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ORIGINAL PAPER

Moderate NaCl alleviates osmotic stress in *Lycium ruthenicum*

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Abstract
Lycium ruthenicum is a salt-accumulating xerophytic species with excellent adaptability to adverse environments. Previous studies showed that a certain amount of NaCl addition promoted plant growth. To reveal the mechanism underlying the positive effect of Na^+ addition on plant growth and investigate the role of moderate NaCl in *L. ruthenicum* drought resistance, the growth, photosynthesis, and K^+ and Na^+ transport-related genes were assessed after being subjected to different NaCl (0–400 mM) treatments and osmotic stresses (−0.5 MPa) in the presence or absence of additional NaCl (50 mM). Compared to the control, 50 mM NaCl strongly boosted the fresh weight, dry weight, relative growth rate, and significantly increased the Na^+ concentrations in roots, stems, and leaves; the K^+ concentrations in roots and leaves also increased significantly. Furthermore, 50 mM NaCl sharply up-regulated the expression of *LrSOS1* in roots and *LrNHX* and *LrVP1* in leaves, while *LrHKT1* was down-regulated in roots, this was the reason why a high quantity of Na^+ accumulated in leaves. *LrAKT1* was up-regulated in roots, and *LrSKOR* decreased first and then increased in roots, whereas *LrSKOR* in leaves remained stable and slightly up-regulated, thereby absorbing a large amount of K^+ through LrAKT1 and transporting to the leaf through LrSKOR. Moreover, external NaCl apparently alleviated the inhibition of osmotic stress in plant growth, and significantly increased Na^+ and K^+ concentrations. It is speculated that moderate NaCl treatment could significantly improve the Na^+ and K^+ concentrations, thus enhancing the osmotic regulation ability of plants, and then improve the photosynthesis and water status of *L. ruthenicum*.

Keywords *Lycium ruthenicum* · Na^+, K^+ accumulation · Na^+ and K^+ transport related genes · Photosynthesis

Introduction

Drought stress is a critical factor that limits plant growth, induces a range of physiological and biochemical responses in plants, and affects all stages of plant development (Hasanuzzaman et al. 2018). In order to adapt to the adverse environment, xerophytes growing in arid areas have evolved their own unique drought resistance mechanisms.

Drought tolerance is a complex trait that is determined by numerous physiological indicators. In order to deal with osmotic stress, plant cells must contain a certain amount of water and maintain a certain turgor pressure to drive extension growth in roots and shoots, which can be maintained through a process called osmotic adjustment (Shabala et al. 2010). Under stress, higher plants usually adopt two methods of osmoregulation. The first method is the synthesis of free amino acids, soluble sugars, and other organic regulatory substances, and the second is the accumulation of more inorganic ions, mainly containing K^+ and Na^+. Under drought stress, K^+ facilitates osmotic adjustment in both the vacuoles and cytosol of numerous species (Shabala 2011). However, Na^+ is a controversial osmoregulant. For most species, Na^+ is not essential in any sense and may even be toxic to plants, but a few plant species such as some halophytes and C4 plants cannot complete their life cycle without it (Maksimovi et al. 2010). The use of Na^+ may be an adaptive mechanism that ensures the survival and reproduction of xerophytes and halophytes in arid or semi-arid environments.

Lycium ruthenicum Murr. belongs to the Solanaceae family and is widely distributed in the salinized desert in the northwest of China. Due to its nutritional, medicinal, and ecological values, *L. ruthenicum* has attracted widespread attention. Over a very short time, it has since become widely

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cultivated in desert areas (Peng et al. 2014; Dai et al. 2019). Furthermore, as the main species in dry areas, *L. ruthenicum* prevents wind erosion and stabilizes sand, and is a plant with strong salt tolerance that is able to grow very well where other plants cannot grow. More surprisingly, *L. ruthenicum* can reduce soil salinization (Dai et al. 2019). Research showed that, the addition of an appropriate amount of NaCl (100 mM NaCl) significantly promoted the growth of *L. ruthenicum*, and under high salt (450 mM NaCl) conditions, when the Na^+ content had increased greatly, the K^+/Na^+ ratio in the stems and leaves reached 28.3 and 22.3, respectively. Moreover, the K^+/Na^+ ratio of *L. ruthenicum* was significantly higher than that in other salt-accumulating plants such as *Kalidium foliatum* and *Nitraria sibirica* (Wang et al. 2011). Therefore, absorbing Na^+ and maintaining a steady balance of K^+ may be an effective strategy used by *L. ruthenicum* to resist adversity. However, the mechanisms for salt resistance while accumulating Na^+ and maintaining K^+/Na^+ homeostasis in *L. ruthenicum* are still unknown.

Generally, drought stress inhibits the uptake and transport of nutrients, including K^+, in plants, especially in glycophytes (Hu and Schmidhalter 2005). Mahouachi (2007) reported that a decreased level of K^+ uptake was observed in banana (*Musa nana*) under drought conditions. Hu et al. (2007) revealed that drought decreased the accumulation of K^+ and Na^+ in blades of wheat (*Triticum aestivum*). In *Beta vulgaris*, the addition of 50 mM NaCl improved resistance against osmotic stress with increased Na^+ concentrations, while the K^+ concentrations in shoots and roots were decreased (Wu et al. 2015). Conversely, *Zygophyllum xanthoxylum* accumulated a large quantity of Na^+ but K^+ concentrations remained unchanged in response to drought with or without the addition of 50 mM NaCl; in contrast, significant reductions in the shoot K^+ concentrations of *Arabidopsis thaliana* were observed under osmotic stress alone or when combined with 5 mM NaCl (Wang et al. 2019). However, very little is known about Na^+ and K^+ changes in *L. ruthenicum* exposed to drought combined with salt.

Here, the growth, photosynthesis, water status, and K^+ and Na^+ transport-related genes were assessed in plants subjected to different NaCl treatments and osmotic stress in the presence or absence of additional NaCl. This work helps elucidate the factors underlying *L. ruthenicum* survival in saline and drought areas.

Materials and methods

Plant growth conditions and treatments

Lycium ruthenicum seeds were collected from Minqin County (101°59′E–104°12′E, 38°08′N–39°26′N), in Gansu Province of northwest China.

