Silicon Improves Ion Homeostasis and Growth of Liquorice (*Glycyrrhiza Uralensis* Fisch. and *Glycyrrhiza Inflata* Bat.) Under Salt Stress by Reducing Plant Na⁺ Uptake

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Abstract

Silicon effectively alleviates the damage caused by salt stress in plants and can improve plant salt tolerance. However, the details of the mechanism by which silicon improves salt tolerance of liquorice are limited, and the effects of foliar application of silicon on different liquorice species under salt stress are not known. Here, the effects of foliar spray of silicon on the growth, physiological and biochemical characteristics, and ion balance of *Glycyrrhiza uralensis* Fisch. and *Glycyrrhiza inflata* Bat. were investigated. High salt stress resulted in the accumulation of a large amount of Na^+ , decreased photosynthetic pigment content, perturbed ion homeostasis, and eventually inhibited the both liquorice species growth. These effects were more pronounced in *G. uralensis*, as *G. inflata* is more salt tolerant than *G. uralensis*. Foliar spraying of silicon effectively reduced the decomposition of photosynthetic pigments, improved gas exchange parameters, and promoted photosynthesis. It also effectively inhibited lipid peroxidation and electrolyte leakage and enhanced osmotic adjustment of plants. Further, silicon application increased the K^+ concentration, reduced Na^+ absorption, transport and accumulation in the plants. The protective effects of silicon were more pronounced in *G. uralensis* than those in *G. inflata*. In conclusion, silicon reduces Na^+ absorption, improves ion balance, and alleviates the negative effects of salt stress in the two liquorice species studied, but the effect is liquorice species-dependent. These findings may inform novel strategies for protecting liquorice plants against salt stress and also provide a theoretical basis for the evaluation of salt tolerance and the scientific cultivation of liquorice.

Introduction

Soil salinisation is the main factor restricting agricultural development worldwide¹. It has been estimated that 20% of the total cultivated land and 33% of the irrigated agricultural land is affected by high salinity². Ion toxicity and osmotic stress are the main manifestations of salt damage in plants³. Ion toxicity causes plant roots and shoots to accumulate many harmful ions, such as Na^+ , under salinity stress. When the ions enter the plant cell, they destroy the water and ion balance, damage the organelle structure, and inhibit plant growth, even leading to plant death⁴. According to some studies, ion toxicity caused by Na^+ is more likely to cause irreversible damage to plant than osmotic stress⁵. Further, in plants, an antagonistic relationship exists between Na^+ and K^+ , whereby high concentrations of Na^+ directly interfere with and inhibit the absorption of K^+ by the plasma membrane⁶. The importance of K^+ in plant growth is second only to that of nitrogen, as the activation of more than 60 plant enzymes depends on K^+ ⁷. Therefore, maintaining a high K^+/Na^+ ratio under salt stress is essential for improving the salt tolerance of plants¹.

Osmotic stress produces high levels of reactive oxygen species (ROS) under salt stress. Excessive ROS destroy macromolecules, lead to lipid peroxidation, eventually inhibit plant growth⁸. ROS clearance is mainly through enzymes and non-enzymatic antioxidant defence systems. Enzymes mainly include superoxide dismutase (SOD) and catalase (CAT), while non-enzymatic antioxidants mainly include the accumulation of compatible penetrants such as proline, soluble sugar, and soluble protein⁹.

Silicon, which is usually absorbed by higher plants in the form of H_4SiO_4 ¹⁰, is the second most abundant element in the Earth's crust after oxygen¹¹. While it is considered a non-essential element for the growth and development of higher plants¹², many studies have shown that silicon plays an important role in alleviating biotic and abiotic stresses in plants¹³⁻¹⁶. Compared with other silicon application methods, foliar spray is the most effective¹⁷⁻¹⁸. Importantly, excessive application of silicon does not cause environmental pollution¹⁰.

Silicon accumulates in the cell wall of plants in the form of SiO_2 , which not only enhances the mechanical function of the cell wall, but also acts as a physical barrier to reduce water loss and improve the plant's ability to resist adverse environmental conditions¹⁹⁻²⁰. Silicon also regulates polyamine metabolism, enhances H^+ -ATPase activity in the plasma membrane and the tonoplast, promotes Na^+ excretion from the cell, and reduces Na^+ translocation and damage to plants²¹⁻²². Further, this element improves the activity of antioxidant enzymes (such as SOD and CAT), reduces electrolyte leakage (EL)²³⁻²⁴, improves cell membrane stability, increases the water content of plant tissues, and improves photosynthetic function to promote plant growth²⁵. Coskun, et al.²⁶ recently proposed the 'apoplastic obstruction hypothesis' model, which explains the mechanism of silicon-mediated plant resistance to biotic and abiotic stresses.

