Proper Nacl Alleviates Osmotic Stress in *Lycium Ruthenicum*

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Research Article

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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 resulted in promoting plant growth. To investigate the mechanism of Na^+ to plant growth and the effect of drought stress, the growth, photosynthesis, water status and K^+, Na^+ transport related genes were subjected to different NaCl treatments and osmotic stress in the presence or absence of additional NaCl were assessed. Compared to the control, 50 mM NaCl strongly boosted the fresh weight, dry weight and relative growth rate of *L. ruthenicum*, and significantly increased the concentration of Na^+, the K^+ concentration in roots and stems remained stable, while which in leaves increased significantly. Furthermore, the addition of 50 mM NaCl sharply up-regulated the expression of *LrSOS1* in roots, *LrNHX* and *LrVP1* in leaves, *LrHKT1* down-regulated in roots, it's the reason why a high quantity of Na^+ was accumulated in leaves under 50 mM NaCl. *LrAKT1* up-regulated in roots, *LrSKOR* decreased first and then increased in roots, whereas *LrSKOR* in leaves remained stable and slightly up-regulated, thereby absorb a large amount of K^+ by LrAKT1 and transport it to the leaf through LrSKOR. Moreover, external NaCl apparently alleviated the inhibition of osmotic stress in plant growth. Compared with the drought treatment, the addition of 50 mM NaCl significantly increased the Na^+ and K^+ content in roots, stems and leaves of *L. ruthenicum*, resulted in a decrease in proline content and no significant change in soluble sugar content, it is speculated that NaCl treatment could significantly improve the Na^+, K^+ concentration, thus enhance the osmotic regulation ability of plants, and then improve the photosynthesis and water status of *L. ruthenicum*.

Introduction

Drought stress is a critical factor that limiting plant growth, it induces a range of physiological and biochemical responses in plants, and affect all stages of plant development (Hussain et al. 2018). However, 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 and determined by numerous physiological indicators. In order to deal with osmotic stress, plant cells must contain a certain amount of water and show certain turgor pressure to drive extension growth in roots and shoots, which can be maintained by a process called osmotic adjustment (Shabala et al. 2010). Under stress, higher plants usually adopt two ways of osmoregulation, one is to synthesize free amino acids, soluble sugars and other organic regulatory substances in plants; the other is to accumulate 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 controversial among these osmoregulants. For most species, Na^+ is not essential in any sense and even 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), and maybe it an adaptive mechanisms that ensure xerophytes and halophytes survival and reproduction in arid or semi-arid environments.

Lycium ruthenicum Murr. belongs to the Solanaceae family, is widely distributed in the salinized desert of northwest of China. Due to its nutritional, medical and ecological values, *L. ruthenicum* has attracted wide attention and become not only a unique but also urgently developed wild plant in the desert area (Peng et al. 2014; Dai et al. 2019). Furthermore, as the main species in dry area, *L. ruthenicum* has the function of preventing wind and fixing sand. Besides, it is one of the three major alkaline soil indicator and pioneer plants in the world, and is a plant with strong salt tolerance which be able to grow very well where the other plants could not grow, more surprising, it is a particular plant which can reduce soil salinization (Dai et al. 2019). Research showed that, proper amount of NaCl (100 mM NaCl) could significantly promote the growth of *L. ruthenicum*, and at high salt (450 mM NaCl) condition, when the Na^+ content increases greatly, K^+/Na^+ ratio in stem and leaf could reach 28.3 and 22.3, respectively; moreover, in three salt-accumulated plants, K^+/Na^+ ratio of *L. ruthenicum* was significantly higher than *Kalidium foliatum* and *Nitraria sibirica* (Wang et al. 2011). Therefore, absorbing Na^+ and maintain the steady balance of K^+ may be an effective strategy for *L. ruthenicum* to resist adversity. However, the mechanisms for drought resistance of Na^+ and maintain K^+/Na^+ homeostasis in *L. ruthenicum* are still unknown. Moreover, the Na^+ and K^+ changes of *L. ruthenicum* exposed to drought combined with salt have not yet been measured.

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

Materials And Methods

Plant growth conditions and treatments

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

The diurnal temperature of the culture chamber: (28±2)°C/(23±2)°C; Time and intensity of illumination: 16 h/d and 600 $\mu mol.m^{-2}.s$.

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

(i) 0, -0.25, -0.5, -1.0 and -1.5 MPa (solution with sorbitol) were treated for 7 days, characterized and photographed. (ii) 0, 50, 100, 200, 300, 400 mM NaCl (NaCl added to nutrient solution) was treated for 7 days, characterized and photographed. (iii) Based on the results of (i) and (ii), seedlings dealt with as follows :1) control (C): normal irrigation 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; 4) drought and salt treatment (D+S): sorbitol solution with a total osmotic potential of -0.5 MPa was added to 50 mM of NaCl with Hoagland nutrient solution. Sampling and determination of the following indicators, each treatment of 6 replicates, each repeated 2 seedlings. The above treatments replace the solution once a day to keep the concentration of the solution relatively constant.

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 get 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. Ion concentration was determined with a flame spectrophotometer (2655-00; Cole Parmer Instrument Co., USA).

Measurement of free proline and soluble sugar

The free proline in *L. ruthenicum* leaves was extracted with sulfosalicylic acid and determined with spectrophotometer. Total sugar measured by anthrone colorimetry.

Determination of osmotic potential and physiological parameters

Leaf was rinsed with deionized water and blotted on filter paper immediately, then frozen in liquid nitrogen and thawed to extract sap by a syringe. The acquired sap was determined with a cryoscopic osmometer (OSMOMAT-030, GONOTECGmbH, Germany). The readings (mmol/kg) were used to calculate the solute potential in MPa (Mega Pascales) with the formula: Ψ_s= -moles of solute × RT, where R =0.008314 and T = 297°C (Yuan et al. 2014).

The Pn, Tr, Gs was measured with Photosynthetic System (L1-6400.LI-COR Biosciences, USA), and WUE = Pn/Tr (Hassine et al. 2009).

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, 24 h. Reactions were performed in a GeneAmp® PCR System 9700 (Applied Biosystems, USA). *LrLEF1α* was used as the reference gene. *LrSKOR*, *LrAKT1*, *LrHKT*, *LrSOS1*, *LrNHX*, *LrAPV1*

are available in the NCBI SRA database (Accession number SRR7700825). Fragment of *LrSKOR*, *LrAKT1*, *LrHKT1;1*, *LrSOS1*, *LrNHX*, *LrAPV1*, *LrLEF1α* was amplified with a pair of primers P1 and P2, P3 and P4, P5 and P6, P7 and P8, P9 and P10, P11 and P12, 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).

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 treatments of NaCl 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 leaves and roots of *L. ruthenicum* under 50 and 100 mM NaCl treatments were significantly better than control and other treatments, and the effect of 50 mM NaCl was the most pronounced one among these treatments (Supplementary Fig. S1).

To further evaluate the effect of NaCl on growth of *L. ruthenicum*, the fresh weight, dry weight, tissue water content and relative growth rate was determined under NaCl treatments (0-400 mM) (Fig. 1). 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 above significantly increased by 126.4%, 60.4% and 47.2%, respectively. It is further suggesting that addition of appropriate NaCl could obviously promote plant growth.

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 roots, stems and leaves treatment were significantly increased (62.5%, 209.6%, 173.7% and 156.1%, 332.1%, 283.7% in roots, stems and leaves under 50 and 100 mM NaCl treatments, respectively) under 50-400 mM NaCl treatment. K^+ in roots and leaves increased significantly (increased by 20.6%, 6.7% and 26.1%, 4.2% under 50 and 100 mM NaCl treatments, respectively), and remained stable in stems; however, with the increase of NaCl concentration, K^+ concentration in various tissues decreased significantly.