Seeds were cleaned with water, soaked for 12 h, and germinated in a culture dish covered with filter paper. After about 10 days, the robust seedlings were selected when the seedlings grew to about 1 cm and transplanted into plastic containers (5 cm^3; two seedlings/container) filled with sand and cultured with adjusted Hoagland nutrient solution (containing 2 mM KNO_3, 0.5 mM KH_2PO_4, 0.5 mM $MgSO_4.7H_2O$, 0.5 mM $Ca(NO_4)_2.4H_2O$, 50 μM H_3BO_3, 10 μM $MnCl_2.4H_2O$, 1.6 μM $ZnSO_4.7H_2O$, 0.6 μM $CuSO_4$, 0.05 μM $Na_2MoO_4.2H_2O$ and 60 μM $FeC_6H_5O_7$).

The seedlings were grown in a greenhouse with day/night temperatures of 28 ± 2 °C/23 ± 2 °C, and the time and intensity of illumination were 16 h/d and 600 μmol·m^{-2}·s, respectively.

Lycium ruthenicum seedlings were cultured with adjusted Hoagland nutrient solution for 30 days. Consistent seedlings were selected and treated as follows:

(i) Plants were treated with 0, − 0.25, − 0.5, − 1.0, and − 1.5 MPa (solution with sorbitol) for seven days, characterized, and photographed.
(ii) Plants were treated with 0, 50, 100, 200, 300, and 400 mM NaCl (NaCl added to nutrient solution) for seven days, characterized, and photographed.
(iii) Based on the results of (i) and (ii), seedlings were divided into treatment groups as follows: (1) control (C): normal irrigation with Hoagland nutrient solution; (2) salt treatment (S): irrigation with Hoagland nutrient solution containing 50 mM NaCl; (3) drought treatment (D): sorbitol solution with a total osmotic potential of − 0.5 MPa prepared from the Hoagland nutrient solution; and (4) drought and salt treatment (D + S): sorbitol solution with a total osmotic potential of − 0.5 MPa added to 50 mM of NaCl with Hoagland nutrient solution. For sampling and assessment of each indicator, each treatment included six replicates, and each replicate consisted of two seedlings. The solution was replaced in the above treatments once a day to keep the concentration of the solution relatively constant. To minimize the effects of possible environmental gradients in the greenhouse, pots were randomly reassigned to new positions every day.

Evaluation of growth, water use efficiency and Na^+ and K^+

After treatments, plant roots were washed twice for 8 min in ice-cold 20 mM $CaCl_2$ to exchange cell wall-bound Na^+; stems or leaves were rinsed in deionized water to remove surface salts (Wang et al. 2007). Plants were separated into roots, stems, and leaves immediately to obtain fresh weights and then dried in an oven at 80 °C for 48 h to obtain dry

weights. Na^+ and K^+ were extracted from dried plant tissues in 100 mM acetic acid at 90 °C for 2 h, and ion concentration was determined with a flame spectrophotometer (2655-00; Cole Parmer Instrument Co., USA).

Measurement of free proline and soluble sugar

Lycium ruthenicum seedlings were washed thoroughly with distilled water and separated into roots, stems, and leaves immediately to obtain fresh weights. The free proline was extracted with sulfosalicylic acid and determined with a spectrophotometer (Wang et al. 2004; Moustakas et al. 2011). Soluble sugar was extracted with a kit following the anthrone method (Suzhou Comin Biotechnology, China) according to the manufacturer's instructions.

Determination of osmotic potential and physiological parameters

For the leaf osmotic potential (Ψ_s), the leaf was rinsed with deionized water and blotted on filter paper immediately, then frozen in liquid nitrogen and thawed to extract sap using a syringe. The acquired sap was examined with a cryoscopic osmometer (OSMOMAT-030, GONOTECGmbH, Germany). The readings (mmol/kg) were used to calculate the solute potential in MPa (megapascals) with the formula: $\Psi_s = -\text{moles of solute} \times RT$, where $R = 0.008314$ and $T = 297$ °C (Yuan et al. 2014).

The net photosynthetic rate (Pn), stomatal conductance (Gs), and transpiration rate (Tr) were measured with a Photosynthetic System (L1-6400.LI-COR Biosciences, USA), and the water use efficiency (WUE) = Pn/Tr (Hassine et al. 2008).

Real-time quantitative RT-PCR

Four-week-old seedlings were treated with adjusted Hoagland nutrient solutions supplemented with additional 50 mM NaCl and sampled after 0, 6, and 24 h. Reactions were performed in a GeneAmp® PCR System 9700 (Applied Biosystems, USA). *LrLEF1α* was used as the reference gene. *LrSKOR*, *LrAKT1*, *LrHKT1*, *LrSOS1*, *LrNHX*, and *LrAPV1* are available in the NCBI SRA database (accession number SRR7700825). Fragments of *LrSKOR*, *LrAKT1*, *LrHKT1*, *LrSOS1*, *LrNHX*, *LrAPV1*, and *LrLEF1α* were amplified with the primer pairs P1 and P2, P3 and P4, P5 and P6, P7 and P8, P9 and P10, P11 and P12, and P13 and P14, respectively (Supplementary Table S1). Real-time PCR was performed using LightCycler® 480 II Real-time PCR Instrument (Roche, Swiss) with 10 µl PCR reaction mixture that included 1 µl of cDNA, 5 µl of 2 × QuantiFast® SYBR® Green PCR Master Mix (Qiagen, Germany), 0.2 µl of forward primer, 0.2 µl of reverse primer and 3.6 µl of nuclease-free water. The expression levels of mRNAs were calculated using the $2^{-\Delta\Delta Ct}$ method (Duan et al. 2015). This experiment was performed three times to confirm the accuracy of the results.

Data analysis

All the data are presented as means with standard errors (SE). All statistical analyses including one-way ANOVA and Duncan's multiple range tests were performed by statistical software (SPSS Ver.17.0, SPSS Inc., Chicago, IL, USA).

Results

Effects of different NaCl treatments on *L. ruthenicum*

We observed the growth of *L. ruthenicum* seedlings under different concentrations of NaCl (0–400 mM). It was found that the growth of *L. ruthenicum* leaves and roots under the 50 and 100 mM NaCl treatments was significantly better than under the control and other treatments, and the effect of 50 mM NaCl was the most pronounced among these treatments (Supplementary Fig. S1).

To further evaluate the effect of NaCl on the growth of *L. ruthenicum*, the fresh weight, dry weight, tissue water content and relative growth rate were determined under NaCl treatments (0–400 mM) (Fig. 1). The results showed that the fresh weight, dry weight, and relative growth rate of *L. ruthenicum* were significantly increased under 50 and 100 NaCl treatments, and 50 mM had the most obvious effect; these indexes significantly increased by 126.4%, 60.4%, and 47.2%, respectively.