Glycyrrhiza uralensis Fisch. and *Glycyrrhiza inflata* Bat. are the basic liquorice plants listed in the Chinese Pharmacopoeia²⁷. Their roots and rhizomes have various pharmacological activities, such as anti-tumour²⁸, anti-viral²⁹ and anti-inflammatory³⁰. Further, liquorice plants survive in extreme environments, such as those characterised by low temperature, drought, and high salinity and alkalinity. Accordingly, the soil of the liquorice habitat is usually mildly, moderately, or severely salinized. The total salt content in the soil suitable for *G. uralensis* growth is 0-0.7% and that suitable for *G. inflata* is 0–1.3%³¹. Consequently, liquorice is an important plant resource for improving saline–alkali soil, and preventing wind and sand erosion in the arid and semi-arid regions of Northwest China³².

Xinjiang is a vast territory and the main producer of liquorice in China. However, 31.1% of the existing cultivated land therein is damaged by salinity, so that 18% of that land is severely salinized, and 33% shows medium-level salinisation, which is detrimental to the growth of liquorice³¹. According to recent studies, at salt concentration exceeding 100 mmol L^{-1} , the germination rate, germination potential, and germination index of *G. uralensis* seeds significantly decrease with a prolonged germination time. The plant height, chlorophyll content, and antioxidant enzyme activity of the seedlings are significantly reduced under these conditions, eventually leading to low biomass and low effective component accumulation in *G. uralensis*³²⁻³³.

The ameliorating effect of silicon application on plants under salt stress has been reported for many commercial crops, such as rice³⁴, wheat³⁵, sunflower³⁶, sorghum³⁷ and maize³⁸, but the mechanisms involved vary among plant species. Further, to date, the understanding of the mechanism by which silicon

improves the salt tolerance of liquorice is limited, and it is not known whether the application of silicon differently affects different liquorice species. In the current study, we focused on *G. uralensis* and *G. inflata* in Xinjiang, and explored the effects of foliar spray of silicon on the growth, physiological and biochemical characteristics, and ion balance of liquorice under salt stress, to clarify the mechanisms whereby silicon alleviates salt stress in these two liquorice species. This study provides a theoretical basis for the evaluation of salt tolerance and the scientific cultivation of liquorice.

Materials And Methods

Plant material and growth conditions.

A pot experiment was performed on the experimental field of the College of Life Science (Shihezi University, China; 86°06'E, 44°30'N). This region experiences a temperate continental climate, with a mean annual rainfall of 166 mm, a mean annual temperature of 25.6 °C, 169 frost-free days, and annual sunshine of 3769 h. *G. uralensis* seeds were collected from Wenquan County (Xinjiang, China; 80°57'E, 44°58'N), and *G. inflata* seeds were collected from Korla (Xinjiang, China; 86°12'E, 41°69'N). The composition of sandy soil was as follows: pH 7.63; silica, 30 mg kg⁻¹; soluble salt, 2.9 g kg⁻¹; total nitrogen, 0.246 g kg⁻¹; total phosphorus, 0.105 g kg⁻¹; total potassium, 5.988 g kg⁻¹; available nitrogen, 42.549 mg kg⁻¹; available phosphorus, 4.231 mg kg⁻¹; available potassium, 82.509 mg kg⁻¹; and soil organic matter, 5.826 g kg⁻¹.

Experimental design.

The treatments were arranged in a completely randomised block design with three replicates. To explore the effects of leaf application of silicon on the two liquorice species under different salt concentrations, the following treatments were chosen for detailed analysis: (1) control (CK); (2) control + 3 mM silicon (CK + Si); (3) 6 g kg⁻¹ NaCl (6S); (4) 6 g kg⁻¹ NaCl + 3 mM silicon (6S + Si); (5) 12 g kg⁻¹ NaCl (12S); and (6) 12 g kg⁻¹ NaCl + 3 mM silicon (12S + Si). Silicon was applied as a foliar spray in the form of potassium silicate (K₂SiO₃). To account for the effect of potassium ions, a corresponding amount of potassium chloride was added to the treatments without silicon.

Ten seeds of liquorice were sown per plastic pot (23.5 × 16 × 18 cm³) containing 5 kg of sandy soil. After the emergence of 2 or 3 true leaves (15 d after sowing), six liquorice seedlings of the same height and growth, robust and disease-free were retained in each pot and were watered every day. NaCl and Si treatments were conducted simultaneously 4 weeks after sowing. Except for the treatments, other management measures were consistent with local field management practices. After 100 d of treatment, the growth parameters and physiological and biochemical characteristics of each liquorice species were determined.

Determination of growth parameters of liquorice plants.

Plant height was measured using a tape measure, and stem thickness was measured using a Vernier calliper. The leaves, stems, and roots of liquorice were washed and placed in an oven at 105 °C for 30 min, dried at 75 °C for 46 h, and weighed.

Determination of gas exchange attributes.

