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Silicon alleviates salt and drought stress of *Glycyrrhiza uralensis* plants by improving photosynthesis and water status

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Abstract

Silicon has been widely reported to have a beneficial effect on improving plant tolerance to biotic and abiotic stresses. However, the mechanisms of Si in mediating responses to simultaneous salt and drought stresses are still poorly understood. *Glycyrrhiza uralensis* Fisch. is classified as a non-Si accumulator and suffered from salt and drought stresses. In this study, we investigated the long-term application of Si on Si content in *G. uralensis* roots, stems and leaves, leaf anatomy, ultrastructure, chlorophyll (Chl) content, gas exchange characteristics, relative water content, and growth of two-year-old plants under different salt and drought stresses. Silicon application resulted in a higher Si uptake in *G. uralensis* roots and more Si accumulation in leaves (especially deposition of Si on cell walls), and Si counteracted the adverse effects induced by salt and drought stresses on the leaf anatomy and ultrastructure. In plants treated with Si, a higher chlorophyll content, net photosynthetic rate and relative water content led to a higher growth rate and dry mass under salt and drought stresses compared with corresponding non-Si treated plants.

Additional key words: chlorophyll, leaf ultrastructure, net photosynthetic rate, relative water content, stomatal conductance, transpiration rate, water use efficiency.

Introduction

Salt and drought stresses are two most critical abiotic threats that affect plant growth and production worldwide (Rizwan *et al.* 2015) and are becoming more serious concerns as world desertification increases (Janz *et al.* 2012). Furthermore, salinization and desertification are rapidly increasing on a global scale and currently affect more than 10 % of arable land which results in more than 50 % reduction in average yields of major crops (Li *et al.* 2010, Ouzounidou *et al.* 2014). Generally, high salt concentration in the soil induces high osmotic potential of soil which results in water deficit within plants firstly, and drought stress usually occurred in the field together with salt stress would further enhance the moisture loss (Farooq *et al.* 2009).

Water deficit in plants usually reduces leaf expansion,

impaired photosynthetic machinery, induces premature leaf senescence leading to reduction in dry matter accumulation (Bijanazadeh and Emam 2010, Aminian *et al.* 2011). In addition, plant tolerance to salt and drought stress is mainly associated with the maintenance of plant water status either by reducing water loss through decreasing transpiration or improving plant root capacity to extract more water through osmotic adjustment. Above all, salt and drought stresses mainly affect the photosynthetic characteristics and the water status of plants (Maghsoudi and Maghsoudi 2008, Shahid *et al.* 2014). They induce the decrease of net photosynthetic rate and chlorophyll (Chl) content, relative water content (RWC), and water use efficiency (WUE) (Peng *et al.* 2006, Jaleel *et al.* 2009, Ouzounidou *et al.* 2014, Abbas *et al.* 2015).

Glycyrrhiza uralensis Fisch. is a very popular herbal plant widely appreciated as Chinese medicine. Moreover,

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Abbreviations: c_i - intercellular CO₂ concentration; Chl - chlorophyll; DAT - days after treatment; E - transpiration rate; FWC - field water content; g_s - stomatal conductance; P_N - net photosynthetic rate; RWC - relative water content; WUE - water use efficiency.

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it is commonly used as additives in tobacco and food worldwide (Egamberdieva *et al.* 2016). It is also very important for wind hampering, sand fixing, and soil formation in arid and semiarid regions (Li and Wang 2002). *G. uralensis* is often cultivated in arid, semiarid, and salt-affected regions. Therefore, improving the tolerance of commercial cultivars of *G. uralensis* to salt and drought stress has become a key target for sustainable development.

At present, there are various physical and chemical ways for improving the plant responses to abiotic stresses. Exogenous Si is one of the important methods. Its uptake by plants has been adopted as an effective strategy for alleviating the negative effects of salt and drought stress and improving salt and drought tolerance of plants (Zhu and Gong 2014). And Si may be a “quasi-essential” element for plants has been increasingly recognized (Pilon-Smits *et al.* 2009). However, its role in plant biology has not been understood clearly. In most cases, the favourable effects of Si seem to originate from reinforcement of the cell walls due to the deposition of Si in the form of amorphous silica and opal phytoliths (Epstein 1999). Meanwhile, its active involvement in a multitude of physiological and metabolic processes is also evident (Moussa 2006). Under drought stress, Si acts not only as a physical or mechanical barrier to minimize transpiration losses but also participates in many processes which improve drought tolerance (Hattori *et al.* 2005). Feng *et al.* (2010) found that Si protected the photosynthetic apparatus by protection of thylakoid membranes and improved pigment content, resulting in higher photosynthetic rate in cadmium stressed cucumber plants. Previous studies by Hattori *et al.* (2008) and Chen *et al.* (2011b) showed that the Si-induced alleviation of osmotic stress in cucumber and drought stress in rice is independent of leaf water status, but Wang *et al.* (2015) found that Si enhances the salt tolerance of cucumber by improving water balance. Above mentioned researchers studied the effects of Si on photosynthesis and water status of plants under every stress individually and in a short term, and they focused on grain crops, vegetables, and fruit trees. Nevertheless, salt and drought stresses usually occurred together and in a long term; the mechanisms of plant tolerance to salt and drought stress are physiologically connected and overlapped as mentioned above.