To explore the pathway of coordinated regulation of Na^+, K^+ channels or transporters in *L. ruthenicum*, the expression patterns of *LrAKT1*, *LrSKOR*, *LrSOS1*, *LrHKT*, *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 increased, while *LrHKT* was down-regulated in roots; moreover, the expression of *LrAPV1* and *LrNHX* were significantly up-regulated in leaves (Fig. 3).

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

As Supplementary Fig.S2 shows that compared with the control, the seedlings of *L. ruthenicum* grew normal under -0.25 MPa, and -0.5, -1.0, -1.5 MPa delayed plant growth. Furthermore, under -1.0 MPa treatment exhibiting poor plant performance and under -1.5 MPa stress treatment, the plant appeared obvious wilting phenomenon, thus -0.5 MPa was chosen as the followed drought treatment.

Moderate concentrations of NaCl alleviates the deleterious impact of water deficit

In order to explore whether 50 mM NaCl could alleviate the drought stress of *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). Compared with the control, as same as the result of NaCl treatment above, plants still grew very well exposed 50 mM NaCl, but drought treatment exhibited a certain inhibitory effect on seedling growth. However, what really caught our attention was that drought plus salt treatment was better than drought treatment, therefore, 50 mM NaCl could alleviate the drought stress of plants.

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

Compared with control, the water content of tissues under salt treatment increased significantly, while under drought treatment which 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). 50 mM NaCl significantly increased the relative growth rate of plants, drought and drought plus salt treatment obviously delayed the relative growth of plants compared to control; but compared with drought treatment, 50 mM NaCl plus drought treatment evidently increased the relative growth rate of plants by 1.8% (Fig. 5d), therefore NaCl could alleviate drought stress.

Compared with control, salt and drought plus salt treatments significantly increased the Na^+ concentration in roots, stems and leaves of *L. ruthenicum*, but had no significant change under drought treatment; salt and drought plus salt treatment also increased K^+ content in leaves and stems. Compared with drought, drought plus salt treatment increased Na^+ (roots, stems and leaves: 54.8%, 385.7% and 155.4%, respectively) and K^+ concentration (roots, stems and leaves: 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 when drought addited salt (Fig. 6c, d).

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

Compared with control, drought or drought plus salt treatment significantly increased the soluble sugar content in leaves, but evidently decreased the soluble sugar content in roots and stems; the soluble sugar content in roots also decreased significantly under salt treatment (Fig. 7b) .

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

Salt treatment significantly increased the net photosynthetic rate (Pn), stomatal conductance (Gs) and transpiration rate (Tr), and drought significantly decreased the net photosynthetic rate (Pn), stomatal conductance (Gs) and transpiration rate (Tr), but significantly increased the water use efficiency (WUE). Compared with drought stress, Pn, Gs and Tr increased evidently under drought plus salt treatment, but WUE decreased significantly (Fig. 9).

Discussion

Proper concentration of NaCl could promote the growth of *L. ruthenicum*

Studies have shown that salt stress could 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 even 6% in 60 cm soil layer. In addition, it has been proved that appropriate of Na^+ could promote the growth of *L. ruthenicum* (Wang et al. 2011), and we confirm this point of view in the present study, 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 detected in other xerophyte and halophyte, such as *Zygophyllum 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 arid environment. In this study, increases of Na^+ concentration were also observed in *L. ruthenicum* when exposed to 50 mM NaCl, which further ascertained that proper NaCl could promote plant growth and development by accumulating a large number of Na^+ in plant (Fig. 2a). Hence, we propose that *L. ruthenicum* should be considered as a salt-accumulating xerophytic species. K^+ is the most important ion that causes the change of osmotic potential of guard cells, which 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 K^+ retain 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 control, the K^+ concentration in leaves increased significantly under 50 mM NaCl

treatment (Fig. 2b). Maybe 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 not only Na^+, but also remained K^+ stable, thus maintain Na^+/K^+ homeostasis, which may be a key determinant for 50 mM NaCl promoting growth of *L. ruthenicum*.

LrHKT1;1, LrSOS1, LrNHX1, LrAVP1, LrSKOR and LrAKT1 synergistically modulate Na^+ and K^+ homeostasis in *L. ruthenicum*

However, under moderate salt treatment, what is the molecular mechanism of regulating Na^+, K^+ homeostasis contributing to the growth of *L. ruthenicum*? We try to make more in-depth analysis. There are four major proteins, HKT (High-affinity K^+ Transporter), SOS1 (Salt Overly Sensitive 1), NHX1 (Tonoplast Na^+/H^+ antiporter) and AVP1 (Vacuolar H^+-ATPase) have been identified in plants are involved in Na^+ transport (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. HKT I 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 involved in long-distance transport of Na^+ from roots to shoots (Ma et al. 2014; Mahi et al. 2019). AtHKT1;1, selectively unloads Na^+ directly from the xylem of roots to xylem parenchyma cells, thus protecting leaves from sodium toxicity (Sunarpi et al. 2005). NHX1 has been suggested to play a major role in sequestration of Na^+ into vacuoles to maintain Na^+ homeostasis, which results in enhancing 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 NHX1 to pump Na^+ into vacuoles(Gaxiola et al. 2001). AKT1 played important roles of 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). Pre-experiment showed that, *LrNHX* and *LrVP1* was preferentially expressed in leaves and barely in roots, *LrSOS1*, *LrAKT1* and *LrHKT1;1* was predominantly expressed in roots rather than leaves, whereas *LrSKOR* mainly expressed in both roots and leaves under normal and 50 mM NaCl condition. Thus, in this study, the mRNA levels of *LrNHX*, *LrVP1* were assayed mainly in leaves, *LrSOS1* and *LrHKT1;1* were in roots, and *LrSKOR* were in both roots and leaves. Compared with plants growing without salt, the addition of 50 mM NaCl sharply up-regulated the expression of *LrSOS1* in roots thereby contributing to a 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* down-regulated in roots, thus, the synergistic effect of LrHKT1;1 and LrSOS1 would result in greater Na^+ loading into the xylem. It maybe the reason why a high quantity of Na^+ was accumulated in leaves under 50 mM NaCl. *LrAKT1* up-regulated in roots, *LrSKOR* decreased first and then increased in roots, whereas *LrSKOR* in leaves remained stable and slightly up-regulated, thereby absorb a large amount of K^+ by LrAKT1 and transport 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 perhaps plays an important role in both ions accumulation and homeostasis thus facilitating *L. ruthenicum* growth.

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

L. ruthenicum is wildly known as a xerophytic species that is distributed mainly over the desert area of Northwestern China. Since Na^+ could promote the growth of *L. ruthenicum*, would it contribute to alleviate drought stress? Furthermore, the effects of Na^+ on the drought tolerance of *L. ruthenicum* were observed. Just as expected, 50mM NaCl added to the drought treatment improved the fresh weight and the relative growth of plants obviously (Figs. 4 and 5), demonstrating that Na^+ has a positive effect on the growth of *L. ruthenicum* under osmotic stress.

