Na⁺ AND K⁺ HOMEOSTASIS IS IMPORTANT FOR SALINITY AND DROUGHT TOLERANCE OF CALLIGONUM MONGOLICUM

JING HU¹, XIAOKE HU¹, HUIRONG DUAN², HUIWEN ZHANG¹ AND QIUSHI YU*¹

¹State Key Laboratory Breeding Base of Desertification and Aeolian Sand Disaster Combating, Gansu Desert Control Research Institute, Lanzhou 730070, China
²Lanzhou Institute of Husbandry and Pharmaceutical Sciences of Chinese Academy of Agricultural Sciences, Lanzhou 730050, China
*Corresponding author’s email: yuqs158@yeah.net; Tel.: +86-931-7606112; Fax: +86-931-7686822

Abstract

Calligonum mongolicum is a typical pioneer xerophyte for sand fixation. To unravel the drought and salt tolerance of C. mongolicum, salt and drought stress were simulated by NaCl and sorbitol treatments respectively, and the accumulation and distribution of Na⁺, K⁺ in C. mongolicum were analyzed. The results showed that the fresh weight, dry weight and relative growth rate of C. mongolicum were increased significantly under the treatment of 25 mM NaCl, suggesting that 25 mM NaCl could promote plant growth. The addition of 50-300 mM NaCl increased sharply Na⁺ concentration in roots and shoots, while K⁺ concentrations remained stable; further study found that the 25-100 NaCl treatment significantly increased the net K⁺ uptake rate. Moreover, compared with moderate salt stress (25 mM NaCl), as the degree of stress intensified (50 and 100 mM NaCl), the ST value improved evidently. Therefore, it is suggested that C. mongolicum is able to maintain K⁺ homeostasis, thus refrain from K⁺ insufficiency brought by the external Na⁺ competition to resist salt stress. Under -0.25 – -1.0 MPa treatments, net Na⁺, K⁺ uptake rate was increased significantly, but Na⁺/K⁺ ratio, ST value and Na⁺, K⁺ relative distribution in tissues of C. mongolicum remained stable, implying that maintaining Na⁺, K⁺ homeostasis may be especially significant for C. mongolicum to deal with drought.

Key words: Calligonum mongolicum, Salt stress, Drought stress, Na⁺ and K⁺ homeostasis.

Introduction

Drought and soil salinization usually coexist, causing serious losses to agricultural production and ecological environment. Most crop plants, which due to long-term cultivation under more favorable conditions, therefore are highly sensitive to salinity and drought and unable to cope with these stresses without compromising growth and development. Xerophytic species from deserts, however, have evolved a series of adaptive strategies related to morphology, biochemistry and genetics to deal with drought and salt stress (Flowers & Colmer, 2008; Ashraf, 2010). To clarify this mechanism, it is urgent to explore the strategy that refer to salt and drought stress resistance in tolerant species or ecological types, which will lay a theoretical foundation to explore and utilize the abundant resources of anti-stress genes contained in desert plants.

Calligonum mongolicum (Supplementary Fig. S1), a typical sand-fixing pioneer shrub, is a widely distributed xerophyte (Polygonaceae) that is resistant to wind erosion, sand burying, cutting, drought and barren in the desert of the arid area (Ren et al., 2002; Fan et al., 2018). Meanwhile, the twigs can be used by livestock making C. mongolicum attractive as economic forage, and moreover, its fruit is ornamental and manifests a valuable species as soil cover and landscape in the barren desert. In recent years, there is increasing interest in exploring this xerophyte, however, data relating to its physiological behavior in arid and saline condition are insufficient.