Our previous study addressed that Si can promote the growth of *G. uralensis* seedlings under salt and drought stresses by regulating antioxidant metabolism, osmoprotectants, ion balance, and so on (Li *et al.* 2016, Zhang *et al.* 2017). These observations are consistent with many other non Si-accumulating plants (Katz 2014, Zhu and Gong 2014). In the present study, we studied the influence of Si on long term salt and drought stressed *G. uralensis* plants by analyzing the changes in Si content, leaf anatomical structure, Chl content, gas exchange parameters, water status, growth, and the dry matter accumulation. We hypothesized that Si application can increase Si content in roots and leaves, keep the integrity of leaves, increase Chl content, net photosynthetic rate and WUE, and thereby enhance growth and dry matter accumulation of *G. uralensis* under salt and drought stresses.

Materials and methods

Plants and experimental design: One-year-old *Glycyrrhiza uralensis* Fisch. plants were collected from Yanchi County, Ningxia Hui Autonomous Region, China. Pot experiment was conducted at Ningxia Medical University during growing season of 2016. Average day/night temperatures were 28/16 °C, a 14-h photoperiod, and natural irradiance. On April 18, 2016, *G. uralensis* plants were transplanted into pots containing sandy loam soil (pH 7.4, containing [g kg⁻¹]: 3.28 organic matter, 0.87 total nitrogen, 2.5 total phosphorus, 0.17 instant potassium, 0.021 instant phosphorus, 0.053 alkaline solution nitrogen, 0.039 silica, and 2.3 NaCl).

Treatments were arranged in a randomized experimental design and included control (CK) containing 2.3 g(NaCl) kg⁻¹(dry soil), salt stress solely (S) containing 6 g(NaCl) kg⁻¹(dry soil) + 60 - 65 % field water content (FWC), salt stress combined with drought stress (SD1) containing 6 g(NaCl) kg⁻¹(dry soil) + 45 - 50 % FWC or (SD2) 6 g(NaCl) kg⁻¹(dry soil) + 30 - 35 % FWC with or without 0.1 g(SiO₂) kg⁻¹(dry soil). Silicon was applied as K₂SiO₃ and a corresponding amount of K (as KCl) was also added to CK to compensate the K content. Drought was imposed by withholding water. The volume of water required to achieve the pot field capacity represented the consumption of water during the previous day. There were three replications per treatment and each replication comprised three pots, each pot included four plants. All pots were randomly arranged and periodically rotated to minimize the effects of environmental heterogeneity. At 70 and 110 d after drought treatment, *G. uralensis* plants were collected for evaluation of various physiological and biochemical parameters and growth parameters. Gas exchange parameters were measured at 90 d after treatment.

Determination of Si content: Si content of *G. uralensis* plants was determined colorimetrically by the molybdate method (Zhang *et al.* 2017). About 0.01 g of finely dry plant samples were placed in 100-cm³ polyethylene tubes previously washed with 0.1 M NaOH and rinsed with demineralised water. NaOH (3 cm³, 50 %) was added to each tube and the suspensions autoclaved for 1 h to oxidize any organic matter present and soluble Si. To develop the colour, 35 cm³ of 200 cm³ dm⁻³ acetic acid was added to 10 cm³ of ammonium molybdate solution, 5 cm³ of 200 cm³ dm⁻³ tartaric acid, 1 cm³ of reducing solution and excess of 200 cm³ dm⁻³ acetic acid to bring the volume to 50 cm³. After mixing, the absorbance was read at 630 nm with a spectrophotometer. Plastic ware rinsed with 0.1 M NaOH for removing silica was used throughout the assay. Different amounts of silicic acid were included as standards for determining Si content of plant samples.

Ultrastructural observations of mesophyll cells: Three leaves per treatment were collected to examine chloroplast ultrastructure in mesophyll cells. A rectangular section of a leaf (3.0 × 1.5 mm) near the center vein of each leaf was removed from a blade. After fixation with 2.5 %

(m/v) glutaraldehyde for 4 h, and washed with 0.1 M phosphate buffer, pH 7.4, leaf cells were post-fixed with 1 % (m/v) osmic acid at 4 °C for 4 h, washed with 0.1 M phosphate buffer, and then dehydrated with acetone gradient (acetone:resin mixtures at 3:1, 1:1, and 1:3, v/v) and then placed in pure resin for 12 h at 37 °C, 12 h at 45 °C, and 48 h at 60 °C. The thin sections were cut from leaf samples with an *LKB-V* ultramicrotome and placed upon 250 mesh grids. Samples were double stained using stem uranyl acetate and lead citrate and then observed and randomly photographed using a *Hitachi-600* (Tokyo, Japan) transmission electron microscope (Xu *et al.* 2008).

Determination of Chl content: Chlorophyll was extracted from 0.3 g small pieces of leaf tissue in 20 cm³ acetone solution (acetone and ethanol, 2:1, v/v). After extraction at room temperature and under dark for 24 h, Chl content in the supernatant was analyzed by measuring absorbance at 663 and 645 nm, respectively, as described by Ming *et al.* (2007): Chl *a* = 12.72 *A*₆₆₃ - 2.59 *A*₆₄₅; Chl *b* = 22.88 *A*₆₄₅ - 4.67 *A*₆₆₃; Chl *a+b* = 20.29 *A*₆₄₅ + 8.05 *A*₆₆₃. All absorbances were measured with a *SP-752PC* spectrophotometer (*Shanghai Spectrum Instruments*, Shanghai, China).