Net photosynthesis (P_n), net transpiration rate (T_r), stomatal conductance (g_s), and intercellular CO₂ concentration (C_i) were recorded between 9.00 a.m., and 12.00 a.m. using a Li-6400 photosynthesis instrument (Li-COR, Lincoln, NE, USA). Three similarly sized healthy and fully expanded leaves from the top of the stem from plants under each treatment were analysed at a leaf temperature of 28 °C, irradiance of 1200 mmol m⁻² s⁻¹, and CO₂ concentration of 400 mmol mol⁻¹.

Determination of chlorophyll content.

The veins of fresh leaves were removed, cut into pieces, and weighed. Then, 10 mL of 80% (v/v) acetone was added to 0.5 g of the plant material and extracted in the dark until the leaves were colourless. Next, the supernatant was obtained after centrifugation at 4000 r min⁻¹ for 10 min. Then we added 4 mL of 80% acetone to 1 ml of supernatant, and the absorbance values of the extracts was determined at 470 nm, 646 nm, and 663 nm using a Shimadzu UV-1900 spectrophotometer (Kyoto, Japan). The contents of chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids were calculated by Lichtenthaler and Wellburn³⁹.

Determination of antioxidant enzyme activities.

Fresh leaves (0.5 g) were added to phosphate buffer solution (pH 7.0), ground into a homogenate, and centrifuged at 10,000 r min⁻¹ for 10 min. The supernatant was diluted to 25 mL using the same buffer solution. The samples were then stored in an ice-box for the determination of SOD and catalase CAT activities. All operations were performed at 0–4 °C. SOD activity was determined using the nitrogen blue tetrazole method⁴⁰, and CAT activity was determined using the colorimetric method⁴¹.

Determination of soluble sugar, soluble protein, and proline contents.

For soluble sugar determinations, fresh leaves (0.2 g) were ground into a homogenate in 6 mL of distilled water, and then incubated in a water bath at 100 °C for 20 min. After cooling, the samples were centrifuged at 3000 r min⁻¹ for 10 min. The extract (1.0 mL) was then mixed with 5 mL of anthrone reagent, and absorbance value at 620 nm was measured by a Shimadzu UV-1900 spectrophotometer (Kyoto, Japan). The soluble sugar content was then calculated using a standard curve of sucrose.

Soluble protein was determined using the Coomassie Brilliant Blue G-250 method⁴². Fresh leaves (0.2 g) were added to phosphate buffer solution (pH 7.0), ground into a homogenate, and centrifuged at 5,000 r min⁻¹ for 10 min. Coomassie Brilliant Blue G-250 reagent was added to 1 mL of the supernatant and the

absorbance was read at 595 nm. The protein content was calculated using a standard curve of bovine serum albumin.

For proline content determinations, fresh liquorice leaves (0.5 g) were placed in 5 mL of 3% sulfosalicylic acid solution, and centrifuged at 5000 r min^{-1} for 10 min. The supernatant (2 mL) was added to 2 mL of glacial acetic acid and 2 mL of acidic ninhydrin reagent, and the mixture was heated in a boiling water bath for 30 min. After cooling, 4 mL of toluene was added, the samples were shaken for 30 s, and centrifuged at 5000 r min^{-1} for 10 min. Using toluene as a blank control, sample absorbance was measured at 520 nm by a Shimadzu UV-1900 spectrophotometer (Kyoto, Japan), and the proline content was calculated using a standard curve⁴³.

Determination of lipid peroxidation and EL.

Lipid peroxidation was determined by measuring the concentration of malondialdehyde (MDA). Liquorice leaves (0.5 g) were homogenised in 5 mL of 0.1% (w/v) trichloroacetic acid solution. After centrifugation at $10,000 \text{ r min}^{-1}$ for 10 min, the supernatant was mixed with 0.5% (w/v) trichloroacetic acid solution, and incubated in a water bath at 100°C for 2 min. The samples were then centrifuged at $10,000 \text{ r min}^{-1}$ for 10 min. The absorbance of sample supernatant at 600 nm, 532 nm, and 450 nm was determined by a Shimadzu UV-1900 spectrophotometer (Kyoto, Japan), and the MDA content was calculated as described by Heath and Packer⁴⁴.

To determine EL, fresh liquorice leaves were cleaned, cut into 2-cm pieces, and placed in a test tube containing 10 mL of distilled water. The samples were shaken on an oscillating table at 25°C for 24 h to determine electrical conductivity 1 (EC_1). The test tube was then placed in a water bath at 100°C for 30 min to determine electrical conductivity 2 (EC_2)⁴⁵. EL was calculated using the following formula: $\text{EL} (\%) = \text{EC}_1 / \text{EC}_2 \times 100$.

Determination of Na^+ and K^+ concentrations, transfer, and absorption.

Dry plant samples (0.1 g; various portions, as specified) were digested in a mixture of nitric acid and perchloric acid (volume ratio 2:1). The leaf and root concentrations of Na^+ and K^+ were measured using a flame photometer (FP640, Shanghai Precision Scientific Instrument Co., Ltd., Shanghai, China). Sodium uptake at the liquorice root surface, and ion (Na^+ and K^+) translocation from the root to the shoot were calculated using the methods described by Yan, et al.⁴⁶ and Ali, et al.³⁸, respectively.