Under osmotic stress, plants can reduce water potential by accumulating a large of solute to maintain water balance and ensure its normal growth. In present study, the addition of 50 mM NaCl under drought stress significantly increased the Na^+ content in roots, stems and leaves of *L. ruthenicum* (Fig. 6a). Compared with the drought treatment, 50 mM NaCl also significantly increased K^+ accumulation in roots, stems and leaves (Fig. 6b). Meanwhile, the distribution ratio of Na^+ and K^+ in various tissues also changed with the addition of appropriate amount of salt, that is, the ion ratio in the root decreased but increased in the shoot (Fig. 6c, d). Therefore, it is speculated that NaCl treatment could significantly improve the Na^+, K^+ concentration, reduced the osmotic potential by 29.7% (Fig. 8), thus enhance the osmotic regulation ability of shoots. In addition to Na^+ and K^+, these substances also 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 single drought treatment, 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).

Last but not least, varieties with high osmotic stress tolerance would maintain higher stomatal conductance under stress condition, resulting in higher CO_2 assimilation rate (James et al. 2008), thus maintaining 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, transpiration capacity of *L. ruthenicum* (Fig. 9a, b, c). which may be the main reason for promoting plant growth and alleviating 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, transpiration rate decreases more than 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. On the other hand, the opening of stomatal guard cells depends on osmotic adjustment ability, which is the main performance 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 stress environment (Zhang et al. 1999; Ramanjulu and Sudhakar 2000). Obviously, compared with the drought treatment, 50 mM NaCl addition reduced the osmotic potential (Fig. 8), thereby enhancing photosynthetic activity, water status and alleviating drought stress.

In conclusion, appropriate concentration of NaCl perhaps through Na^+ and K^+ transporters to accumulate and maintain ions homeostasis thus facilitating *L. ruthenicum* growth. Moreover, moderate NaCl alleviate drought stress by increasing Na^+, K^+ content and photosynthesis.

Declarations

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Conflict of Interest Statement 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.

References

1. Apse MP, Aharon GS, Snedden WA, Blumwald E (1999) Overexpression of a vacuolar Na^+/H^+ antiport confers salt tolerance in Arabidopsis. Science 285: 1256-1258
2. Brini F, Hanin M, Mezghani I, Berkowitz GA, Masmoudi K (2007) Overexpression of wheat Na^+/H^+ antiporter TNHX1and H^+-pyrophosphatase TVP1improve salt-and drought-stress tolerance in Arabidopsis thaliana plants. J Exp Bot 58:301-308
3. Chen Z, Newman I, Zhou M, Mendham N, Zhang G, Shabala S (2005) Screening plants for salt tolerance by measuring K^+ flux: a case study for barley. Plant Cell Environ 28: 1230-1246
4. Dai F, Li A, Rao S, Chen J (2019) Potassium transporter LrKUP8 is essential for K^+ preservation in *Lycium ruthenicum*, a salt-resistant desert shrub. Genes 10(8)
5. Davenport RJ, Munoz-Mayor A, Jha D, Essah PA, Rus A, Tester M (2007) The Na^+ transporter AtHKT1;1 controls retrieval of Na^+ from the xylem in Arabidopsis. Plant Cell and Environ 30: 497-507
6. Debez A, Saadaoui D, Ramani B, Ouerghi Z, Koyro HW, Huchzermeyer B, Abdelly C (2006) Leaf H^+-ATPase activity and photosynthetic capacity of *Cakile maritima* under increasing salinity. Environ Exp Bot 57: 285-295

7. Duan HR, Ma Q, Zhang JL, Hu J, Bao AK, Wei L, Wang Q, Luan S, Wang SM (2015) The inward-rectifying K^+ channel SsAKT1 is a candidate involved in K^+ uptake in the halophyte *Suaeda salsa* under saline condition. Plant Soil 395(1-2): 173-187
8. Epstein E, Bloom A (2005) Mineral nutrition of plants: Principles and perspectives (Sinauer, Sunderland, MA).
9. Farkhondeh R, Nabizadeh E, Jalilnezhad N (2012) Effect of salinity stress on proline content, membrane stability and water relations in two sugar beet cultivars. International Journal of Agricultural and statistical sciences 2(5):385-392
10. Franks PJ, Buckley TN, Shope JC, Mott KA (2001) Guard cell volume and pressure measured concurrently by confocal microscopy and the cell pressure probe. Plant Physiol 125: 1577-1584
11. Gaxiola RA, Li J, Undurraga S, Dang LM, Allen GJ, Alper SL, Fink GR (2001) Drought- and salt-tolerant plants result from overexpression of the AVP1 H^+-pump. Proc Natl Acad Sci USA 98:11444-11449
12. Gaymard F, Pilot G, Lacombe B, Bouchez D, Bruneau D, Boucherez J, Michaux-Ferrière N, Thibaud J, Sentenac H (1998) Identification and disruption of a plant Shaker-like outward channel involved in K^+ release into the xylem sap. Cell 94:647-655
13. Hasanuzzaman, Mirza, Bhuyan, Borhannuddin MHM, Nahar, Kamrun, Hossain, Shahadat M, Mahmud, Jubayer A, Hossen, Masud, Abdul Awal Chowdhury, Moumita, Fujita, Masayuki (2018) Potassium: a vital regulator of plant responses and tolerance to abiotic stresses. Agronomy 8(3)doi:10.3390/agronomy8030031
14. Hassine AB, Ghanem ME, Bouzid S, Lutts S (2008) An inland and a coastal population of the Mediterranean xero-halophyte species *Atriplex halimus* L. differ in their ability to accumulate proline and glycinebetaine in response to salinity and water stress J Exp Bot 59:1315-1326
15. Hessinia K, Martínez JP, Gandour M, Albouchi A, Soltani A, Abdelly C (2009) Effect of water stress on growth, osmotic adjustment, cell wall elasticity and water-use efficiency in *Spartina alterniflora*. Environ Exp Bot 67:312-319
16. James RA, von Caemmerer S, Condon AG, Zwart AB, Munns R (2008) Genetic variation in tolerance to the osmotic stress component of salinity stress in durum wheat. Funct Plant Biol 35: 111-123
17. Li JP, Yang XG, Fu H, Zhang BL (2005) The content and distribution characteristics of some osmotic adjusting materials in three species of desert plants in Alashan Desert of Northwest China. Pratacul Sci 22:35-38
18. Ma Q, Hu J, Zhou Xi, Yuan H, Tanweer Kumar, Luan S, Wang S (2017) ZxAKT1 is essential for K^+ uptake and K^+/Na^+ homeostasis in the succulent xerophyte *Zygophyllum xanthoxylum*. Plant J 90(1):48-60
19. Ma Q, Li YX, Yuan HJ, Hu J, Wei L, Bao AK, Zhang JL, Wang SM (2014) ZxSOS1 is essential for long-distance transport and spatial distribution of Na^+ and K^+ in the xerophyte *Zygophyllum xanthoxylum.*Plant Soil 374: 661-676