Measurement of gas exchange characteristics: Net photosynthetic rate (*P_N*), stomatal conductance (*g_s*), transpiration rate (*E*), and intercellular CO₂ concentration (*c_i*) of *G. uralensis* leaves were measured between 10:00 and 12:00 h on three consecutive days (20 - 22 August) using a portable photosynthesis system (*CI-310*, *CID Bio-Science*, Camas, WA, USA), using an infrared gas analyzer. Six similar, healthy, fully expanded leaves on the top of the stem in each treatment were measured at a leaf temperature of 20 ± 2 °C, an air humidity of 14 ± 2 %, an irradiance of 570 μmol m⁻² s⁻¹, and CO₂ concentration of 420 μmol mol⁻¹. The WUE was calculated as the ratio of *P_N* and *E*.

Determination of water status: Fully expanded third leaf from the top of the main stem of three plants from

each treatment were selected to measure the RWC. After measuring the fresh mass (FM), leaves were placed in distilled water for 24 h at 4 °C in darkness and the water saturated mass (WSM) was recorded. The samples were oven-dried at 60 °C until constant mass was attained and leaf dry mass (DM) was determined. RWC was calculated based on the formula suggested by Teulat *et al.* (2003) as follows: RWC [%] = [(FM - DM) / (WSM - DM)] × 100.

Measurement of growth parameters: At the end of the experiment, the plant height, root height, root diameter, and stem diameter were recorded from three replicates of each treatment. The plant height was measured from the base of the stem, at the soil level, to the terminal bud of the main stem. The stem and root diameter were measured with a digital micrometer. Leaf area was obtained with a *CI-203* leaf area scanner (*CID Bio-Science*, Camas, WA, USA). All plants were taken out of the soil and separated into leaves, stems, and roots. The tissues were first cleaned using distilled water. Then the sample was oven-dried at 60 °C for 48 h to constant mass and weighted.

Statistical analysis: All experimental data were analyzed by *ANOVA* and Pearson's correlation analysis using *SPSS 17.0* software (*SPSS*, Chicago, IL, USA). Significant differences were tested using the least significant difference (LSD) test at *P* < 0.05. Mean values and standard errors (SEs) were presented (*n* = 6).

Results

At 70 d after treatment (DAT), Si content in *G. uralensis* roots significantly decreased under SD2 and Si content in stems significantly decreased under all treatments without Si addition compared to control (Table 1). At 110 DAT, Si content in roots and leaves significantly decreased under SD1 and SD2 compared with control when Si absence. However, Si addition significantly affected Si content in roots, stems, and leaves, and this effect was

Table 1. Effect of Si [0.1 g(SiO₂) kg⁻¹(dry soil)] on Si content in roots, stems, and leaves of *Glycyrrhiza uralensis* plants under control conditions (CK), salinity, and drought stress [6 g(NaCl) kg⁻¹(soil) + 60 - 65 % field water content (FWC) (S), 6 g(NaCl) kg⁻¹(soil) + 45 - 50 % FWC (SD1), 6 g(NaCl) kg⁻¹(soil) + 30 - 35 % FWC (SD2)]. Means ± SEs, *n* = 6; in the same column different capital letters indicate significant differences among different treatments without Si at 0.05 probability, and different lower case letters indicate significant differences among different treatments after Si application at 0.05 probability; * stands for significant differences at 0.05 probability between two treatments with or without Si.

Treatments	Si content in roots [mg g ⁻¹ d.m.]		Si content in stems [mg g ⁻¹ d.m.]		Si content in leaves [mg g ⁻¹ d.m.]	
	70 DAT	110 DAT	70 DAT	110 DAT	70 DAT	110 DAT
CK	4.23 ± 0.30A	4.55 ± 0.11A	3.50 ± 0.15A	3.21 ± 0.15A*	4.48 ± 0.25AB	4.65 ± 0.17A
CK+Si	4.38 ± 0.26a	4.14 ± 0.26a	3.25 ± 0.17a	2.59 ± 0.03ab*	4.61 ± 0.08ab	4.66 ± 0.40a
S	4.06 ± 0.25A*	3.75 ± 0.24AB	2.95 ± 0.19B	3.47 ± 0.18A	4.65 ± 0.11A	4.80 ± 0.12A*
S+Si	4.56 ± 0.07a*	3.93 ± 0.09a	3.22 ± 0.30a	3.29 ± 0.19a	4.91 ± 0.17a	5.19 ± 0.06a*
SD1	3.41 ± 0.18AB*	3.43 ± 0.10B	2.55 ± 0.13B*	3.70 ± 0.07A*	4.29 ± 0.08AB*	4.11 ± 0.11B*
SD1+Si	3.55 ± 0.07a*	3.67 ± 0.16a	3.08 ± 0.13a*	2.96 ± 0.02a*	4.47 ± 0.02ab*	4.40 ± 0.11a*
SD2	3.13 ± 0.14B*	3.12 ± 0.04B*	2.76 ± 0.08B*	3.18 ± 0.19A*	4.03 ± 0.04B*	4.08 ± 0.04B*
SD2+Si	3.78 ± 0.20*a	3.55 ± 0.16a*	3.21 ± 0.13a*	2.06 ± 0.00b*	4.24 ± 0.00b*	4.34 ± 0.07a*

different depending on the treatments and development stages (Table 1). The Si addition significantly increased Si content in roost under all stressed conditions at 70 DAT and under SD2 at 110 DAT. Si addition significantly increased Si content in stems under SD1, SD2 at 70 DAT, while significantly decreased Si content in stem under CK, SD1, and SD2 at 110 DAT. However, the Si addition significantly increased Si content in leaves under all treatments except for S at 70 DAT.