Statistical analysis.

The data were analysed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was used to test the effect of different treatments on each index in the same liquorice species ($P < 0.05$), and Duncan's multiple comparisons test was used to determine significant differences between different treatments of the same liquorice. When the variances were not uniform, the data were

converted to logarithm or square-root values before analysis. All figures were prepared using Origin 9 (OriginLab, Northampton, MA, USA).

Results

Plant growth.

Salt stress significantly inhibited the growth of the two liquorice species. The inhibitory effect on plant growth was more pronounced in *G. uralensis* than that in *G. inflata*. In particular, under the 12S treatment, the plant height, root dry weight, and shoot dry weight of *G. uralensis* decreased by 55%, 88%, and 84%, respectively, compared with the corresponding CK values; for *G. inflata*, these indicators decreased by 41%, 86%, and 83%, respectively, in the same comparison. In addition, under the 12S treatment, the plant height, root dry weight, and shoot dry weight of *G. inflata* were 57%, 39%, and 42% higher, respectively, than those of *G. uralensis* (Table 1). This inhibitory effect was alleviated by silicon (Table 1). Under the 12S + Si treatment, the plant height, root dry weight, and shoot dry weight of *G. uralensis* increased by 74%, 81%, and 74%, respectively, compared with the corresponding 12S values; for *G. inflata*, these indicators increased by 19%, 48%, and 49%, respectively, in the same comparison. Furthermore, the application of silicon with the CK treatment increased the growth indicators of the two liquorice species.

Gas exchange attributes.

The P_n , g_s , T_r and C_i parameters of liquorice plants were generally negatively affected (Fig. 1). Under the 6S treatment, the P_n , g_s and T_r of *G. uralensis* were reduced by 54%, 64%, and 62%, respectively, compared with the corresponding CK values; for *G. inflata*, the corresponding parameters were reduced by 26%, 22%, and 21%, respectively, in the same comparison. Under the 12S treatment, the P_n , g_s , T_r and C_i of *G. inflata* were decreased by 70%, 76%, 77%, and 20%, respectively, compared with the corresponding CK values; for *G. uralensis*, the corresponding parameters were also significantly decreased, except for C_i , which was increased by 41% in the same comparison. In addition, the P_n , g_s , and T_r of *G. inflata* were 148%, 277%, and 267% higher than those of *G. uralensis* under the 6S treatment, respectively. Nevertheless, compared with the 6S values, the 6S + Si treatment increased the P_n , g_s , T_r and C_i of *G. uralensis* by 78%, 59%, 55%, and 33%, respectively; it increased the corresponding parameters of *G. inflata* by 29%, 15%, 15%, and 15%, respectively. By contrast, the application of silicon with the 12S treatment reduced the C_i of *G. uralensis* (16%).

Chlorophyll concentration.

Compared with the CK values, 12S treatment significantly reduced the chlorophyll content of the two liquorice species (Fig. 2). Under the 12S treatment, the chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoid contents of *G. uralensis* were reduced by 65%, 53%, 63%, and 65%, respectively, compared with the corresponding CK values; for *G. inflata*, the corresponding parameters were reduced by 51%, 53%, 52%, and 57%, respectively, in the same comparison. Further, under the 12S treatment, the chlorophyll *a*,

chlorophyll *b*, total chlorophyll, and carotenoid contents of *G. inflata* were 44%, 22%, 40%, and 24% higher than those of *G. uralensis*, respectively. The application of silicon with the 12S treatment resulted in an increase in the chlorophyll *a* (49% and 30%), chlorophyll *b* (33% and 5%), total chlorophyll (46% and 26%), and carotenoid (66% and 50%) contents of *G. uralensis* and *G. inflata*, accordingly. Further, the application of silicon with the CK treatment also increased the chlorophyll content of the two liquorice species.

Antioxidant enzyme activities.

With the increasing salt concentration, the activities of SOD and CAT in *G. inflata* were significantly increased, while those in *G. uralensis* increased initially and then decreased (Fig. 3). Compared with the corresponding CK values, the SOD and CAT activity in *G. uralensis* increased by 28% and 128%, respectively, under the 6S treatment; the corresponding parameters of *G. inflata* increased by 9% and 62%, respectively, in the same comparison. Compared with the 6S values, the SOD and CAT activities in *G. inflata* increased by 14% and 64%, respectively, under the 12S treatment; the corresponding parameters in *G. uralensis* decreased by 12% and 22%, respectively, in the same comparison. In addition, under the 12S treatment, the SOD and CAT activities in *G. inflata* were 20% and 69% higher than those of *G. uralensis*, respectively. Compared with the salt treatment, the application of silicon significantly increased the SOD and CAT activities in the two liquorice species ($P < 0.05$).

Soluble sugar, soluble protein, and proline contents.