Drought and salt resistance is a highly complicated web including some interacting properties. Osmotic adjustment (OA) is generally regarded as one of adaptive strategies that plants have evolved, which involve in reducing osmotic potential (Ψₒ) by conserving solutes when exposed to drought (Ma et al., 2012; Ramanjulu & Sudhakar, 2000). K⁺ is thought to be the primary osmolyte, which is accumulated in direct response to water deficit in many plants (Mengel & Arneke, 1982). Furthermore, a few plants, such as Zygophyllum xanthoxyllum, Atriplex canescens and Sesuvium portulacastrum also accumulate a great number of Na⁺ for osmoregulation (Glenn et al., 1998; Slama et al., 2007; Wu et al., 2011). However, for most of higher plants, Na⁺ is the main source of ion-damage (Rains & Epstein, 1967; Shabala & Cuin, 2008). Once the toxic Na⁺ enters the cytoplasm in which most metabolic processes occur, it will interfere with the normal physiological and biochemical processes of plants, inhibit plant growth and even lead to death (Munnis & Tester, 2008). Meanwhile, the concentration of K⁺ in soil ranges from 0.2-10 mmol/L, but high external Na⁺ concentration also impedes the absorption of K⁺ by plants, resulting in K⁺ deficiency (Zhang et al., 2010). Nevertheless, some plants can sustain the balance of ion homeostasis by enhancing the selective absorption of K⁺/Na⁺ in roots and regulating these ions transport and distribution in the plant to resist and evade salt damage (Zhu, 2003). However, studies on the accumulation and distribution of K⁺, Na⁺ in the desert xerophytes C. mongolicum under salt and drought conditions have not been explored.

Therefore, for elucidating the strategy of drought and salt resistance in C. mongolicum, this present work was initiated to characterize its K⁺ and Na⁺ accumulation when exposed to various treatments of NaCl (0-300 mM), osmotic stress (0 - -1.5 MPa).
Four weeks old *Calligonum mongolicum* seedlings were treated as follow: (i) Nutrient solution supplemented with additional 0, 25, 50, 100, 200 or 300 mM NaCl for 6 d. (ii) Modified Hoagland solution was added without or with extra sorbitol where the osmotic potential was 0, -0.25, -0.5, -1.0 and -1.5 MPa for 6 d. Each treatment contained of 5 nutritive cubes consisting 2 seedlings.

**Determination of growth, Na\(^+\) and K\(^+\) concentrations:**

After treatments, seedling roots were washed twice for 8 min with cold 20 mM CaCl\(_2\) to exchange cell wall-bound Na\(^+\); shoots were rinsed in deionized water to remove surface salts (Maathuis & Sanders, 2001; Wang et al., 2007). Plant was divided into root and shoot, and then tissue fresh weight was determined. Tissues were then immediately dried in an oven at 80°C. After 72 h, tissue dry weights were measured. Na\(^+\) and K\(^+\) were extracted from dried plant tissues in 100 mM acetic acid at 90°C for 2 h, and use flame spectrophotometer (2655-00; Cole Parmer Instrument Co., USA) to determine the concentration. Tissue water content was calculated as follow: tissue water content = (leaf fresh weight - leaf dry weight)/ leaf dry weight. The relative growth rate (RGR) was estimated by the method as described by Ma et al., (2012).

Na\(^+\) and K\(^+\) concentration was calculated, net selective transport capacity for K\(^+\) over Na\(^+\) (ST) = (K\(^+\)/Na\(^+\) in shoots)/(K\(^+\)/Na\(^+\) in roots) (Ma et al., 2014; Yuan et al., 2015). Net uptake rate (NUR) = [A whole plant Na\(^+\) (or K\(^+\)) content between salt-treated plant and BT plant]/root fresh weight/∆ time, where BT means before treatments (Wang et al., 2009). Na\(^+\) (or K\(^+\)) relative distribution (%) = Na\(^+\) (or K\(^+\)) content in each tissue/Na\(^+\) (or K\(^+\)) content in the whole plant (Ma et al., 2014; Yuan et al., 2015).

**Data analysis:** Results are presented as means with standard errors (SE). Data analyses were performed one-way ANOVA by statistical software (SPSS Ver.17.0, SPSS Inc., Chicago, IL, USA).

**Results**

**Appropriate NaCl stimulate the growth of *C. mongolicum***: Compared with the control (no additional NaCl), 25 mM NaCl added notably enhanced the fresh and dry weights by 111.6% and 99.3%, respectively. With increase of the external NaCl concentrations, the fresh and dry weights decreased gradually, and reached a lowest value when the NaCl concentration was 300 mM (Fig. 1a, b). No remarkable difference in tissue water content was observed under 0-100 mM NaCl treatments, while it was significantly reduced when NaCl concentration reached 200 and 300 mM (Fig. 1c). As to relative growth rate (RGR), under the treatment of 25 mM NaCl was substantially higher (303.3%) than others besides control, whereas with an rise of NaCl concentration, the RGR of *C. mongolicum* decreased gradually, and when plants were confronted with 200 to 300 mM NaCl its RGR was apparently lower than that of other treatments (Fig. 1d).