Salinity combined with drought induced alterations in the anatomical structure of *G. uralensis* as compared to control. SD1 and SD2 increased the number of mesophyll cells and changed cell shape (long palisade cells were found under S, whereas palisade cells changed to foamy cells under SD1, SD2) (Fig. 1). Although some plastoglobuli were found in mesophyll cells under S, the cells essentially appeared normal in leaves from control and S treatments (Fig. 2). However, under SD1 and SD2 it was observed that 1) the structure of chloroplast and mitochondria was badly damaged and contained numerous plastoglobuli; 2) the thylakoid network was incomplete, and the number of grana decreased as a result of thylakoid membranes degradation; and 3) the mitochondria became swollen, and their inner membranes were severely degraded (Fig. 2). However, addition of Si alleviated the structural changes of *G. uralensis* induced SD1 or SD2. Under the S stress,

the cell arrangement of leaves was slightly loose, the number of capsule cells increased, and more of the stomata were formed near the lower epidermis by Si addition. Under SD1 and SD2 stress, the cells were arranged in a neat arrangement, foamy palisade cells were blocked, and there were more chloroplasts in palisade cells and spongy cells by Si addition (Fig. 1). Addition of Si maintained a well-preserved internal lamellar system in the chloroplasts under SD1 or SD2, and the chloroplasts contained less osmiophilic plastoglobuli and more starch grains (Fig. 2). They were still closely associated with the cell wall with few exceptions and showed an organized membrane system and most of the mitochondrial membrane structure was complete, with a few mitochondria membrane intact, but the ridge disappeared (Fig. 2).

Chlorophyll content in leaves was significantly affected by salinity combined with drought stress, developmental stages, and Si addition. Without Si addition, SD2 caused an increase in Chl *a* and Chl *a+b* content, while a decrease in Chl *b* thus increase in Chl *a/b* ratio compared with control at 70 DAT and at 110 DAT, SD1 caused a decrease in Chl *b* content, whereas Chl *a/b* ratio significantly increased under SD1 and SD2 (Figs. 3 and 4). Under control condition, Si addition significantly increased Chl *a* content while decreased Chl *b* content at 70 DAT. Under S stress, Si addition significantly increased Chl *b* and Chl *a+b*

Fig. 1. Effect of Si [0.1 g(SiO₂) kg⁻¹(dry soil)] on microstructure (magnification 400) of *Glycyrrhiza. uralensis* leaves under control conditions (CK) salinity (S), and drought stresses [6 g(NaCl) kg⁻¹(soil) + 60 - 65 % field water content (FWC), 6 g(NaCl) kg⁻¹(soil) + 45 - 50 % FWC (SD1), 6 g(NaCl) kg⁻¹(soil) + 30 - 35 % FWC (SD2)].

Fig. 2. Effect of Si on cellular ultrastructure of *Glycyrrhiza uralensis* leaves under control conditions (CK), salinity (S), and drought stress (SD1 and SD2) (for detail see Fig. 1) CW - cell wall, Ch - chloroplast, GL - thylakoid, Go - Golgi apparatus, Mi - mitochondrion, N - nucleus, P - plastoglobulus, SG - starch granule, V - vacuole (magnification 30000).

at 110 DAT. Under SD1 stress, Si addition significantly decreased Chl *b* and Chl *a+b* content at 70 DAT and significantly increased Chl *a+b* content and Chl *a/b* ratio at 110 DAT. Under SD2 stress, Si addition significantly

increased Chl *a*, Chl *b*, and Chl *a+b* content and Chl *a/b* ratio except for Chl *a/b* ratio at 70 DAT (Figs. 3 and 4).

The gas exchange parameters (E , P_N , c_i , and g_s) gradually decreased with the increasing salt and drought

Fig. 3. Effect of Si on chlorophyll *a* and chlorophyll *b* content in *Glycyrrhiza uralensis* leaves under control conditions (CK), salinity (S), and drought stress (SD1 and SD2) (for detail see Fig. 1). Means $\pm$ SEs, $n = 6$, different letters within different treatments indicate significant differences at 0.05 probability; * stands for significant differences at 0.05 probability between two treatments with or without Si.

Fig. 4. Effect of Si on chlorophyll *a+b* content and chlorophyll *a/b* in *Glycyrrhiza uralensis* leaves under control conditions (CK), salinity (S), and drought stress (SD1 and SD2) (for detail see Fig. 1). Means $\pm$ SEs, $n = 6$, different letters within different treatments indicate significant differences at $\alpha = 0.05$; * - significant differences at $\alpha = 0.05$ between two treatments with or without Si.

Table 2. Effect of Si on growth characteristics of *Glycyrrhiza uralensis* plants under control, and salt and drought stresses. See Table 1 for detail.