The accumulation of Na^+ and K^+ in plants was also analyzed under 0–400 mM NaCl treatments (Fig. 2). Compared with the control, the concentrations of Na^+ in the roots, stems, and leaves treatment were significantly increased (by 62.5%, 209.6%, 173.7%, respectively, under the 50 mM NaCl treatment and by 156.1%, 332.1%, 283.7%, respectively, under the 100 mM NaCl treatment) under 50–400 mM NaCl treatment. K^+ in roots and leaves increased significantly (by 20.6% and 6.7%, respectively, under the 50 mM NaCl treatment, and by 26.1% and 4.2%, respectively, under the 100 mM NaCl treatment), and remained stable in stems; however, with the increase of NaCl concentration (200–400 mM), K^+ concentration in various tissues decreased significantly.

To explore the pathway of coordinated regulation of the Na^+ and K^+ channels or transporters in *L. ruthenicum*, the expression patterns of *LrAKT1*, *LrSKOR*, *LrSOS1*, *LrHKT1*, *LrAPV1*, and *LrNHX* in *L. ruthenicum* were analyzed. After NaCl treatment for 6 and 24 h, *LrSOS1* and *LrAKT1* expression increased gradually, *LrSKOR* decreased first and then

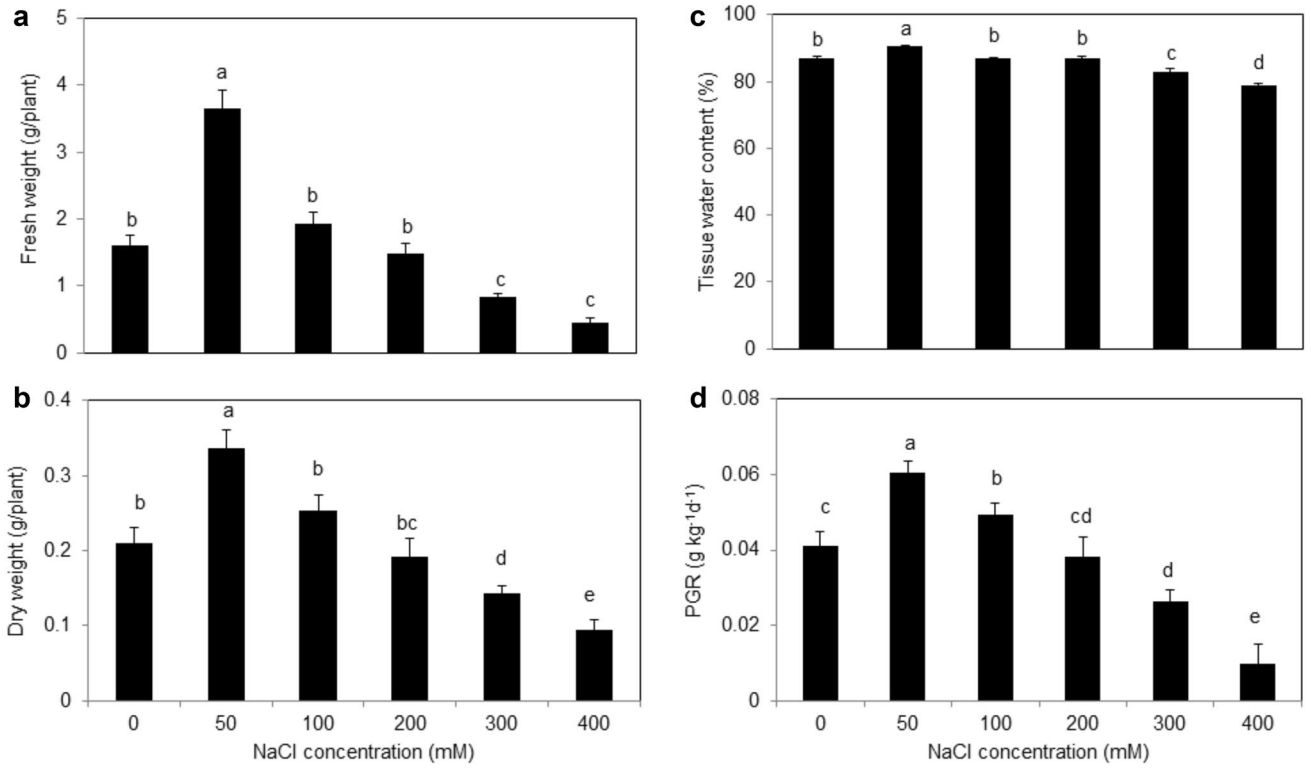

Fig. 1 Fresh weight (**a**), dry weight (**b**), tissue water content (**c**) and relative growth rate (**d**) of *L. ruthenicum* under the treatments of NaCl (0, 50, 100, 200, 300 and 400 mM) for seven days. Values are means ± SE (n = 6) and bars indicate SE. Different letters within the same column indicate significant difference at $P < 0.05$ (Duncan test), the same below

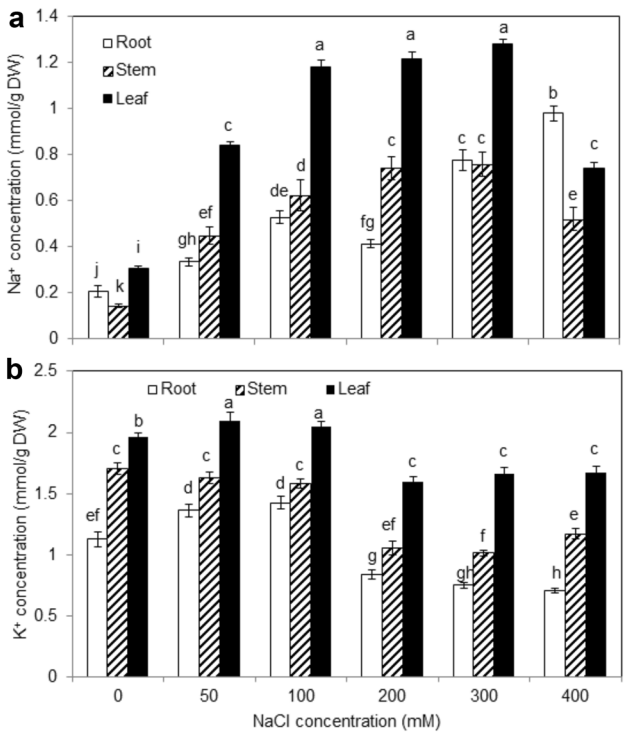

Fig. 2 Na^+ concentration (**a**) and K^+ concentration (**b**) of *L. ruthenicum* under the treatments of 0, 50, 100, 200, 300, 400 mM NaCl for seven days

increased, and *LrHKT1* was down-regulated in roots; moreover, the expressions of *LrAPV1* and *LrNHX* were significantly up-regulated in leaves (Fig. 3).

