leaves of sugarcane. *Sugar Technol.* 17, 41–48. <https://doi.org/10.1007/s12355-014-0337-y>.
- Zhang, W.J., Yu, X.X., Li, M., Lang, D.Y., Zhang, X.H., Xie, Z.C., 2018. Silicon promotes growth and root yield of *Glycyrrhiza uralensis* under salt and drought stresses through enhancing osmotic adjustment and regulating antioxidant metabolism. *Crop Prot.* 107, 1–11. <https://doi.org/10.1017/j.cropro.2018.01.005>.
- Zhou, H.Y., Bao, A.K., Du, B.Q., Wang, S.M., 2012. The physiological mechanisms underlying how eremophyte *Reaumuria soongorica* responds to severe NaCl stress. *Pratacultural Sci.* 29 (1), 71–75 (in Chinese).