20. Ma Q, Yue LJ, Zhang JL, Wu GQ, Bao AK, Wang SM (2012) Sodium chloride improves photosynthesis and water status in the succulent xerophyte *Zygophyllum xanthoxylum*. Tree Physiol 32: 4-13
21. Maathuis F J M, Sanders D (2010) Mechanisms of potassium absorption by higher plant roots. Physiol Plantarum 96(1):158-168
22. Mahi, H. E. , Javier Pérez-Hormaeche, Luca AD, Villalta I, Espartero J, Francisco Gámez-Arjona, Fernandez JL, Bundo M, Mendoza I, Mieulet D (2019) A critical role of sodium flux via the plasma membrane Na^+/H^+ exchanger sos1 in the salt tolerance of rice. Plant Physiol 180(2):pp.00324.DOI: 10.1104/pp.19.00324
23. Maksimovi I, Putnik-Deli M, Gani I, Mari J, Žarko Ilin (2010) Growth, ion composition, and stomatal conductance of peas exposed to salinity. Cent Eur J Biol 5(5):682-691
24. Martínez JP, Lutts S, Schanck A, Bajji M, Kinet JM (2004) Is osmotic adjustment required for water-stress resistance in the Mediterranean shrub *Atriplex halimus* L.? J Plant Physiol 161:1041-1051
25. Peng Q, Liu H, Shi S, Li M (2014) *Lycium ruthenicum* polysaccharide attenuates inflammation through inhibiting TLR4/NF-KB signaling pathway. Int J Biol Macromol 67:330-335
26. Rahnama, Afrasyab, James, Richard A, Poustini, Kazem, & Munns, Rana (2010) Stomatal conductance as a screen for osmotic stress tolerance in durum wheat growing in saline soil. Funct Plant Biol 37(3): 255-263
27. Ramanjulu S, Sudhakar C (2000) Proline metabolism during dehydration in two mulberry genotypes withcontrasting drought tolerance. J Plant Physiol 157: 81-85
28. Shabala S (2011) Physiological and cellular aspects of phytotoxicity tolerance in plants: the role of membrane transporters and implications for crop breeding for waterlogging tolerance. New Phytol 190: 289-298
29. Shabala S , Pottosin I I (2010) Potassium and Potassium-Permeable Channels in Plant Salt Tolerance. Ion Channels and Plant Stress Responses: 87-110
30. Shabala S, Cuin TA (2008) Potassium transport and plant salt tolerance. Physiol Plantar133:651-669
31. Shi H, Quintero FJ, Pardo JM, Zhu JK (2002) The putative plasma membrane Na^+/H^+ antiporter SOS1 controls long distance Na^+ transport in plants. Plant Cell 14: 465-477
32. Slama I, Ghnaya T, Messedi D, Hessini K, Labidi N, Savoure A, Abdelly C (2007) Effect of sodium chloride on the response of the halophyte species *Sesuvium portulacastrum* grow in mannitol-induced water stress. J Plant Res 120: 291-299
33. Sunarpi HT, Motoda J, Kubo M, Yang H, Yoda K, Horie R, Chan WY, Leung HY, Hattori K, Konomi M, Osumi M, Yamagami M, Schroeder JI, Uozumi N (2005) Enhanced salt tolerance mediated by AtHKT1 transporter-induced Na^+ unloading from xylem vessels to xylem parenchyma cells. Plant J 44:928-938
34. Wang L, Mi Y, Lin H (2011) Effect of salt stress on ion absorption and distribution of two Lycium seedlings. Acta Prataculturae Sinica 20(4):129 -136

35. Wang SM, Zhang JL, Flowers TJ (2007) Low-affinity Na^+ uptake in the halophyte *Suaeda maritima*.Plant Physiol 145:559-571

36. Yuan HJ, Ma Q, Wu GQ, Wang P, Hu J, Wang SM (2014) ZxNHX controls Na^+ and K^+ homeostasis at whole-plant level through feedback regulating the expression of genes involved in their transport in *Zygophyllum xanthoxylum*, Ann Bot 115(3): 495-507

37. Zhang J, Nguyen H, Blum A (1999) Genetic analysis of osmotic adjustment in crop plants. J Exp Bot 50: 291-302

38. Zhang M, Cao Y, Wang Z, Wang ZQ, Shi J, Liang X, Song W, Chen Q, Lai J, Jiang C (2017) A retrotransposon in an hkt1 family sodium transporter causes variation of leaf Na^+ exclusion and salt tolerance in maize. New Phytol 217(3): 1161-1176

Figures

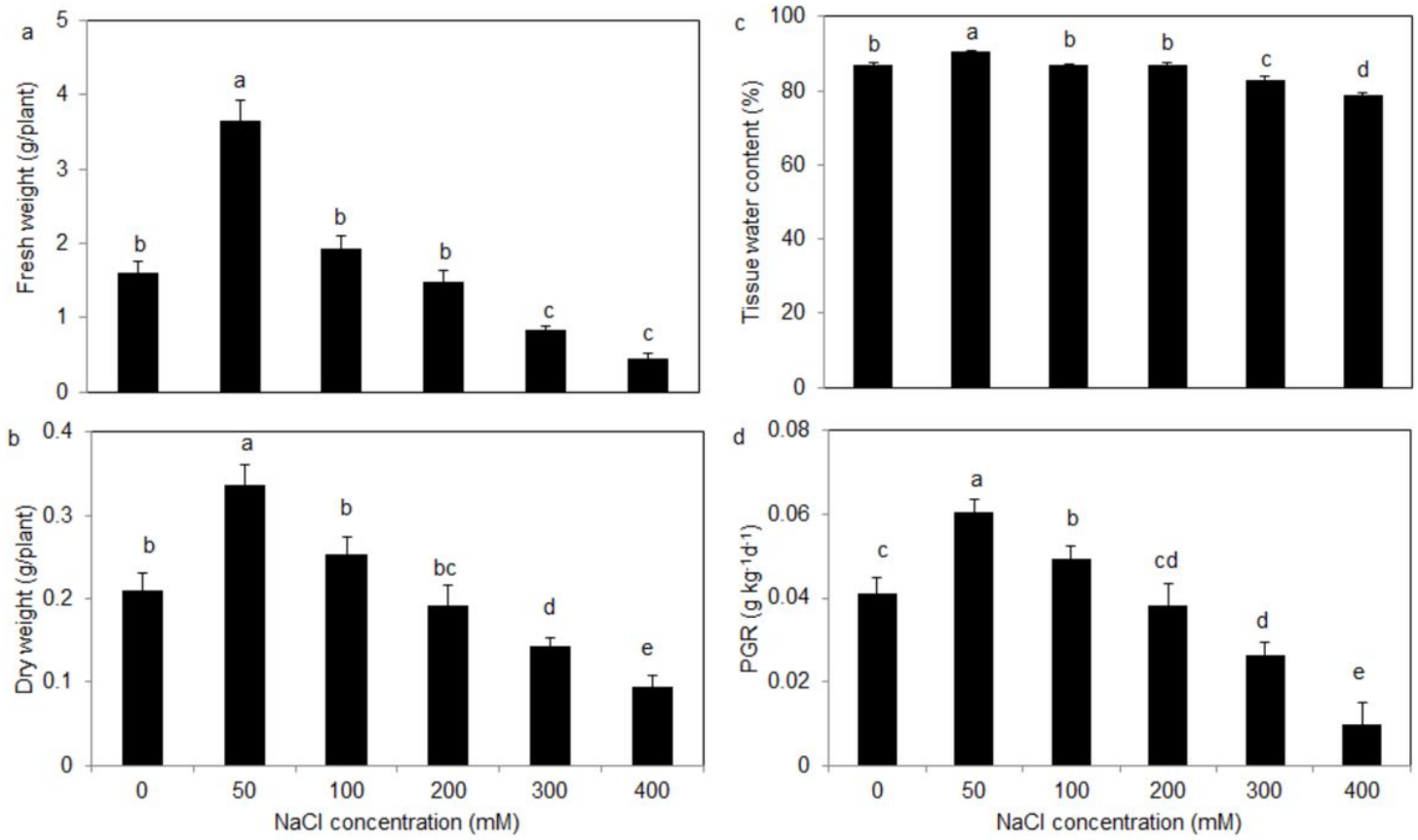

Figure 1

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 7 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.