Effects of drought treatment on the growth and development of *L. ruthenicum*

As shown in Supplementary Fig. S2, the seedlings of *L. ruthenicum* grew normally under − 0.25 MPa, and − 0.5, − 1.0, and − 1.5 MPa delayed plant growth. Furthermore, plants exhibited poor performance under − 1.0 MPa treatment and obvious wilting under the − 1.5 MPa stress treatment. Therefore, − 0.5 MPa was selected for the following drought treatment.

Moderate concentrations of NaCl alleviate the deleterious impact of water deficit

In order to explore whether 50 mM NaCl could alleviate drought stress in *L. ruthenicum* seedlings, salt (S: 50 mM NaCl), drought (D: − 0.5 MPa), and drought plus salt (D + S: 50 mM NaCl + − 0.5 MPa) treatments were carried out in this study (Fig. 4). As in the results of NaCl treatments above, plants still grew very well when exposed to 50 mM NaCl, but drought treatment exerted an inhibitory effect

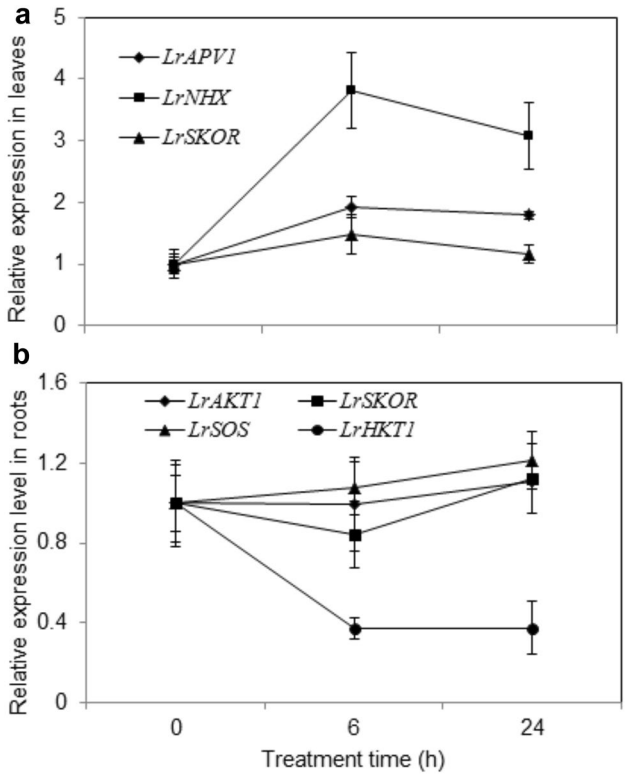

Fig. 3 Expression of *LrAKT1*, *LrSKOR*, *LrSOS1*, *LrHKT1* in root and *LrAPV1*, *LrNHX*, *LrSKOR* in leaf of *L. ruthenicum* under 50 mM NaCl concentrations. Real-time qPCR analysis of *LrAKT1*, *LrSKOR*, *LrSOS1*, *LrHKT1* in roots and *LrAPV1*, *LrNHX*, *LrSKOR* in leaves of four-week-old plants treated with various 50 mM NaCl for 6 or 24 h. *LrEF1α* was used as an internal control. The results shown represent qPCR analysis of the cDNA synthesized from three experiments. Values are means ± SE (n = 3) and bars indicate SE. Columns with different letters indicate significant differences at $P < 0.05$ (Duncan's test)

on seedling growth. However, when salt was added to the drought, plants grew better than under drought treatment alone.

The fresh and dry weight of *L. ruthenicum* plants increased significantly under 50 mM NaCl compared with the control, and the fresh weight decreased significantly under the − 0.5 MPa and 50 mM NaCl plus − 0.5 MPa treatments, but the dry weight did not change significantly; However, the fresh weight under the 50 mM NaCl plus − 0.5 MPa treatment was significantly higher than that of the − 0.5 MPa treatment alone (Fig. 5a, b).

Compared with the control, the water content of tissues under salt treatment increased significantly, while the water content of tissues under drought treatment decreased significantly, and the water content of the plants under drought plus salt treatment was significantly higher than that under drought treatment alone (Fig. 5c). The addition of 50 mM NaCl significantly increased the relative growth rate of plants, and the drought and drought plus salt treatments obviously delayed the relative growth of plants compared to the control. However, compared with drought treatment, the 50 mM NaCl plus drought treatment evidently increased the relative growth rate of plants by 1.8% (Fig. 5d).

In comparison with the treatment without additional NaCl (0 mM), the salt and drought plus salt treatments significantly increased the Na^+ concentrations in roots, stems, and leaves of *L. ruthenicum*, but there were no significant changes under drought treatment. The salt and drought plus salt treatments also increased K^+ content in leaves and stems. Compared with drought alone, the drought plus salt treatment increased Na^+ (roots, stems and leaves by 54.8%, 385.7%, and 155.4%, respectively) and K^+ concentrations (roots, stems, and leaves by 95.3%, 3.7%, and 59.4%, respectively) (Fig. 6a, b). Further analysis showed that the distribution ratio of Na^+ and K^+ in the root decreased and increased in the shoot under the drought plus salt treatment (Fig. 6c, d).

The proline contents in roots and stems increased significantly under salt treatment to 5.1 and 5.7 times those of the control, respectively; the proline content was also significantly increased in stems under the drought treatment, but there was no obvious change under the drought plus salt treatment (Fig. 7a).