The soluble sugar, soluble protein, and proline contents in the leaves of *G. uralensis* first increased and then decreased with an increasing salt concentration (Fig. 4). Compared with the corresponding CK values, the 6S treatment increased the soluble sugar content of *G. uralensis* and *G. inflata* by 101% and 61%, soluble protein content by 97% and 38%, and proline content by 107% and 99%, respectively. However, under the 12S treatment, the soluble sugar, soluble protein, and proline contents of *G. uralensis* decreased by 30%, 17%, and 20%, respectively, compared with the corresponding 6S values. In addition, under the 12S treatment, the soluble protein and proline contents of *G. inflata* were 47% and 29% higher than those of *G. uralensis*, respectively. Compared with the absence of silicon, application of silicon significantly reduced the proline content of the two liquorice species ($P < 0.05$), and increased the soluble sugar and soluble protein contents. Further, the application of silicon with the 12S treatment resulted in a 19% decrease of the proline content of *G. uralensis*, and a 53% and 20% increase in the soluble sugar and soluble protein contents, respectively, in this species.

Lipid peroxidation and EL.

Salt treatment significantly increased the MDA and EL of the two liquorice species ($P < 0.05$; Fig. 5). Under the 6S and 12S treatments, the MDA of *G. uralensis* increased by 82% and 161%, respectively, and EL increased by 49% and 141%, respectively, compared with the corresponding CK values. For the same conditions, the MDA of *G. inflata* increased by 52% and 118%, respectively, and EL increased by 25% and 68%, respectively, compared with the corresponding CK values. Further, under the 12S treatment, the MDA

and EL of *G. uralensis* were 36% and 48% higher than those of *G. inflata*, respectively. Furthermore, the application of silicon with the 12S treatment resulted in the reduction of the MDA of *G. uralensis* by 33% and that of EL by 16%; for *G. inflata*, the MDA was reduced by 19% and EL was reduced by 22%.

Accumulation of Na⁺ and K⁺ in the root and leaf.

The accumulation of Na⁺ in the root and leaf of the two liquorice species increased significantly with an increasing salt concentration, with a simultaneous significant decrease in the accumulation of K⁺ (Fig. 6). Under the 12S treatment, Na⁺ levels in the root and leaf of *G. uralensis* increased by 109% and 265%, respectively, while K⁺ levels decreased by 31% and 58%, respectively, compared with the corresponding CK values. Under the same conditions, Na⁺ levels in *G. inflata* increased by 181% and 246%, accordingly, and K⁺ levels decreased by 37% and 37%, accordingly, compared with the corresponding CK values. In addition, under the 6S treatment, the Na⁺ and K⁺ levels in the root, and K⁺ levels in the leaf of *G. inflata* were 44%, 27%, and 50% higher, respectively, than those in *G. uralensis*. However, without silicon application (the CK, 6S, and 12S treatments), the Na⁺ accumulation in the leaf of *G. uralensis* was 49%, 35%, and 57% higher than that in *G. inflata*, respectively. The application of silicon with the 6S treatment significantly reduced Na⁺ accumulation in the leaf of *G. uralensis* (27%), and increased K⁺ accumulation in the leaf of this species (28%).

Ion translocation and uptake.

Salt treatment significantly increased Na⁺ transport (root–leaf) and Na⁺ uptake on the root surface and decreased the K⁺/Na⁺ ratio in both liquorice species (Fig. 7). Under the 6S and 12S treatments, compared with the corresponding CK values, Na⁺ transport in *G. uralensis* increased by 56% and 73%, respectively; Na⁺ uptake on the root surface increased by 1.81- and 22-fold, respectively; K⁺ transport (root–leaf) decreased by 30% and 40%, respectively; and the K⁺/Na⁺ ratio decreased by 59% and 81%, respectively. In addition, the K⁺ transport and K⁺/Na⁺ ratio in *G. inflata* were 18% and 72% higher than those of *G. uralensis* under the 6S treatment, respectively, while the Na⁺ transport and Na⁺ uptake on the root surface in *G. uralensis* were 95% and 85% higher than those of *G. inflata*, respectively. While the application of silicon decreased the Na⁺ transport, and increased the K⁺ transport and K⁺/Na⁺ ratio, it only significantly affected the Na⁺ uptake on the root surface under high salt concentration, decreasing the Na⁺ uptake by 52% in *G. uralensis* and 46% in *G. inflata*.

Discussion

Salt stress is one of the main adverse environmental conditions faced by plants¹. It inhibits plant growth and reduces plant yields, mainly because of ion toxicity and osmotic stress, and even leads to plant death⁴⁷. According to many studies, silicon can be used to effectively alleviate the harmful effects of salt stress on plants and promote plant growth in saline environments⁴⁸⁻⁴⁹. In the current study, we analysed the growth indicators, gas exchange parameters, chlorophyll content, osmotic regulators, membrane

damage indicators, antioxidant enzyme activity, and ion balance in two liquorice species. We showed that silicon application alleviated the negative effects of salt stress to a certain extent with the extent of the mitigating effect depending on the liquorice species. Although some investigations on the effect of silicon on the growth of liquorice under salt stress have been reported⁵⁰⁻⁵², this is the first report demonstrating that foliar spray with silicon enhances the salt tolerance of *G. uralensis* and *G. inflata*.