	Plant height [cm]		Root length [cm]		Root diameter [mm]	
	70 DAT	110 DAT	70 DAT	110 DAT	70 DAT	110 DAT
CK	44.96 ± 0.53A	45.24 ± 1.34A	34.70 ± 1.03A	39.40 ± 1.33A	9.04 ± 0.20A	10.25 ± 0.23A
CK + Si	45.12 ± 0.42a	49.32 ± 1.65a	35.76 ± 1.50a	38.79 ± 2.04a	9.07 ± 0.20a	10.75 ± 0.18a
S	41.73 1.73A	42.64 ± 1.28AB*	30.50 ± 0.19B*	33.98 ± 0.89B	8.66 ± 0.32A	9.16 ± 0.14B*
S + Si	44.79 ± 0.62a	47.29 ± 1.07a*	34.21 ± 0.085a*	35.36 ± 1.21ab	9.05 ± 0.65a	10.56 ± 0.55a*
SD1	32.36 ± 1.61B*	40.54 1.19B	25.09 ± 1.84C	32.09 ± 0.92B	8.03 ± 0.45A	8.77 ± 0.29AB*
SD1 + Si	38.66 ± 1.83b*	40.29 ± 0.12b	26.55 0.70b	34.30 ± 0.94ab	8.43 ± 0.08a	9.20 ± 0.44b*
SD2	32.74 ± 0.14B*	34.41 0.77C	13.00 ± 0.20D*	30.44 ± 0.94B	7.82 ± 0.19A*	7.97 ± 0.33C*
SD2 + Si	37.63 ± 1.54b*	36.12 ± 1.82a	16.47 0.64c*	32.22 ± 1.12b	8.47 ± 0.28a*	8.76 ± 0.07b*

	Leaf area [mm ²]		Stem diameter [mm]	
	70 DAT	110 DAT	70 DAT	110 DAT
CK	8.28 0.23A	9.89 ± 0.78A	2.46 ± 0.065A	2.31 ± 0.03A*
CK + Si	7.75 ± 0.57a	10.43 ± 1.00a	2.56 ± 0.52ab	2.54 ± 0.08a*
S	4.94 ± 0.44B	9.02 ± 0.24AB*	2.36 ± 0.04A*	2.19 ± 0.08AB
S + Si	6.03 ± 0.25a	10.34 ± 0.22a*	2.65 0.09a*	2.32 ± 0.17a
SD1	3.77 ± 0.29B*	8.64 ± 0.37AB*	2.04 0.04B*	2.10 ± 0.01B*
SD1 + Si	6.17 ± 0.72a*	10.05 ± 0.29ab*	2.37 0.04b*	2.44 ± 0.09a*
SD2	4.62 ± 0.46B*	7.24 ± 0.24B*	2.11 0.01*B	2.1± ± .11B
SD2 + Si	6.62 ± 0.66a*	7.97 0.27b*	2.31 ± 0.07b*	2.24 ± 0.03a

Table 3. Effect of Si on leaf dry mass, stem dry mass, root dry mass, and total dry mass of *Glycyrrhiza uralensis* plants under control, and salt and drought stresses. See Table 1 for detail.

	Leaf dry biomass [g pot ⁻¹]		Stem dry biomass[g pot ⁻¹]	
	70 DAT	110 DAT	70 DAT	110 DAT
CK	11.00 ± 1.11A	7.93 ± 1.51A	9.24 ± 0.05A	11.31 ± 0.05A*
CK + Si	10.69 ± 1.15b	8.65 ± 0.56a	10.22 ± 0.27b	13.10 ± 0.84a*
S	12.01 ± 0.62A	5.11 ± 0.14B*	9.00 ± 0.34A*	9.04 ± 0.61B*
S + Si	11.97 ± 1.68a	5.94 ± 0.09b*	11.24 ± 0.12a*	11.35 ± 0.68a*
SD1	6.58 ± 0.19B*	4.84 ± 0.11B	6.11 ± 0.67B	5.95 ± 0.36C
SD1 + Si	7.70 ± 0.18c*	4.62 ± 0.53b	6.63 ± 0.13c	6.41 ± 0.67b
SD2	3.29 ± 0.49C*	1.14 ± 0.03C	3.96 ± 0.28C*	4.05 ± 0.14D*
SD2 + Si	6.36 ± 0.49c*	1.38 ± 0.15c	6.08 ± 0.55c*	4.74 ± 0.21b*

	Root dry mass [g pot ⁻¹]		Total dry mass [g pot ⁻¹]	
	70 DAT	110 DAT	70 DAT	110 DAT
CK	34.70 ± 1.03A	58.97 ± 5.02A	54.94 ± 2.19A	78.21 ± 6.58A
CK + Si	35.76 ± 1.50a	57.35 ± 1.95a	56.67 ± 2.92a	79.09 ± 3.34a
S	30.50 ± 0.19B*	52.31 1.05AB	51.51 ± 1.16A*	66.45 ± 1.79B
S + Si	34.21 ± 0.09a*	53.65 ± 1.26a	57.42 1.88a*	70.94 ± 2.33b
SD1	25.09 ± 0.36C*	46.08 ± 0.44B*	37.78 1.22B	56.87 ± 0.91C
SD1 + Si	26.55 ± 0.70b*	48.36 ± 0.36b*	40.88 ± 1.01b	59.38 ± 1.57c
SD2	13.04 ± 0.25D*	27.03 ± 0.19C*	20.29 1.01C*	32.22 ± 0.35D*
SD2 + Si	16.47 ± 0.64b*	30.37 0.13c*	28.91 ± 1.68c*	36.38 ± 0.48d*

stress except for c_i which only significantly decreased under SD2 (Fig. 4). Si addition significantly increased gas exchange parameters under all stress conditions except

for c_i under S stress (Fig. 5). The RWC of *G. uralensis* leaves significantly decreased under SD1 and SD2 compared with control and reached the least value under