Compared with the control, drought or drought plus salt treatment significantly increased the soluble sugar content in leaves, but evidently decreased the soluble sugar content

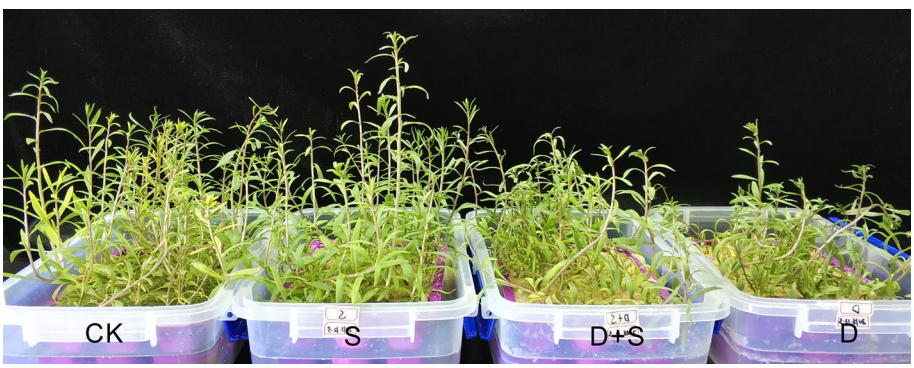

Fig. 4 Control (CK), 50 mM NaCl (S), − 0.5 MPa (D) and 50 mM NaCl+ − 0.5 MPa (D + S) for seven days effect on the growth of *L.ruthenicum*

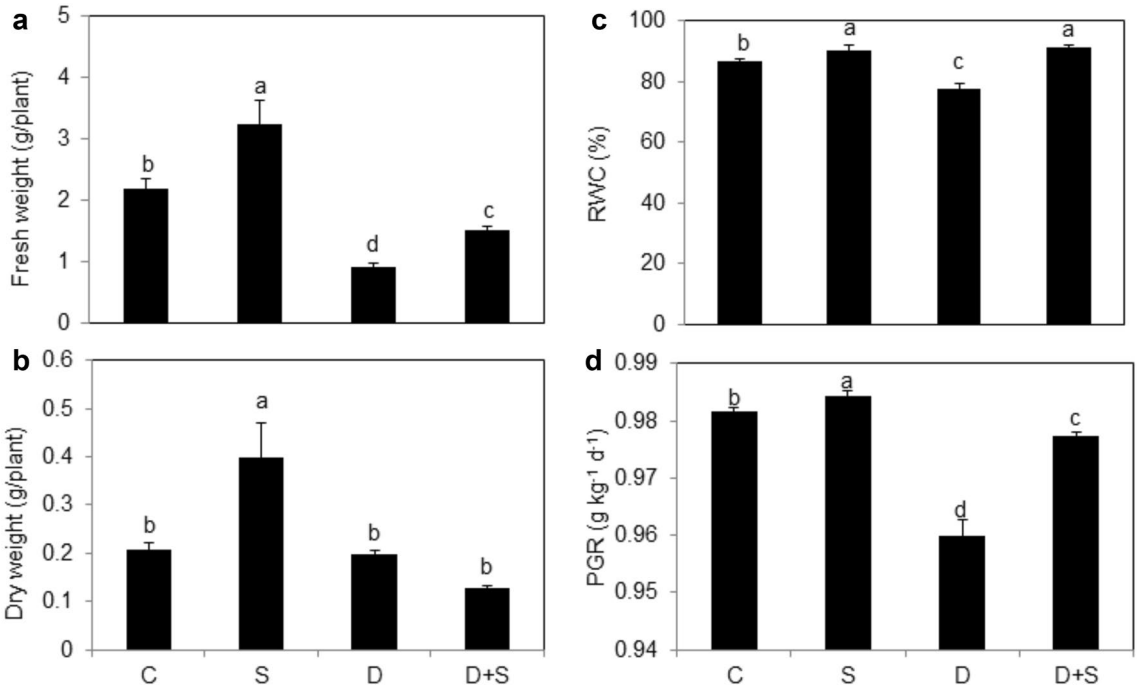

Fig. 5 Fresh weight (**a**), dry weight (**b**), tissue water content (**c**) and relative growth rate (**d**) of *L. ruthenicum* under the treatments of Control (C), 50 mM NaCl (S), − 0.5 MPa (D) and 50 mM NaCl + −0.5 MPa (D + S) for seven days

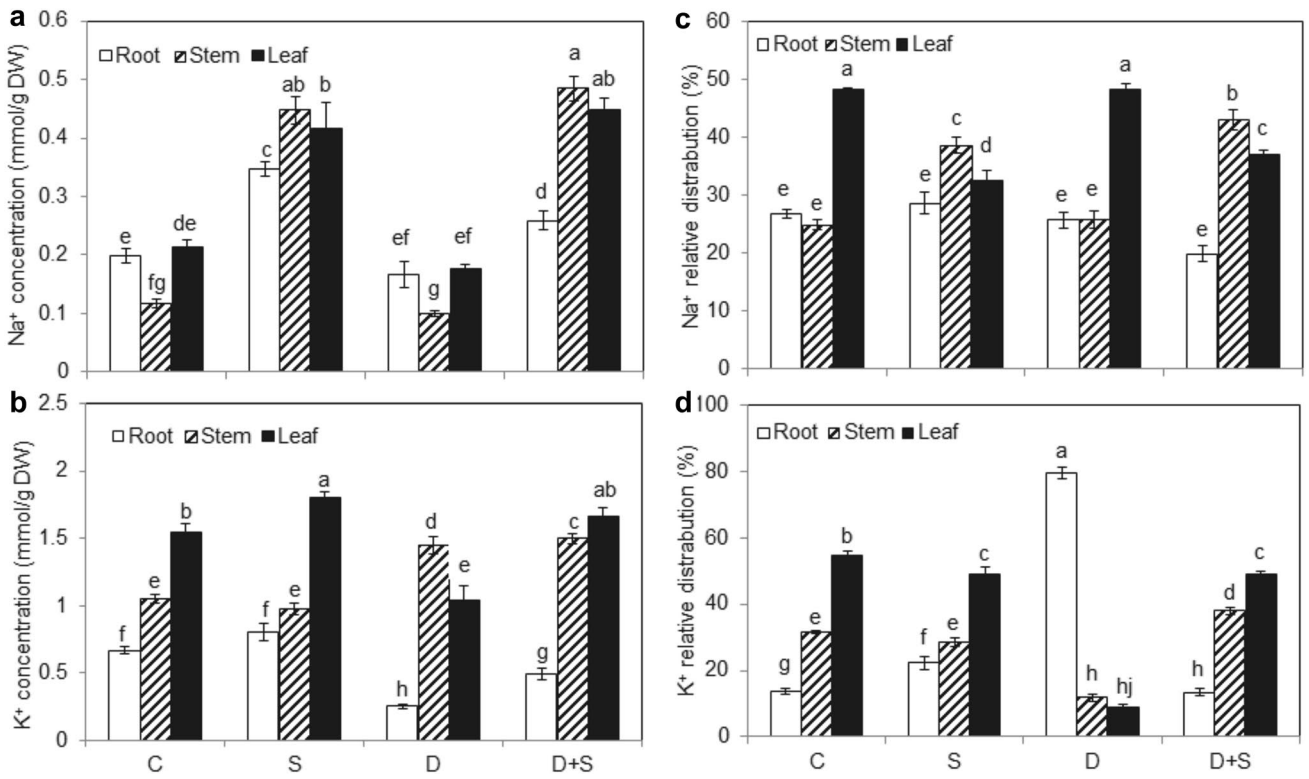

Fig. 6 Na^+ concentration (**a**) and K^+ concentration (**b**) of *L. ruthenicum* under the treatments of Control (C), 50 mM NaCl (S), − 0.5 MPa (D) and 50 mM NaCl + −0.5 MPa (D + S) for seven days