**Supplementary Fig. S1:** *Calligonum mongolicum*. The photographs show the landscape, the natural habitat and the morphological peculiarities of *C. mongolicum*. These wild *C. mongolicum* plants grew in Minqin County (101°59´E-104°12´E, 38°08´N-39°26´N), in Gansu Province of northwest China. This area is largely surrounded by the Badain Jaran Desert in the northwest and the Tengger Desert in the east. The climate is arid desert with an average annual precipitation of 116.5 mm and an average temperature of 7.8°C and has 27.4 gale-days per year at wind velocity ≥17 m/s with an annual mean wind speed of 2.4 m/s.

**Materials and Methods**

**Experiment materials culture and stress imposition:**

The experiment material was *Calligonum mongolicum*, seeds were collected from Minqin County, in Gansu Province of northwest China. Seeds were immersed in 98% H\(_2\)SO\(_4\) for 10 minutes and rinsed 8-10 times with water, then cultured with wetted vermiculite at room temperature. For about 10 days after the seeds germination, uniform seedlings were chosen out and transferred to plastic containers (5 cm\(^3\), 2 seedlings/container) filled with sand and irrigated with modified Hoagland nutrient solution (Hu et al., 2016). Solutions were renewed every 2 days. The temperature of greenhouse was 28±2°C/23±2°C (light/dark: 16/8 h), the luminous intensity was about 600 μmol/(m\(^2\)*s) and the relative humidity was about 65%.
K⁺ and Na⁺ accumulation under NaCl treatment: Na⁺ concentration in roots was abnormally enhanced under 50-300 mM NaCl; however, in shoots, a notable decrease in Na⁺ was exhibited when *C. mongolicum* seedlings cultivated in addition of 25 mM NaCl solution compared with no additional NaCl, whereas then increased gradually, and was significantly and reached peak value under 300 mM NaCl treatment. By contrast, 25-200 mM extra NaCl hardly affected K⁺ concentrations in both roots and shoots (Fig. 2a, b).

ST value was significantly reduced when exposed to NaCl, while compared with 25 mM NaCl treatment, additional 50 and 100 mM NaCl abysically up-regulated ST value (Fig. 2c). Compared with control, 50-300 and 100-300 mM NaCl dramatically improved Na⁺/K⁺ ratio in roots and shoots, respectively (Fig. 2d).

Furthermore, research indicated that net Na⁺ uptake rate was dramatically elevated when exposed to NaCl, and achieved a maximum at 200 mM NaCl (Fig. 3a). Interestingly, 25-100 mM NaCl treatment significantly raised the net K⁺ uptake rate (Fig. 3b).

Moreover, the Na⁺, K⁺ relative distribution was also determined. Under 25-200 mM NaCl treatment, Na⁺ relative distribution in roots was reduced significantly, while in shoot increased significantly. K⁺ relative distribution in roots was substantially declined compared to control under 50-100 mM NaCl, and then which remained relatively stable with the rise of external NaCl concentration (200-300 mM NaCl). On the contrary, the K⁺ relative distribution in shoot reaches the maximum under the treatment of 50 mM NaCl, and then decreases (Fig. 3c, d).

Osmotic stress influenced the growth of *C. mongolicum*: Under the treatment of -0.25 - -1.5 MPa, the fresh weight of plants was decreased significantly (Fig. 4a). However, dry weight gradually increased when exposed to the osmotic -0.25 - -1.0 MPa and then decreased significantly under -1.5 MPa condition (Fig. 4b). The tissue water content was significantly decreased under osmotic stress of -0.25 - -1.5 MPa, whereas the relative growth rate remained stable under the treatment of -0.25 - -1.0MPa (Fig. 4c, d).

K⁺ and Na⁺ accumulation under osmotic stress: Na⁺ and K⁺ accumulation, K⁺/Na⁺, ST value, Na⁺ and K⁺ ratio in tissues, Net Na⁺ and K⁺ uptake rate as well as Na⁺ and K⁺ relative distribution when plants exposed to additional sorbitol was also determined.