SD2 (Fig. 6). The WUE significantly decreased under SD2 (Fig. 7). However, the Si addition could maintain better water status (higher RWC) and increased WUE under all stresses and especially under SD1 and SD2 (Figs. 6 and 7). The Si content in stems positively correlated with E , P_N , c_i , and RWC, while Si content in leaves positively correlated with g_s and WUE (Table 1 Suppl.).

Morphological parameters (root length, root diameter, plant height, stem diameter, and leaf area) and the dry matter accumulation (leaf dry mass, stem dry mass, root dry mass, and total dry mass) were reduced in combined stress when compared with control and reached minimum value under SD2 (Tables 2 and 3). The Si addition markedly changed the growth of *G. uralensis* plants. Especially under S stress, the Si addition significantly increased root length, stem diameter, and root and total dry mass at 70 DAT, as well as significantly increased root diameter,

leaf area, and leaf dry mass at 110 DAT. Under SD1 stress, the Si addition significantly increased plant height and leaf dry mass at 70 DAT and significantly increased root length and root diameter at 110 DAT, while it significantly increased stem diameter, leaf area and root dry mass both 70 and 110 DAT. Under SD2 stress, the Si addition significantly increased all morphological parameters at 70 DAT, but root diameter and leaf area also at 110 DAT. The dry matter accumulation was significantly increased by Si addition during the experiment except for leaf dry mass which only increased at 70 DAT (Tables 2 and 3). In this study, we also found a positive correlation between growth parameters, photosynthesis, and water status. Specifically, leaf dry mass and leaf area were positive correlation with E , P_N , c_i , g_s , and WUE. Stem dry mass was positive correlation with gas exchange parameters and RWC. The root dry mass positively correlation with P_N , g_s ,

Fig. 5. Effect of Si on net photosynthetic rate (A), transpiration rate (B), stomatal conductance (C), and intercellular CO_2 concentration (D) of *Glycyrrhiza uralensis* leaves under control conditions (CK), salinity (S), and drought stress (SD1 and SD2) (for detail see Fig. 1) Means $\pm$ SEs, $n = 6$, different letters within different treatments indicate significant differences at 0.05 probability; * stands for significant differences at 0.05 probability between two treatments with or without Si.

Fig. 6. Effect of Si on relative water content of *Glycyrrhiza uralensis* leaves under control conditions (CK), salinity (S), and drought stress (SD1 and SD2) (for detail see Fig. 1) at 70 and 110 days after treatment. Means $\pm$ SEs, $n = 6$, different letters within different treatments indicate significant differences at 0.05 probability; * stands for significant differences at 0.05 probability between two treatments with or without Si.

WUE, and RWC and total dry mass positively correlated with E, P_N, c_i, and WUE (Table 2 Suppl.).

Discussion

All plants grown in soil contain Si in their tissues, and Si content in plants varies greatly among species and genotypes (Ma and Yamaji 2008). *G. uralensis* was weak in Si absorption rate, but *G. uralensis* plants can accumulate a certain amount of Si with the extension of the absorption time (Zhang *et al.* 2017). It is commonly accepted that Si is absorbed by plant root in the form of soluble silicic acid. After being absorbed, silicic acid is then transported to the shoot *via* xylem (Ma and Yamaji 2008). Silicic acid finally polymerizes and precipitates, forming “opal phytoliths” in the cell wall, intercellular spaces, and trichomes (Cooke and Leishman 2011, Mazumdar 2011). A significant decrease of Si content in tomato roots was observed under salt stress by Muneer and Jeong (2015). In this study, Si content in *G. uralensis* roots and leaves was decreased by SD1, SD2 treatments at 70 and 110 DAT and Si content in stem only at 70 DAT (Table 1). These results indicated that these stresses decreased Si absorption by roots and further inhibited transportation to shoot *via* xylem. Si addition resulted in markedly increased Si content in roots and leaves while decreased it in stems under SD1 and SD2 at 110 DAT (Table 3), which was similar to the previous studies in soybean (Li *et al.* 2004), tomato (Cao *et al.* 2015), and wheat (Ma *et al.* 2016). The higher content of Si under SD1 and SD2 in plants roots and leaves might be due to deposition of Si in cell walls and endodermis, which can reduce the translocation of NaCl (Liang *et al.* 2005). The Si applied also could strengthen the membranes of plant cells, change their permeability, and maintain the integrity of cellular structures (Figs. 1 and 2). In addition, Ma *et al.* (2016) find that application of Si in the field can alleviate damage of photosynthesis induced by drought stress in wheat. This study further confirms that Si content in stem and leaf of *G. uralensis* was positively correlated with photosynthetic parameters and water status (Table 1 Suppl.). These results indicated that Si addition enhanced