Both the medium-salt (6S) and high-salt (12S) treatments significantly reduced the biomass of the two liquorice species, with the lowest values obtained at high salt concentrations (Table 1). Under the same salt treatment, the growth of *G. inflata* was better than that of *G. uralensis*, indicating that *G. inflata* is more salt tolerant than *G. uralensis*. The increase in Na^+ under salt treatment hindered the photosynthesis by reducing photosynthetic pigment levels and further reducing the photosynthetic rate¹⁷, thereby inhibiting plant growth and reducing the biomass (Table 1). Consistent with the findings for mung bean⁸ and cucumber⁵³, salt stress reduced chlorophyll concentration and carotenoid levels in the leaves of the two liquorice species (Fig. 2). In addition, under high salt stress, the P_n , T_r and g_s of the two liquorice species were also significantly reduced. However, the C_i of *G. uralensis* was significantly increased under high salt stress, indicating that when the salt concentration exceeds the tolerance limit of a plant, the stomatal protection mechanism is damaged, and non-stomatal factors play a major role in photosynthesis⁵⁴. Further, foliar spray with silicon significantly increased chlorophyll and carotenoid levels in plant leaves under salt stress^{17, 55}. These observations suggest that silicon promotes the biosynthesis of photosynthetic pigments under salt stress. This may be attributed to the notion that silicon alleviates the damage to chloroplast under salt stress⁵¹, enhances RuBisCo protein expression, and promotes the synthesis of chlorophyll and carotenoids⁴⁸. Similarly, foliar spraying with silicon alleviated the negative effects of salt stress on gas exchange parameters in the two liquorice species (Fig. 1). The promoting effect was different for the different liquorice species. In the current study, the effect of silicon was more pronounced for *G. uralensis* (Fig. 2).

Under salt stress, the damage to plant cell and the decrease in photosynthesis are mainly caused by excessive absorption and accumulation of Na^+ ^{17, 35}. Under high salt stress, the ability of the root system of *G. uralensis* to intercept Na^+ was significantly decreased, and the accumulation of Na^+ in the root and leaf increased, which led to disturbance in the synthesis of photosynthetic pigments⁵⁶, a decrease in the net photosynthetic rate, and a significant reduction of plant biomass (Figs 1a, 2, and Table 1). This indicates that the ability of the root to intercept Na^+ was limited under these conditions. In high-salt environments, the accumulation of Na^+ was increased in roots and the transport ability of Na^+ was enhanced toward the shoot, thus increasing Na^+ accumulation in the leaf and damaging mesophyll cells⁵⁷. When the Na^+ concentration in the leaf exceeds 1.3 mg g^{-1} , the chloroplast structure is destroyed, chlorophyll degradation accelerates, and photosynthesis is inhibited¹⁴. In addition, the transportation and absorption of Na^+ on the root surface of *G. uralensis* were 1.95 and 1.84 times higher than those in *G. inflata* under medium-salinity conditions. This indicates that the ability of *G. inflata* roots to intercept Na^+ is greater than that of *G. uralensis*, which might account for the relatively high salt tolerance of *G. inflata*.

K⁺ is the key regulator of cell homeostasis⁷ and plays an important role in inducing cell elongation, maintaining osmotic regulation in plants, and promoting photosynthesis⁵⁸⁻⁵⁹. Therefore, excessive Na⁺ levels often lead to K⁺ deficiency. Al-Huqail, et al.⁴⁸ reported that high salt concentrations significantly increase the Na⁺ content in maize and greatly reduce the K⁺ content, resulting in an increased Na⁺/K⁺ ratio and plant growth inhibition. In *G. uralensis*, compared with the application of silicon in the soil, foliar spray alone effectively increased K⁺ levels and reduced Na⁺ levels⁵⁰. This may be associated with the upregulation of genes involved in potassium uptake (*OsAKT1* and *OsSHAK1*) and xylem load (*OsSKOR*) by silicon³⁴, which promote the increase of K⁺ transport and increase the H⁺-ATPase activity, forming a mechanical barrier to reduce Na⁺ transport⁶⁰. Therefore, we believe that the capacity of silicon to enhance K⁺-selective transport and increase the K⁺/Na⁺ ratio might be one of the main mechanisms to improve plant growth and productivity under salt stress, which is in agreement with the findings of previous reports⁶¹. In addition, silicon not only reduced the Na⁺ transfer and damage to the shoot under salt stress, but also decreased the Na⁺ absorption by the root in the two liquorice species, which is consistent with the previous findings in rice⁶² and chickpea⁶³. There are at least two possible explanations: (1) silicon deposition on the root cell wall enhances the mechanical strength of the cell wall, thus reducing Na⁺ absorption¹¹; and (2) silicon decreases Na⁺ accumulation in the root apex and cortex by upregulating the expression of *ZmSOS1* and *ZmSOS2* transporters⁶⁴. Taken together, the reduction of Na⁺ uptake and transport may be a potential mechanism of silicon-mediated enhancement of salt tolerance of liquorice.