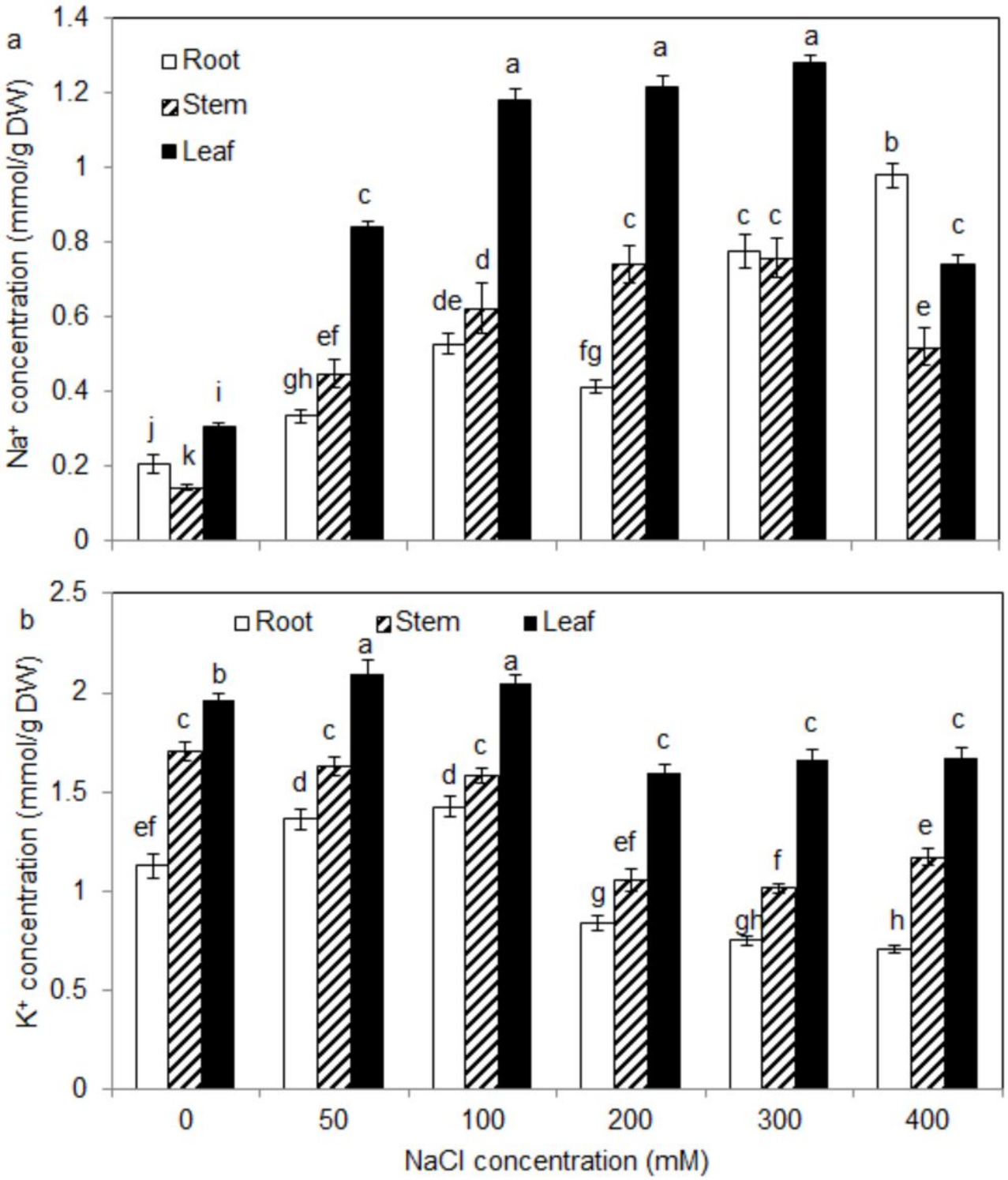

Figure 2

Na+ concentration (a) and K+ concentration (b) of L. ruthenicum under the treatments of Control (C), 50, 100, 200, 300, 400 mM NaCl for 7 days.

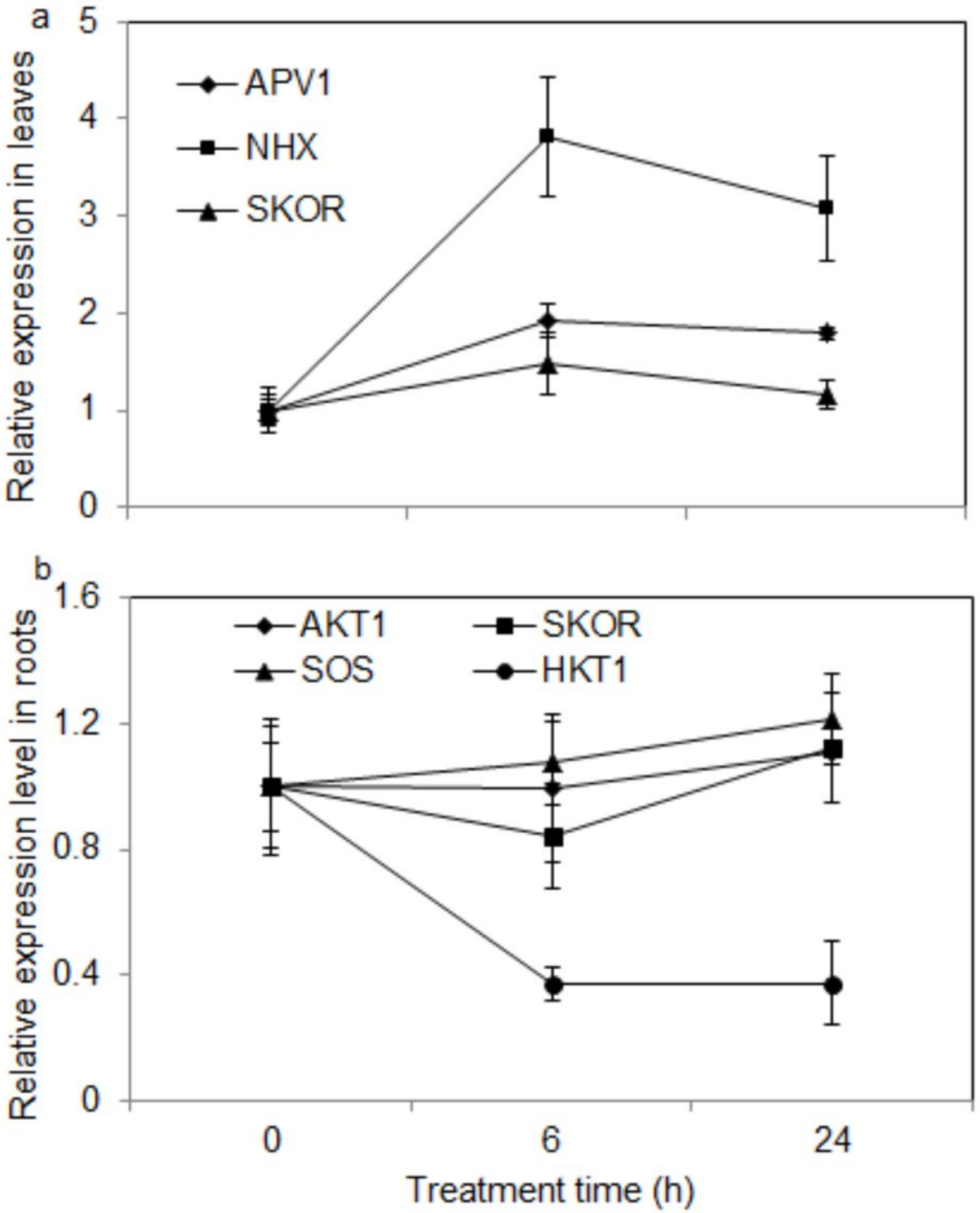

Figure 3

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 4-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)