uptake, transport and accumulation of Si, which played a significant role in improving the growth and dry matter accumulation of *G. uralensis* plants. Plant leaves are very sensitive to environmental changes and salt and drought stresses significantly changed leaf structure: the mesophyll cells became smaller and irregular in shape, with more influence on spongy tissue than on palisade tissues. The addition of Si inhibited the degeneration of membrane systems, mesophyll cells were lined regularly, and vascular area increased slightly (Fig. 1). In this process, palisade tissue cells contain more larger chloroplasts arranged relatively close, which can greatly improve the photosynthetic rate of plants, but can also reduce the evaporation of water (Keremu and Hou 2006). Some studies have found that the more developed main vascular bundle, the higher water transport efficiency. Therefore, the developed vascular bundle structure may ensure water and nutrient transport and has the function of water conservation and storage (He *et al.* 2005). The previous results showed that water deficit and heat stress in rice caused deformed mesophyll cells, but some of the cells could maintain the normal shape by Si addition (Agarie *et al.* 1998), which was similar to the present results.

Chloroplast is the first organelle of mesophyll cells which could be damaged during the salt and drought stresses (Noode'n *et al.* 2004). Generally, salt- and drought-induced lipid peroxidation results in a massive reorganization of leaf chloroplast ultrastructure (Bejaoui *et al.* 2016), which is considered to be a good indicator of plant status under stress conditions (Xu *et al.* 2009). Previous study found that the number of chloroplast and intercellular space decreased dramatically, and the disintegrated chloroplast envelope together with the loosen and swollen grana thylakoids can be observed under salt and drought stressed *G. uralensis* seedlings (Liu *et al.* 2010), these changes are similar to that observed in the present study under salt combined with drought stress (Fig. 2). It has been reported that the number and size of plastoglobuli increases due to thylakoid membranes degeneration (Olmos *et al.* 2007), which was corresponded with more plastoglobuli found under SD2 than that under SD1 (Fig. 2). And the disorganization of thylakoid membranes is correlated to the H₂O₂ accumulation in chloroplasts (Oksanen *et al.* 2005). In addition, changed shape of many chloroplasts from lenticular to round or oval under SD1 and SD2 (Fig. 2) suggests an altered function of them. Also Zellnig *et al.* (2004) observed that stress-impaired growth in leaves of drought-sensitive spinach lines is accompanied with the reduced length and abnormal shape of chloroplasts. Interestingly, it was evident that Si content in leaves of *G. uralensis* increased by Si addition (Table 1), which would be deposited on cell walls (Liang *et al.* 2005). And Si applied could strengthen the membranes of plant cells by depositing around the cell walls and changing their permeability under SD1 and SD2 (Fig. 2). As the light reactions of photosynthesis occur in the grana, a close relationship is expected between thylakoid membrane integrity and photosynthetic activity (Chen *et al.* 2011a). Also, the mitochondria were intact and possessed electron-dense bodies after Si addition which would improve the

Fig. 7. Effect of Si on water use efficiency of *Glycyrrhiza uralensis* leaves under control conditions (CK), salinity (S), and drought stress (SD1 and SD2) (for detail see Fig. 1) at 70 and 110 days after treatment. Means $\pm$ SEs, $n = 6$, different letters within different treatments indicate significant differences at 0.05 probability; * stands for significant differences at 0.05 probability between two treatments with or without Si.

energy metabolism of *G. uralensis* under drought and salt stresses. In addition, more and larger starch grains after Si addition were observed in the present study. These results suggested that Si application helped to maintain the ultrastructure of *G. uralensis* leaves, which enabled a normal physiological functions of the plant exposed to salt combined with drought stress.

The decline in Chl content under salt or drought has been considered to be a typical symptom of pigment photooxidation and chlorophyll degradation (Hajihashemi and Ehsanpour 2013). Ping *et al.* (2015) observed a reduction in Chl content in apple under progressive drought stress. Decreased or unchanged Chl content during drought stress has been reported in some species, depending on the duration and severity of drought (Silva *et al.* 2012). In our study, Chl *a* content increased under SD2 at 70 DAT while Chl *b* content decreased under SD1 and SD2 at 110 DAT. Correspondingly, Chl *a/b* significantly increased under SD1 and SD2 at 110 DAT (Fig. 3), indicating that Chl *b* was more sensitive than Chl *a* to stress similarly as found Sultana *et al.* (1999). In addition, Chl *a+b* content increased under SD1 at 70 DAT while declined under SD2 at 110 DAT. The Si application markedly affected chlorophyll content in *G. uralensis* leaves in a stress degrees and developmental stage dependent manner. Generally, at 70 DAT, Si addition increased Chl *a* content under control and SD2 but decreased Chl *b* under control and SD1. At 110 DAT, Si addition increased Chl *a* and Chl *a+b* content under SD2 and Chl *b* under S and SD2. This indicates that in the course of stress time, Si addition increased Chl content mainly under SD2 and thus increased Chl *a/b* under SD1 and SD2. These results are similar to Si-induced increase in Chl *a* and Chl *b* content under drought stress (Maghsoudi *et al.* 2016). Combined with the above results, Si could maintain the structural integrity of mesophyll cells and chloroplasts to a certain extent and increase the amount of chloroplasts (Figs 1, 2, and 3).