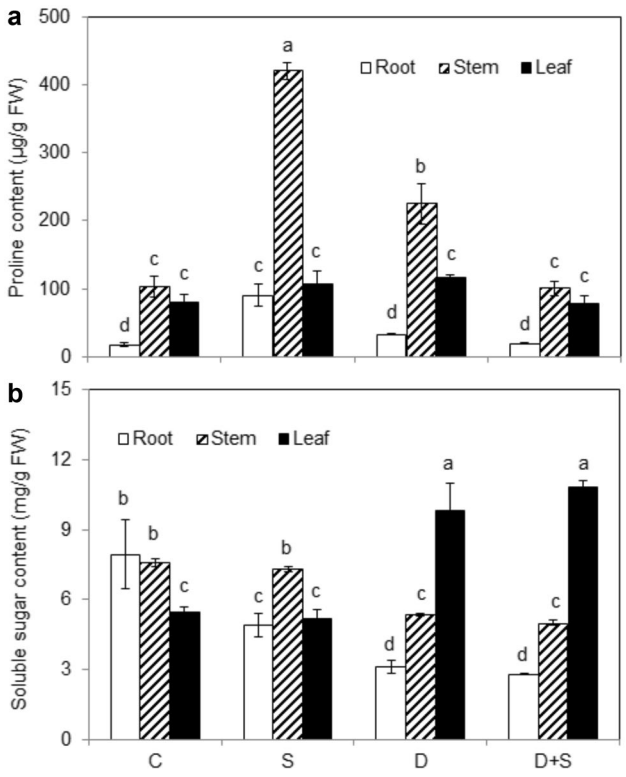

Fig. 7 Proline content (**a**) and soluble sugar content (**b**) of *L. ruthenicum* under the treatments of Control (C), 50 mM NaCl (S), − 0.5 MPa (D) and 50 mM NaCl + − 0.5 MPa (D + S) for seven days

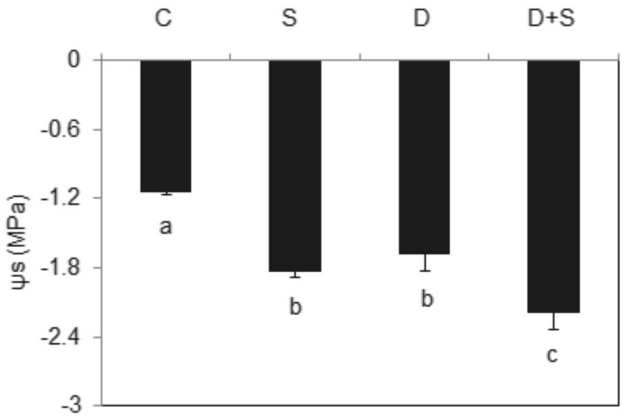

Fig. 8 Osmotic potential of *L. ruthenicum* under the treatments of Control (C), 50 mM NaCl (S), − 0.5 MPa (D) and 50 mM NaCl + − 0.5 MPa (D + S) for seven days

in roots and stems; the soluble sugar content in roots also decreased significantly under salt treatment (Fig. 7b).

The osmotic potential of plants decreased significantly under salt, drought, or drought plus salt treatments in comparison with the control, nevertheless, while that under the drought plus salt treatment was significantly lower than that of the drought treatment (Fig. 8).

Salt treatment significantly increased the Pn, Gs, and Tr, and drought significantly decreased the Pn, Gs, and Tr, but significantly increased the WUE. Compared with drought stress, the Pn, Gs, and Tr increased under the drought plus salt treatment, but the WUE decreased significantly (Fig. 9).

Discussion

An appropriate concentration of NaCl could promote the growth of *L. ruthenicum*

Studies have shown that salt stress can inhibit the growth and development of salt-sensitive plants and reduce their photosynthesis and respiration (Maksimovi et al. 2010). However, *L. ruthenicum* grew well when the salt content was 6% in the 60 cm soil layer. In addition, it has been proven that appropriate addition of Na^+ can promote the growth of *L. ruthenicum* (Wang et al. 2011). This finding was validated in the present study, in which 50 mM NaCl strongly boosted the fresh weight, dry weight, and relative growth rate of *L. ruthenicum* (Supplementary Fig. S1, Fig. 1). Similar results were found in other xerophytes and halophytes, such as *Z. xanthoxylum* and *Atriplex halimus* (Martínez et al. 2004). Ma et al. (2012) and Slama et al. (2007) found that *Z. xanthoxylum* and *Sesuvium portulacastrum* could absorb a large amount of Na^+ and use it as a main osmotic adjustment substance in an arid environment. In this study, increases in Na^+ concentration were also observed in *L. ruthenicum* when exposed to 50 mM NaCl, which further indicated that proper NaCl addition could promote plant growth and development by causing the accumulation of a large amount of Na^+ in plants (Fig. 2a). Hence, we propose that *L. ruthenicum* should be considered a salt-accumulating xerophytic species. K^+ is the most important ion that causes changes in the osmotic potential of guard cells, and is involved in regulating the physiological processes of cell water absorption and stomatal movement (Epstein and Bloom 2005; Maathuis and Sanders 2010). In addition, the ability to retain K^+ in plant tissues under saline conditions seems to be central to salinity tolerance (Shabala and Cuin 2008). In general, K^+ content often decreases under stress conditions, especially when Na^+ is abundant in plants (Hasanuzzaman et al. 2018). Interestingly, our study showed that compared with the control, the K^+ concentration in leaves increased significantly under the 50 and 100 mM NaCl treatments (Fig. 2b). This may be because rapid plant growth and development require large K^+ fluxes to provide this ion to the growing tissues, and consequently, mild salt treatments result in an increased rate of K^+ uptake (Chen et al. 2005). Therefore, it is speculated that NaCl treatment could significantly improve Na^+ and keep K^+ stable, thus maintaining Na^+/K^+ homeostasis, which may be