In high-salt environments, plants accumulate high levels of Na⁺. This leads to excessive accumulation of ROS, perturbing the balance between their production and elimination, and resulting in cellular oxidative damage⁸. MDA and EL are the main indexes used to evaluate the severity of oxidative cell damage⁶². MDA is an oxidation product of membrane lipids and accumulates when plants are subjected to oxidative stress. Na⁺ accumulation results in the production of high levels of ROS, which destroy the cell membrane structure, leading to an increased MDA content and EL. For instance, salt-induced oxidative damage leads to the rupturing of the plasma membrane in maize, leading to lipid peroxidation³⁸. We here showed that both medium and high-salt treatments increased the MDA content and EL in the two liquorice species, especially in *G. uralensis* (Fig. 5), indicating major damage of the cell membrane in these liquorice species. Of the two species, the damage was more pronounced in *G. uralensis*. However, the MDA content and EL in the two liquorice species decreased after foliar spraying with silicon. These observations suggest that silicon may counteract the membrane damage caused by salt stress in liquorice. In addition, silicon can alleviate reactive oxygen damage by maintaining membrane integrity and activating the antioxidant defence system⁶⁵. In the current study, silicon enhanced SOD and CAT activities in the two liquorice species under salt stress (Fig. 3), which was consistent with the observations in mung bean⁸ and mustard⁶⁶. This effect may be related to the upregulation of genes encoding antioxidant enzymes⁶⁷. We also observed that silicon application enhanced the SOD and CAT activities in *G. uralensis* to a greater extent than those in *G. inflata*. Therefore,

the regulation of the plant antioxidant system by silicon upon salt stress is different for the two different liquorice species.

The alleviating effect of silicon described above was not only associated with the increased antioxidant enzyme activity, but also with the accumulation of osmotic regulators in plants⁴⁸. Accordingly, upon salt stress, plants produce and accumulate compatible organic solutes as a means of osmotic adjustment, to maintain the normal physiological and biochemical characteristics of intracellular water, and to impede the damage to the cell membrane^{54, 68}. By contrast, under high-salt stress, the soluble sugar, soluble protein, and proline contents in the leaf of *G. uralensis* were decreased, indicating that high-salt stress greatly affected the ability of *G. uralensis* to respond to the adverse conditions, and inhibited plant growth⁵⁰. Proline is a key osmoprotectant and can reduce the damage caused by ROS, reduce lipid peroxidation, and protect protein and membrane structures⁵⁴. In the current study, the application of silicon reduced the proline content in liquorice, which was consistent with the findings in sunflower³⁶ and pelargonium²⁴. In the presence of silicon, proline was degraded, and used as a source of carbon and nitrogen in plants recovering from stress, as well as a membrane stabiliser and a free-radical scavenger, to reduce lipid peroxidation and EL^{15, 18}. Therefore, reduction of membrane damage may be another mechanism whereby silicon improves the salt tolerance of plants. However, the mechanism for obtaining silicon to improve plant salt tolerance is a complex process. In addition to many physiological and biochemical aspects, further research is required on protein and gene expression analysis.

Conclusion

High salinity greatly inhibited the growth and development of the two liquorice species studied herein. Under salt stress, the morphological characteristics (plant height, stem diameter, and biomass), photosynthetic characteristics (gas exchange parameters and photosynthetic pigments), antioxidant enzyme activities (SOD and CAT), ion homeostasis (K^+ and Na^+ transport, and K^+/Na^+ ratio), and osmotic adjustment (e.g. proline) in *G. inflata* were better than those in *G. uralensis*. These observations indicate that *G. inflata* is more salt tolerant than *G. uralensis*. Foliar application of silicon effectively reduced the absorption of Na^+ , improved ion balance, alleviated membrane damage, and promoted the growth of the two liquorice species. Further, the response of *G. uralensis* to silicon was more pronounced than that of *G. inflata*, indicating that the protective effect of silicon is different for different liquorice species. This study provides a theoretical basis for the evaluation of salt tolerance in and scientific artificial cultivation of liquorice in the future.

Declarations

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Conflict of interest All authors declared no conflict of interest.

Authors' contributions SZH and PXZ conceived the study and participated in its design. SZH, DXX, and CXJ contributed samples and carried out the experiments. SZH, CMX, and PXZ analysed the data. SZH, PXZ and WSM drafted the manuscript. The authors have approved of publication, and there is no conflict of interest. All the authors equally approve of publication.