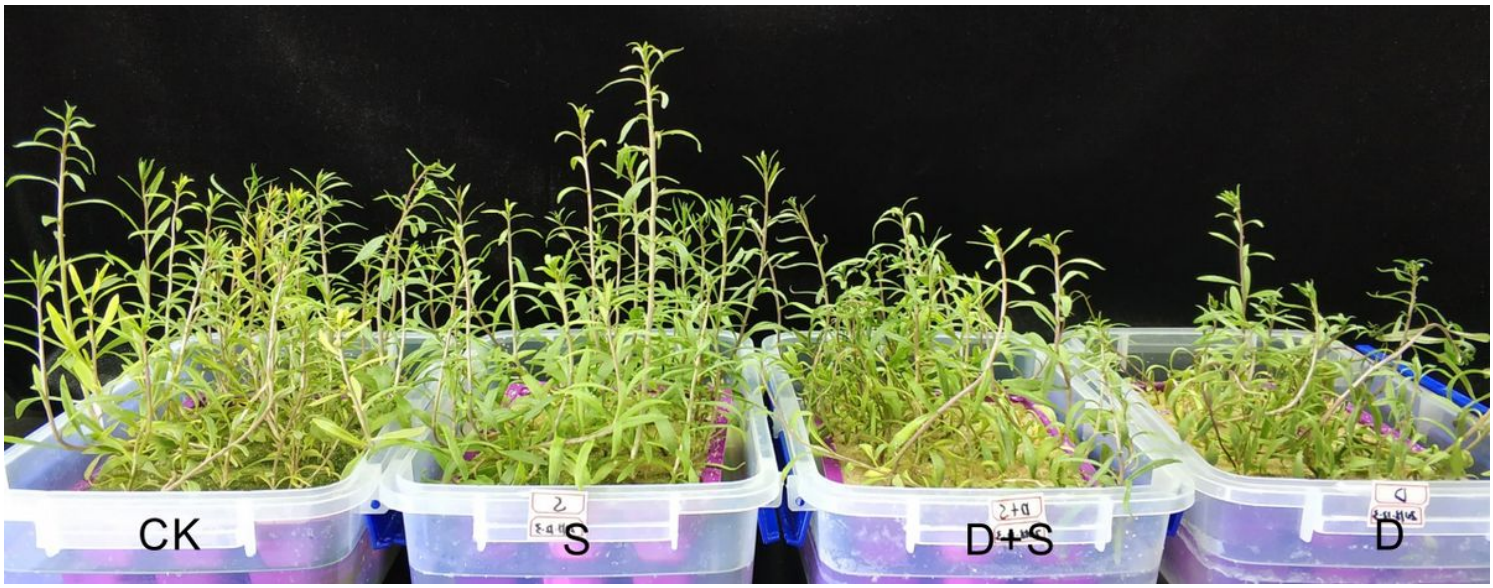

Figure 4

Control (C), 50 mM NaCl (S), -0.5 MPa (D) and 50 mM NaCl +-0.5 MPa (D+S) for 7 days effect on the growth of L.ruthenicum

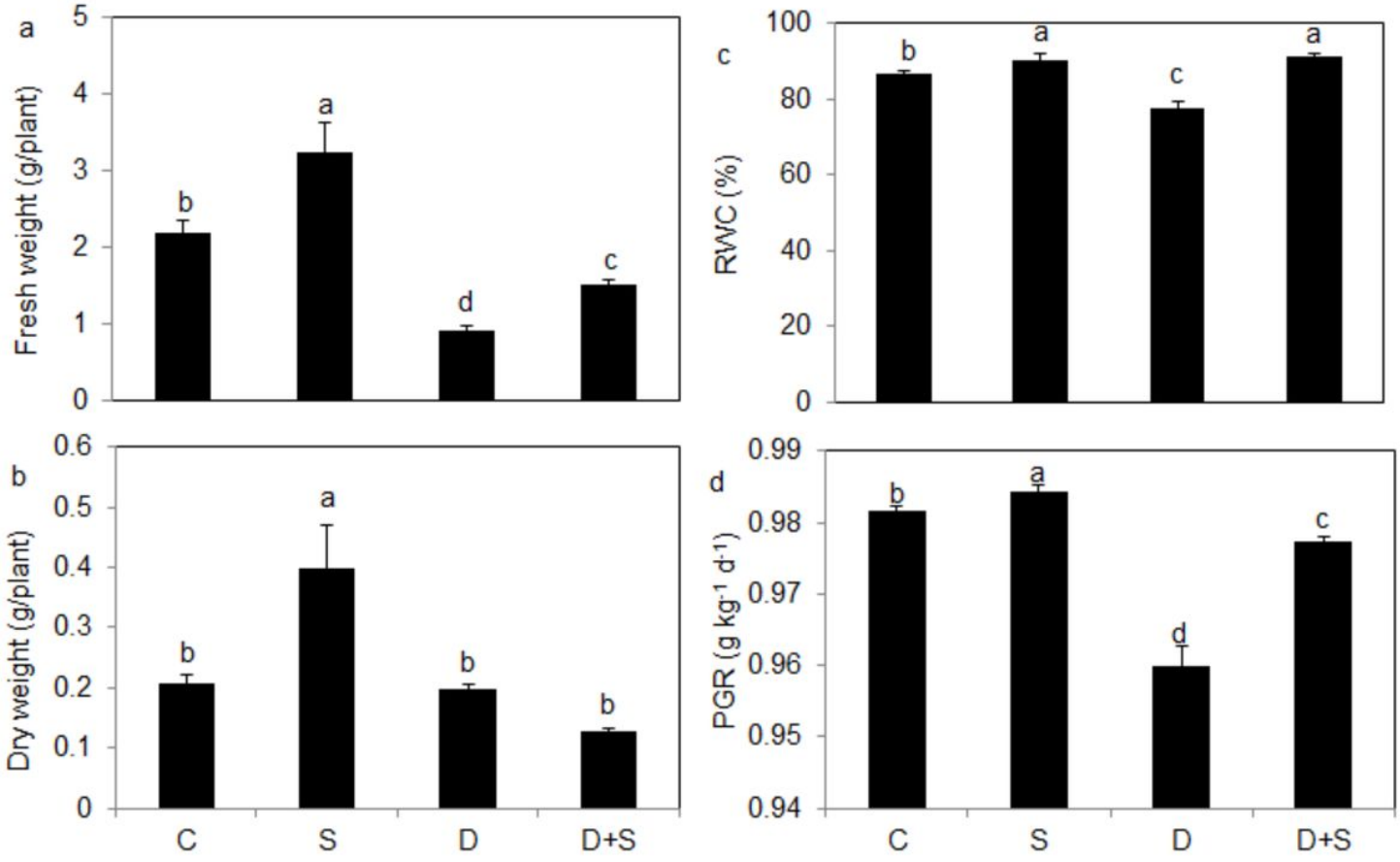

Figure 5

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 7 days.