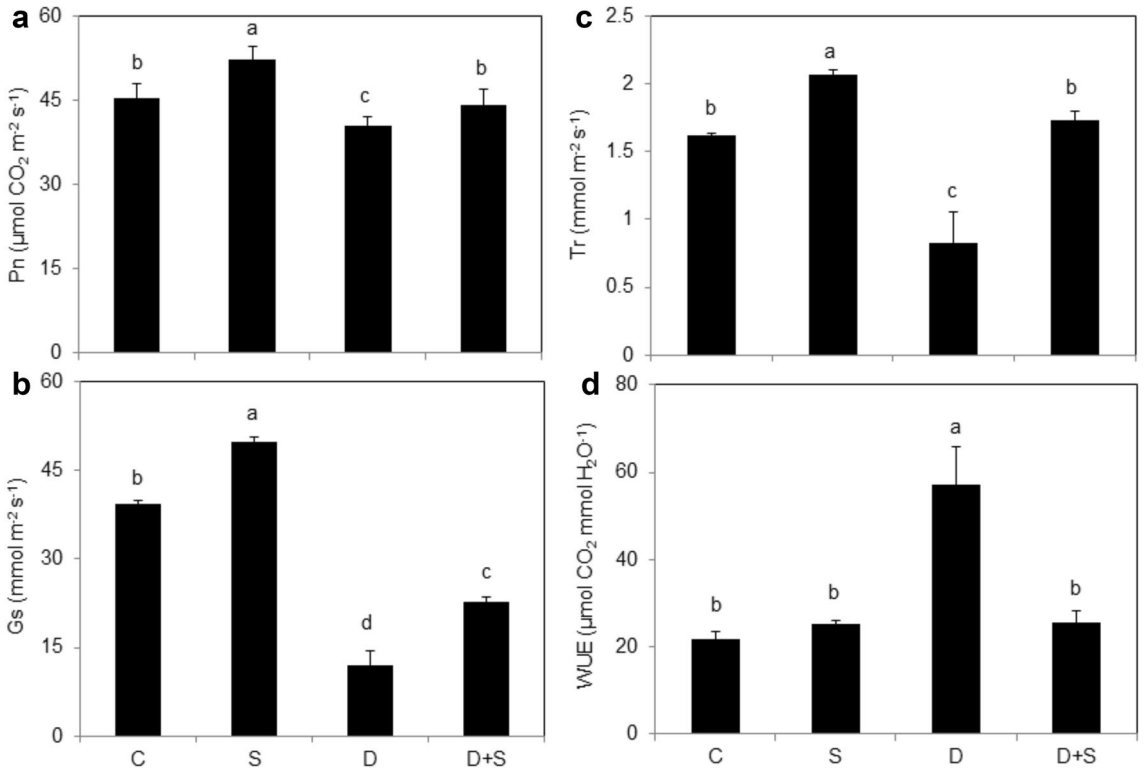

Fig. 9 **a** The net photosynthetic rate (Pn), **b** stomatal conductance (Gs), **c** transpiration rate (Tr) and **d** water use efficiency (WUE) of plants under Control (C), 50 mM NaCl (S), − 0.5 MPa (D) and 50 mM NaCl + − 0.5 MPa (D + S) for seven days

a key determinant for 50 and 100 mM NaCl (50 mM NaCl in particular) promoting the growth of *L. ruthenicum*.

LrHKT1, LrSOS1, LrNHX, LrAVP1, LrSKOR and LrAKT1 synergistically modulate Na^+ and K^+ homeostasis in *L. ruthenicum*

Under moderate salt treatment, what is the molecular mechanism of the regulation of Na^+ and K^+ homeostasis that contributes to the growth of *L. ruthenicum*? A more in-depth analysis was carried out to address this question. Four major proteins involved in Na^+ transport HKT1 (High-affinity K^+ Transporter 1), SOS1 (Salt Overly Sensitive 1), NHX (Tonoplast Na^+/H^+ antiporter), and AVP1 (Vacuolar H^+-ATPase 1) have been identified in plants (Gaxiola et al. 2001; Shi et al. 2002; Brini et al. 2007; Davenport et al. 2007), whereas AKT1 (Arabidopsis K^+ transporter 1) and SKOR (Stelar K^+ outward rectifying channel) are related to K^+ transport. HKT1 and SOS1 have been suggested to have opposite roles in controlling Na^+ delivery to shoots by mediating Na^+ influx and efflux, respectively (Zhang et al. 2017). SOS1 is involved in the long-distance transport of Na^+ from roots to shoots (Ma et al. 2014; Mahi et al. 2019). AtHKT1 selectively unloads Na^+ directly from the xylem of roots to xylem parenchyma cells, thus protecting leaves from sodium toxicity (Sunarpi et al. 2005). NHX has been suggested to play a major role in the sequestration of Na^+ into vacuoles to maintain Na^+ homeostasis, which enhances plant salt tolerance (Apse et al. 1999; Brini et al. 2007; Yuan et al. 2014). AVP1 is involved in the establishment and maintenance of an electrochemical potential gradient between the cytoplasm and the vacuole, thus promoting Na^+/H^+ antiporters such as NHX to pump Na^+ into vacuoles(Gaxiola et al. 2001). AKT1 plays important roles in K^+ uptake and modulating Na^+ transport in plants (Ma et al. 2017). SKOR is involved in loading K^+ into xylem for its transport from roots to shoots (Gaymard et al. 1998). Preliminary experiments showed that *LrNHX* and *LrVP1* were preferentially expressed in leaves and had very low levels of expression in roots; *LrSOS1*, *LrAKT1*, and *LrHKT1* were predominantly expressed in roots rather than leaves; and *LrSKOR* was mainly expressed in both roots and leaves under normal and 50 mM NaCl conditions. Thus, in this study, the mRNA levels of *LrNHX* and *LrVP1* were assayed mainly in leaves, *LrSOS1* and *LrHKT* were in roots, and *LrSKOR* was in both roots and leaves. Guo et al. (2012) proposed a model to explain the coordinated functions of SOS1 and HKT in regulating Na^+ and K^+ homeostasis in plants. Under mild salinity, when Na^+ accumulation in leaves is likely below the capacity to sequester Na^+ into vacuoles, it

appears that the transport activities of SOS1 outweigh those of HKT at the plasma membrane of the xylem parenchyma cells (XPCs), and Na^+ is loaded into the transpiration stream (Guo et al.2012). Similar mechanisms may have occurred in the current study. Compared with plants grown without salt, the addition of 50 mM NaCl sharply up-regulated the expression of *LrSOS1* in roots thereby contributing to the continual loading of Na^+ into the transpiration stream. The expression of *LrNHX* and *LrVP1* in leaves was up-regulated under 50 mM NaCl, which would facilitate sequestering a large quantity of Na^+ into vacuoles. *LrHKT1* was down-regulated in roots; thus, the synergistic effect of LrHKT1 and LrSOS1 would result in greater Na^+ loading into the xylem. This may have been the reason why a high quantity of Na^+ was accumulated in leaves under 50 mM NaCl. *LrAKT1* was up-regulated in roots, and *LrSKOR* decreased first and then increased in roots. *LrSKOR* in leaves remained stable and was slightly up-regulated, thereby absorbing a large amount of K^+ through the action of LrAKT1 and transporting it to the leaf through LrSKOR. Taken together, under 50 mM NaCl treatment, these transporters were well coordinated to mediate Na^+ and K^+ transport and may have played an important role in both ion accumulation and homeostasis thus facilitating *L. ruthenicum* growth.