Under the treatment of -0.25 - -1.0 MPa, the Na⁺, K⁺ concentrations in plant tissues, ST values, Na⁺/K⁺ ratio (Fig. 5), the net absorption rates of Na⁺ and Na⁺ or K⁺ relative distribution in various tissues all remained stable (Fig. 6a, c, d); nevertheless, the net absorption rates of K⁺ increased evidently (Fig. 6b).

Discussion

Maintaining K⁺ homeostasis is important for *C. mongolicum* to improve salt tolerance: The most direct response of plants to stress is inhibitive growth, and it is a convenient way to evaluate the effects of stress on plants is based on the quantification of inhibition on their growth (Borsai et al., 2018). In this study, it was found that *C. mongolicum* could grow normally under 25-200 mM NaCl treatment, while opposed to 300-400 mM NaCl, the growth of plants were restrained and wither or even died. Biomass is a favored indicator as salt tolerance (Bao et al., 2009). In our study, the fresh weight, dry weight, tissue water content and RGR of *C. mongolicum* under 25 mM NaCl were evidently higher than those under other NaCl treatments (Fig. 1), indicating that 25 mM NaCl could stimulate the growth of *C. mongolicum*. Is that, Na⁺ promotes plant growth as a nutrient may be due to its osmotic adjustment function, similar effects of Na⁺ were found in *Beta vulgaris* and *Zygophyllum xanthoxylum* (Wakeel et al., 2011; Yue et al., 2012). Moreover, studies showed that, under 200 mM NaCl treatment, the dry weight of salt-tolerant plant of *B. vulgaris* was decreased by only 20%, moderate salt-tolerant plant of cotton decreased by about 60%, whereas that of salt-sensitive plants such as legumes died under the same treatment (Greenway & Munns, 1980; Cordovilla, 1994; Delgado et al., 1994; Tate, 1995). In this study, at the same NaCl level, the dry weight of *C. mongolicum* was decreased by about 20%, while higher than 200 mM NaCl concentration arrested growth of plants (Fig. 1), this provide an evidence that *C. mongolicum* with excellent adaptability to salt environment.

K⁺ plays an important role in the regulation of enzymatic activation, protein synthesis, photosynthesis, cell osmotic regulation and stomatal movement (Mäser et al., 2002). High salinity prevents plant absorbing mineral nutrition, and as Na⁺ and K⁺ is alike in radius, physicochemical properties and transport mechanisms, thus Na⁺ usually competitively inhibits the absorption and transport of nutrient K⁺ in plants (Shabala & Cuin, 2008; Horie et al., 2009). Consequently once excessive Na⁺ is accumulated in most of plants, the concentration of K⁺ will decrease sharply (Zhang et al., 2010). Interestingly, in our study, 50-300 mM NaCl treatment markedly enhanced Na⁺ content in roots and shoots of *C. mongolicum*, while K⁺ concentration remained stable (Fig. 2). Therefore, it is speculated that maintaining K⁺ homeostasis is an important countermeasure to resist salt stress in *C. mongolicum*. Further analysis showed that 25-100 mM NaCl treatment apparently improved net K⁺ uptake rate (Fig. 4). Meanwhile, compared with mild salt treatment (25 mM NaCl), ST value increased significantly along with external NaCl concentration increased (50 to 100 mM NaCl). Previous study has pointed out ST indicates the selective transport ability of K⁺ and Na⁺, and the larger the value, the greater ability of roots to restrict Na⁺, promote K⁺ transport to shoots (Wang et al., 2002). We found that when Na⁺ concentration in plants increased sharply under salt treatment, *C. mongolicum* could still satisfy itself normal growth and development by adjusting K⁺, Na⁺ selective transport system, thus avoiding deficiency of K⁺ in tissues caused by excessive Na⁺, which may be one of the important salt-resistant strategies of *C. mongolicum*. 
Fig. 1. Fresh weight (a), dry weight (b), tissue water content (c) and relative growth rate (d) of *C. mongolicum* with NaCl treatments. Values are means ± SE (*n* = 5) and bars indicate SE. Columns with different letters indicate significant differences at *p*≤0.05, according to Duncan’s multiple range test.