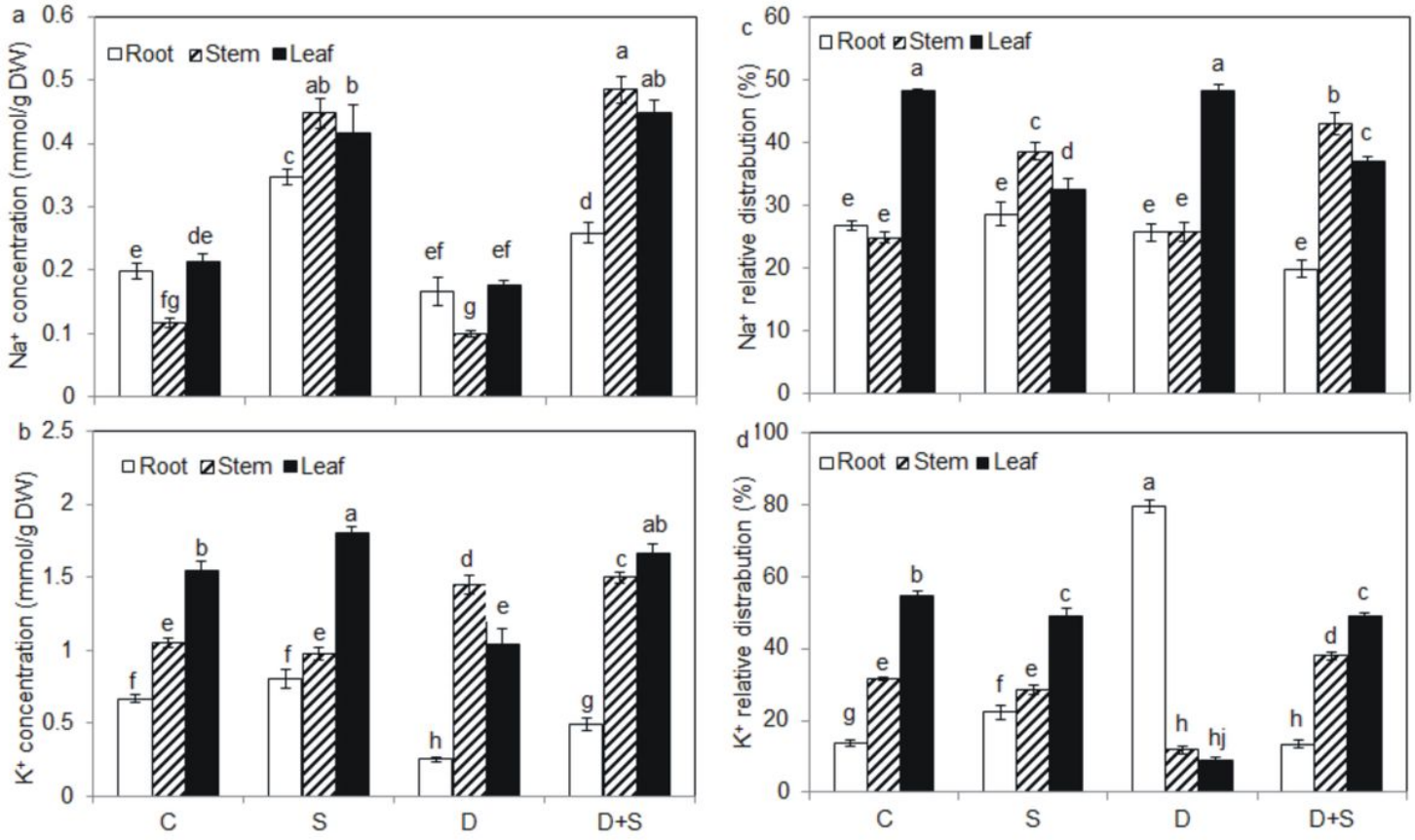

Figure 6

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 7 days.

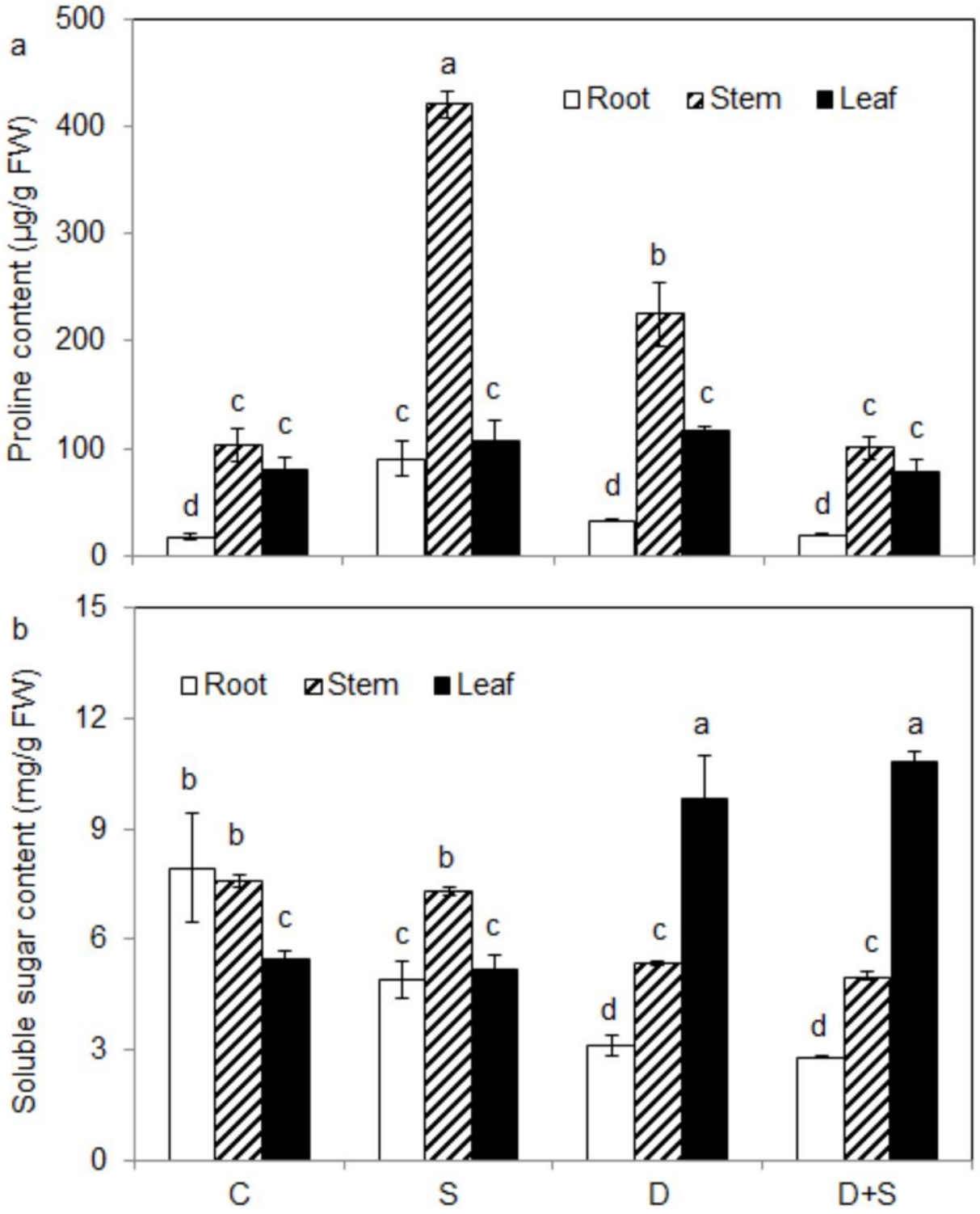

Figure 7

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 7 days.