Appropriate NaCl could enhance the drought resistance of plants by increasing photosynthesis and reducing osmotic potential

Lycium ruthenicum is wildly known as a xerophytic species that is distributed mainly throughout the desert area of northwestern China. Because Na^+ can promote the growth of *L. ruthenicum*, does it contribute to the alleviation of drought stress? To address this question, the effects of Na^+ on the drought tolerance of *L. ruthenicum* were observed. As expected, 50 mM NaCl added to the drought treatment improved the fresh weight and relative growth of plants (Figs. 4 and 5), demonstrating that Na^+ had a positive effect on the growth of *L. ruthenicum* under osmotic stress.

Under osmotic stress, plants can reduce water potential by accumulating a large amount of solute to maintain water balance and ensure normal growth. Commonly, the over-accumulation of Na^+ in leaves and roots leads to a decline in K^+ concentration in glycophytes (Yamaguchi and Blumwald 2005; Silva et al. 2015; Song et al. 2017). In *B. vulgaris*, the addition of 50 mM NaCl improved resistance against osmotic stress with increased Na^+ concentrations, but the K^+ concentrations in shoots and roots were decreased by 14% and 27%, respectively (Wu et al. 2015). However, in xerophyte *Z. xanthoxylum*, under water stress, the addition of 50 mM NaCl dramatically improved the Na^+ concentration in leaves by 232% while having no visible impact on the K^+ concentration (Ma et al. 2012).Surprisingly, in the present study, the addition of 50 mM NaCl under drought stress significantly increased the Na^+ content in the roots, stems, and leaves of *L. ruthenicum* (Fig. 6a). The addition of 50 mM NaCl also significantly increased the K^+ accumulation in the roots, stems, and leaves (Fig. 6b). Thus, maintaining K^+ homeostasis in leaves is a key protective strategy of *L. ruthenicum* to survive in harsh environments. Furthermore, the distribution ratio of Na^+ and K^+ in various tissues also changed with the addition of an appropriate amount of salt; that is, the ion ratio in the roots decreased but increased in the shoots (Fig. 6c, d). Therefore, it is speculated that NaCl treatment could significantly improve the Na^+ and K^+ concentration and reduce the osmotic potential by 29.7% (Fig. 8), thus enhancing the osmotic regulation ability of shoots. Na^+ and K^+ uptake are competitive processes in most higher plants (Maathuis 2014), but such a competition did not occur in *L. ruthenicum*. One possible reason is that *L. ruthenicum* sequesters Na^+ into the vacuoles in the leaf under osmotic stress (when moderate levels of Na^+ are present), which would require the coordinated up-regulation of other osmoticum levels, such as K^+, in the cytoplasm to maintain the osmotic potential balance (Møller and Tester 2007). In addition to Na^+ and K^+, these substances include some small molecular organic compounds, such as proline and soluble sugar, which are the important osmotic factors of osmoregulation (Farkhondeh et al. 2012). In this study, compared with the drought treatment alone, the addition of 50 mM NaCl resulted in a decrease in proline content and no significant change in soluble sugar content (Fig. 7), suggesting that they did not play a key role in the decrease of osmotic potential compared with drought. Similar results were also observed in the other xerophytes, such as *Nitraria sibirica* and *Z. xanthoxylum* (Li et al. 2005; Ma et al. 2012).

Lastly, species with high osmotic stress tolerance would maintain higher stomatal conductance under stress conditions, resulting in higher CO_2 assimilation rates (James et al. 2008), thus maintaining a sufficient carbon supply in growing leaves (Rahnama et al. 2010). Studies have shown that the photosynthetic rate of leaves is positively correlated with stomatal conductance (Debez et al. 2006; Slama et al. 2007), and stomatal conductance depends on the opening degree of stomatal guard cells (Franks et al. 2001). In this study, the addition of NaCl increased the photosynthetic rate, stomatal conductance, and transpiration capacity of *L. ruthenicum* (Fig. 9a, b, c), which may be the main reason that it promoted plant growth and alleviated drought stress. Moreover, stomatal conductance is much more sensitive to soil water than photosynthesis, and transpiration water consumption is closely dependent on stomata. Therefore, when *L. ruthenicum* plants are subjected to drought stress, the transpiration rate decreases more than the net photosynthetic rate, leading to the increase of water use efficiency (Fig. 9d). In addition, it also demonstrated that the promoting effect of NaCl on the

growth of *L. ruthenicum* was partly due to the promotion of stomatal guard cell opening. However, the opening of stomatal guard cells depends on the osmotic adjustment ability, which is the main mechanism of drought tolerance of plants; that is, plants actively accumulate solute to increase the concentration of cell liquid and reduce its osmotic potential, so as to maintain water in the plant and adapt to water-stressed environments (Zhang et al. 1999; Ramanjulu and Sudhakar 2000). Obviously, compared with the drought treatment, the addition of 50 mM NaCl reduced the osmotic potential (Fig. 8), thereby enhancing the photosynthetic activity and water status and alleviating drought stress.

In conclusion, our results demonstrate that under moderate NaCl conditions, Na^+ and K^+ transporters are likely to coordinate the regulation of Na^+ and K^+ absorption and transport, and may play an important role in the accumulation and homeostasis of both ions, thus facilitating *L. ruthenicum* growth. In addition, proper Na^+ addition had a positive effect on the growth of *L. ruthenicum* under osmotic stress, and moderate NaCl alleviated drought stress by increasing Na^+ and K^+ content and photosynthetic activity. However, much more work is required to fully understand the molecular mechanism of how the addition of appropriate amounts of NaCl alleviates osmotic stress.

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Declarations

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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