The influence of drought stress is usually perceived as a decline in photosynthetic rate. When the amount of available soil water is moderately or severely limiting, the first response of plants is to close their stomata to minimize water loss, and decreases the influx of CO₂ into the leaves. This is accompanied by decreases in *g_s*, *E*, and *P_N*. In this study, *E*, *P_N*, and *g_s* were concomitantly decreased under salinity combined with drought stress (Fig. 5). These results suggested that the suppression of *P_N* could be generally attributed to stomatal limitation rather than non-stomatal limitation (Zhang *et al.* 2013). These findings are in agreement with previous studies in melon and rice (Huang *et al.* 2011, Porcel *et al.* 2015). The application of Si reduced the impact of these stresses which was manifested by improved gas exchange parameters. A similar effect of Si was documented for salt stressed tomato (Haghighi and Pessarakli 2013) and cotton (Farooq *et al.* 2013). In the present study, Si significantly elevated *P_N* under all stressed conditions. This could have been due to increase in leaf area, Chl content, and number and size of stomata (Fig. 1 and 2). In addition, this finding was similar to those of Da-Cunha and Do-

Nascimento (2009) who studied cadmium stressed maize plants and found that Si resurrected the photosynthetic potential due to improved integrity of the photosynthetic apparatus (Fig. 1 and 2). The *c_i* can be used to discriminate between changes in *P_N* resulting from stomatal limitation or non-stomatal limitations. From our results, the increase in *P_N* caused by Si addition maybe resulted from stomatal limitations because the Si application significantly enhanced the *g_s* under SD1 and SD2 (Fig. 5) which could be associated with the increase in the number of stomata and stomatal size (Fig. 1 and 2). The positive impacts of Si on plants under stress conditions were often associated with transpiration reduction (Gao *et al.* 2006). However, our study showed that *E* in leaves of stressed *G. uralensis* was significantly increased by Si addition (Fig. 5). The similar phenomenon was observed in sorghum (Hattori *et al.* 2005) and rice (Chen *et al.* 2011b) under drought stress. Also Sonobe *et al.* (2011) investigated the diurnal changes of gas exchange parameters in sorghum grown hydroponically and found that Si induces higher *g_s*, *P_N*, and *E* than in untreated plants under water stress.

The RWC has been reported as a good indicator of salt and drought stresses tolerance (Kraus *et al.* 1995). The WUE for plants is very important, especially when they are subjected to drought. The salinity and drought can reduce WUE in wheat (Gong and Chen 2012) and *Sorghum bicolor* (Yin *et al.* 2013). In the present study, RWC was declined under both SD1 and SD2. This result also in accordance with previous results in tobacco (Dobra *et al.* 2010). In addition, a significant decrease of WUE was observed under SD2, while significantly improvement was observed after Si treatment. Similarly, Si improves the water status of drought stressed wheat (Ding *et al.* 2007) and drought and UV-B radiation stressed soybean seedlings (Shen *et al.* 2010). In the present study, the enhanced WUE in Si-treated stressed plants indicates an augmentation in carbon assimilation rate, which resulted in better plant growth under stressed conditions (Fernandez-Garcia *et al.* 2014). Our results also indicated that Si increased RWC of *G. uralensis*, which is in agreement with the findings of Shen *et al.* (2010) and the improvement of growth could have also been due to this fact. It is suggested that Si could decrease cuticular and stomatal transpiration (Gao *et al.* 2006). However, Liang *et al.* (2008) proposed that accumulation of polar monosilicic acid and/or polymerized silicic acid in epidermal cell walls may form hydrogen bonds between water and hydrated silica, therefore, making water molecules less easily to escape from leaf surface. In the present study, both *g_s* and *E* of *G. uralensis* leaves increased by Si application under salinity combined with drought stress, indicating that Si might regulate root water uptake and transport in response to water loss and maintain water balance in *G. uralensis* plants under stress conditions. Which can be confirmed by improvement of RWC. These results are in accordance with previous findings in sorghum (Hattori *et al.* 2005) and pepper (Lobato *et al.* 2009).

The most common and significant effect of salt and drought stresses on plants is the inhibition of growth and biomass production (Kavas *et al.* 2015). Our results

showed that SD1 and SD2 decreased *G. uralensis* growth and biomass compared with control, and this adverse effect was partly reversed by Si addition (Table 2 and 3), which is consistent with previous findings in sugarcane (Ashraf *et al.* 2010), *G. uralensis* seedlings (Zhang *et al.* 2017), and canola (Hashemi *et al.* 2010). The salinity alone had no significant effect on growth and biomass of *G. uralensis* plants compared with control (Table 2 and 3), indicating that *G. uralensis* is resistant to moderate salt stress (Yang *et al.* 2006). We also found that the beneficial effect of Si on growth and biomass was strongest SD2 at 70 DAT. Furthermore, we also found a strong positive correlation between growth parameters and photosynthesis parameters and water status (Table 2 Suppl.).

Based on the results of this study, it could be concluded that under salinity combined with drought, Si application resulted in markedly increased of Si content in leaves due to its deposition on cell walls. The Si application helped to maintain the integrity of anatomical structure, to improve Chl content, and to increase P_N, E, g, and WUE. All of the above results ultimately led to higher growth and biomass production of *G. uralensis* plants under salinity combined with drought stress after Si application.

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