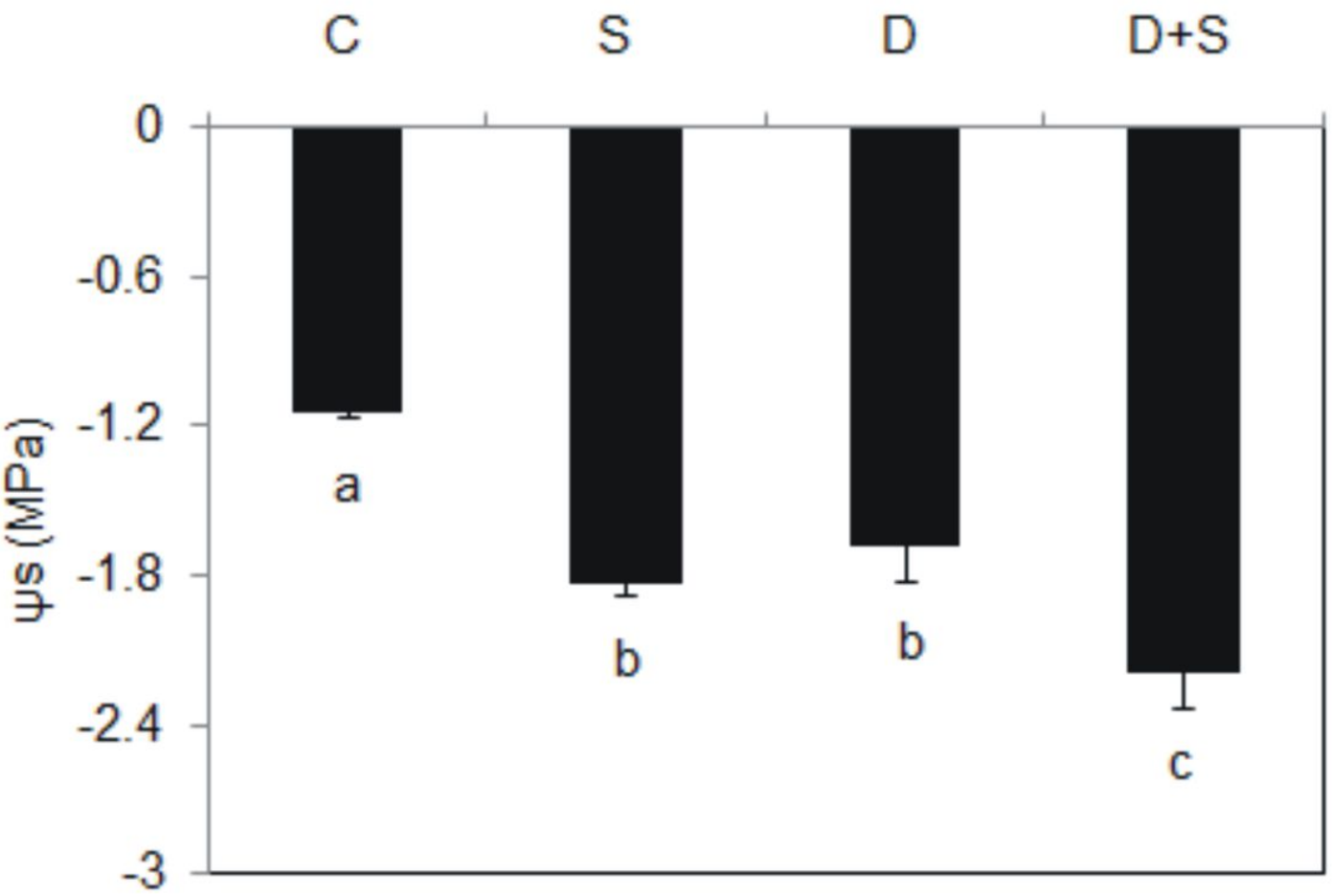

Figure 8

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 7 days.

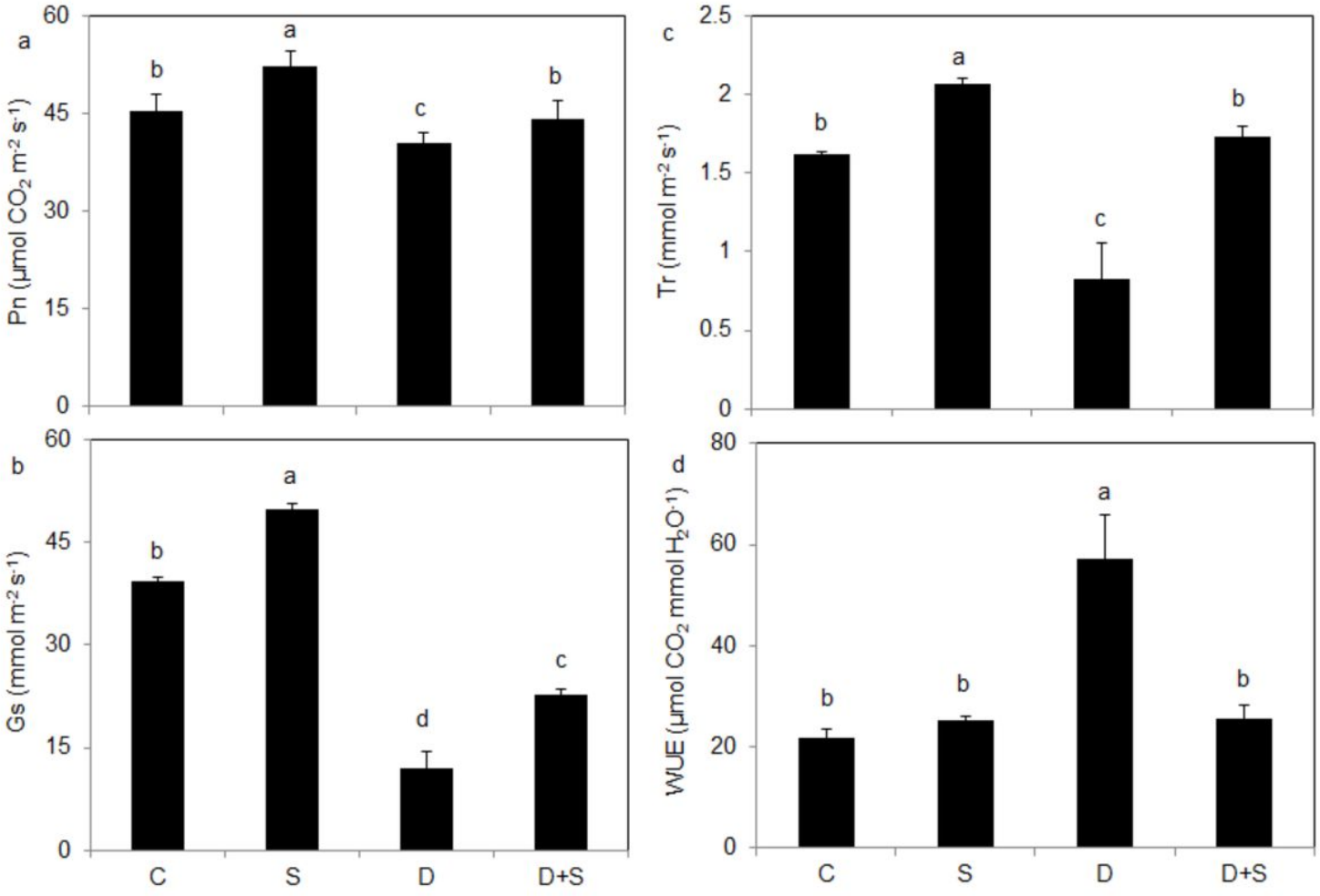

Figure 9

(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 7 days.

Supplementary Files

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