Compliance statement for experimental materials Liquorice is a widely distributed species in China. Xinjiang is the main producing area of liquorice in China. Seed of *G. uralensis* was collected from Wenquan County (Xinjiang, China; 80°57'E, 44°58'N), and that of *G. inflata* was collected from Korla (Xinjiang, China; 86°12'E, 41°69'N). A pot experiment was performed on the experimental field of the College of Life Science (Shihezi University, China; 86°06'E, 44°30'N). Therefore, all operations comply with relevant institutional, national, and international guidelines and legislation.

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Tables

Table 1 Effects of silicon on the growth of *Glycyrrhiza uralensis* and *G. inflata* under salt stress

Cultivar	Treatment	Plant height	Stem diameter	Root DW	Shoot DW
		(cm)	(mm)	(g)	(g)
<i>G. uralensis</i>	CK	25.53 ± 0.88b	2.06 ± 0.11b	3.09 ± 0.10b	2.34 ± 0.09a
	6S	18.6 ± 0.72c	1.75 ± 0.06bc	1.77 ± 0.05c	1.67 ± 0.03b
	12S	11.43 ± 0.06d	1.48 ± 0.07c	0.36 ± 0.01d	0.38 ± 0.01d
	CK + Si	33.20 ± 1.42a	2.95 ± 0.18a	3.85 ± 0.40a	2.51 ± 0.11a
	6S + Si	26.30 ± 0.06b	2.15 ± 0.12b	2.16 ± 0.08c	1.74 ± 0.05b
	12S + Si	19.87 ± 0.34c	1.89 ± 0.16b	0.66 ± 0.02d	0.66 ± 0.02c
<i>G. inflata</i>	CK	30.47 ± 0.78b	2.31 ± 0.04b	3.54 ± 0.04b	3.20 ± 0.09a
	6S	22.33 ± 0.94d	1.97 ± 0.02c	2.80 ± 0.05d	2.26 ± 0.03b
	12S	18.00 ± 1.26d	1.67 ± 0.02d	0.51 ± 0.02f	0.53 ± 0.01c
	CK + Si	38.97 ± 0.47a	2.87 ± 0.21a	4.24 ± 0.07a	3.42 ± 0.21a
	6S + Si	26.83 ± 1.19c	2.45 ± 0.01b	3.40 ± 0.02c	2.34 ± 0.22b
	12S + Si	21.33 ± 0.93d	1.84 ± 0.05cd	0.75 ± 0.02e	0.80 ± 0.01c

Data are presented as the mean ± SE (n = 3). Different letters indicate significant differences between the values for the same index for the same liquorice species under different treatments (P < 0.05). DW, dry weight.

Figures

Figure 1

Effects of salinity and silicon on net photosynthetic rate (P_n , a), stomatal conductance (g_s , b), transpiration rate (Tr , c), and intercellular CO_2 concentration (C_i , d) in the two liquorice species. Data are presented as the mean $\pm$ SE ($n = 3$). Different letters indicate significant differences between the values for one index for the same liquorice species under different treatments ($P < 0.05$)

Figure 2

Effects of salinity and silicon on chlorophyll a (a), chlorophyll b (b), total chlorophyll (c), and carotenoid content (d) in the two liquorice species. Data are presented as the mean $\pm$ SE (n = 3). Different letters indicate significant differences between the values for one index for the same liquorice species under different treatments ($P < 0.05$)

Figure 3

Effects of salinity and silicon on superoxide dismutase activity (SOD, a) and catalase activity (CAT, b) in the two liquorice species. Data are presented as the mean $\pm$ SE (n = 3). Different letters indicate significant differences between the values for one index for the same liquorice species under different treatments (P < 0.05)

Figure 4

Effects of salinity and silicon on soluble sugar content (a), soluble protein content (b), and proline content (c) in the two liquorice species. Data are presented as the mean $\pm$ SE ($n = 3$). Different letters indicate significant differences between the values for one index for the same liquorice species under different treatments ($P < 0.05$)

Figure 5

Effects of salinity and silicon on malondialdehyde content (MDA, a) and electrolyte leakage (EL, b) in the two liquorice species. Data are presented as the mean $\pm$ SE ($n = 3$). Different letters indicate significant differences between the values for one index for the same liquorice species under different treatments ($P < 0.05$)

Figure 6

Effects of salinity and silicon on Na⁺ concentration in the root (a) and leaf (b), and K⁺ concentration in the root (c) and leaf (d) in the two liquorice species. Data are presented as the mean $\pm$ SE (n = 3). Different letters indicate significant differences between the values for one index for the same liquorice species under different treatments (P < 0.05)

Figure 7

Effects of salinity and silicon on Na⁺ translocation (a), K⁺ translocation (b), K⁺/Na⁺ ratio (c), and Na⁺ uptake at root surface (d) in the two liquorice species. Data are presented as the mean $\pm$ SE (n = 3). Different letters indicate significant differences between the values for one index for the same liquorice species under different treatments (P < 0.05)