Fig. 2. Na⁺ (a), K⁺ (b) concentration, ST values (c) and Na⁺/K⁺ ratio (d) in *C. mongolicum* after 144 h without (C) and with NaCl treatments. Values are means ± SE (*n* = 5) and bars indicate SE. Columns with different letters indicate significant differences at *p*≤0.05, according to Duncan’s multiple range test.
Fig. 3. Net Na\(^+\) (a), K\(^+\) (d) uptake rate and Na\(^+\) (c), K\(^+\) (d) relative distribution in tissues of *C. mongolicum* after 144 h without and with NaCl treatments. Values are means ± SE (*n* = 5) and bars indicate SE. Columns with different letters indicate significant differences at *p*≤0.05, according to Duncan’s multiple range test.

Fig. 4. Fresh weight (a), dry weight (b), tissue water content (c) and relative growth rate (d) of *C. mongolicum* with osmotic stress treatments. Values are means ± SE (*n* = 5) and bars indicate SE. Columns with different letters indicate significant differences at *p*≤0.05, according to Duncan’s multiple range test.
Fig. 5. Na$^+$ (a), K$^+$ (b) concentration, ST values (c) and Na$^+$/K$^+$ ratio (d) in *C. mongolicum* with osmotic stress treatments. Values are means ± SE (n = 5) and bars indicate SE. Columns with different letters indicate significant differences at $p \leq 0.05$, according to Duncan’s multiple range test.

Fig. 6. Net Na$^+$ (a), K$^+$ (b) uptake rate, Na$^+$ (c), K$^+$ (d) relative distribution of *C. mongolicum* with osmotic stress treatments. Values are means ± SE (n = 5) and bars indicate SE. Columns with different letters indicate significant differences at $p \leq 0.05$, according to Duncan’s multiple range test.
Then, the question-under salt condition, how does C. mongolicum regulating K\(^+\) and Na\(^+\) transport system? The possible explanations are as follows. It has been reported 

References


Greenway, H. and R. Munns. 1980. Mechanisms of salt tolerance of the cultivated tomato Responses of

Na\(^+\) AND K\(^+\) HOMEOSTASIS IS IMPORTANT FOR SALINITY AND DROUGHT TOLERANCE

Maintaining Na\(^+\), K\(^+\) homeostasis may be an effective strategy to resist drought in C. mongolicum: Under the treatment of -0.25- -1.0 MPa, C. mongolicum could grow normally; while the treatment of -1.5 MPa causes C. mongolicum to wilt and almost die. In addition, -0.25 - -1.0 MPa treatment increased plant dry weight and relative growth rate, indicating that C. mongolicum had strong drought tolerance (Fig. 4).

Keeping adequate K\(^+\) nutrition is essential for plants to adapt to drought (Cakmak, 2005). Furthermore, studies have shown that plants have greater demand for K\(^+\) when they are subjected to drought stress (Cakmak et al., 1999). Moreover, increasing K\(^+\) can effectively alleviate the damage caused by drought stress to plants, but K\(^+\) uptake capacity often decreases significantly under drought stress (Hu & Schmidhalter, 2005). Wang et al., (2019) found that with the prolongation of osmotic stress (-0.5 MPa) treatment time, the shoots K\(^+\) concentration in Arabidopsis thaliana decreased significantly, while the shoots K\(^+\) concentration in Z. xanthoxylum remained stable. Preserving K\(^+\) stability in shoots is one of the important strategies for drought tolerance of Z. xanthoxylum (Hu et al., 2016). Studies pointed out that sorbitol solution would lead to serious membrane hyperpolarization, contributing to the increase of K\(^+\) absorption (Shabala, 2002, 2009). In this study, - 0.25 - -1.0 MPa treatment significantly increased the net K\(^+\) uptake rate (Fig. 6b), indicating that under drought stress, C. mongolicum could improve its drought resistance by regulating the K\(^+\) transport system. Further analysis showed that Na\(^+\)/K\(^+\) ratio, ST value and relative distribution ratio did not change significantly under drought stress. Therefore, it is proposed that maintaining Na\(^+\), K\(^+\) homeostasis might be an efficient mechanism for C. mongolicum to resist drought stress.

Acknowledgements

This research was supported by the Opening Foundation of the State Key Laboratory Breeding Base of Desertification and Aeolian Sand Disaster Combating, Gansu Desert Control Research Institute (GSDC201501), the Natural Science Foundation of Gansu Province (20JR5RA093), the National Natural Science Foundation of China (32060376 and 31700338).


