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Role of Silicon in Alleviating Salt-Induced Toxicity in White Clover

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Abstract To understand the role of silicon (Si) in alleviating sodium (Na) toxicity in *Trifolium repens* L. (white clover), the changes of biochemical and physiological parameters were investigated in four-week-old white clover seedlings exposed to 0 or 120 mM NaCl with or without 1.5 mM Si for 7 days. Results showed that added Si alone did not have any effects on the growth and Na^+, K^+ accumulations in white clover plants compared to the control (no added Si and NaCl). However, in the presence of NaCl, additional Si significantly enhanced the selective transport capacity for K^+ over Na^+ that contributed to reduced Na^+ uptake and increased K^+ uptake by roots, thereby improving its growth and K^+/Na^+ homeostasis in white clover. This study would provide a way for improving salt tolerance in important legume white clover forage.

Keywords Salt tolerance · Silicon · Selective transport capacity for K^+ over Na^+ · K^+/Na^+ homeostasis

Soil salinity is a major constraint of crop productivity because it reduces yield and limits expansion of agriculture onto uncultivated land (Flowers and Yeo 1995). *Trifolium repens* L. (white clover) is one of the most commonly cultivated legume forages due to its high protein content which makes its productivity essential for sheep meat, beef cattle and dairy production industries (Wang et al. 2010). In addition, the high nitrogen fixing ability of white clover makes it adaptable to a wide range of soil and environmental conditions and combines well with many perennial grasses (Tang et al. 2010). However, *T. repens* is a salt-sensitive species (Rogers et al. 1993) and suffers a yield decline at soil salinity levels of around 2 dS m^{-1} (Mehanni and Repsys 1986). Therefore, the objective of cultivating a salt tolerant *T. repens* has long been pursued. To overcome the toxicity of salinity in plant species, many curative and management practices have been adopted. One of these methods is to apply exogenous silicon (Si) in salinity soil.

Si is the second most abundant element on the surface of the earth and can particularly alleviate both biotic and abiotic stresses in higher plant, although it has not been listed among the generally essential elements of plants (Epstein 1994; Liang et al. 1996). Si is known to be involved in plant protection against salt stress. Yeo et al. (1999) reported that under salt stress, the deposition and polymerization of Si in the endodermis and rhizodermis blocked Na^+ influx through the apoplastic pathway in the roots of rice, thereby reducing the entrance of Na^+ without significantly affecting the overall transpiration flux and plant growth. Romero-Aranda et al. (2006) found that silicate crystals deposited in the epidermal cells form a barrier that reduces water loss through cuticles and improves water relation in tomato plant tissue which contributes to salt dilution within plants, mitigating salt toxicity effects. In addition, the increased uptake and transport of K^+ and decreased uptake and transport of Na^+ from roots to shoots in plants may be attributable to Si-induced stimulation of the root plasma membrane (PM) H^+-ATPase under salt stress (Liang et al. 2005, 2006). This indicated that restriction of Na^+ influx either into root cells or into the xylem stream is one way of regulating the optimum

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cytosolic K^+/Na^+ ratio (Chinnusamy et al. 2005). Taken together, the responses vary depending not only on Na and Si concentrations but also on plant species. However, Si involved in alleviating Na toxicity in *T. repens* has not been explored.

The objective of this study was to reveal the role of Si in the resistance to salt stress. In view of this, effects of Si on the growth parameters and Na^+, K^+ accumulations in *T. repens* plants exposed to 0 or 120 mM NaCl with or without 1.5 mM Si was investigated.

Materials and Methods

Seeds of white clover were sterilized with sodium hypochlorite solution (5 %) for 5 min and rinsed thoroughly with distilled water, then were germinated on moistened filter paper for 7 days at 25°C in the dark. When the plumule emerged, seedlings were selected for uniformity and were subsequently transferred into plastic containers (7 cm × 7 cm × 7 cm) filled with 0.6 L modified Hoagland solution (2 mM KNO_3, 1 mM $Ca(NO_3)_2$, 0.5 mM $MgSO_4$, 0.25 mM $NH_4H_2PO_4$, 11.9 μM Fe-citrate, 11.5 μM H_3BO_3, 1.25 μM $MnSO_4$, 0.2 μM $ZnSO_4$, 0.075 μM $CuSO_4$, and 0.025 μM $NH_4Mo_7O_{24}$) for 4 weeks. The nutrient solution was adjusted to pH 6.8 with 0.1 M HCl/NaOH, and was renewed every 2 days. Plants were grown in a growth chamber at a day/night cycle 16/8 h, at 22/18°C, respectively, a relative humidity between 50 % and 60 %, and a light intensity of 120 μmol m^{-2} s^{-1} PAR.

Si was added to the Hoagland solution as K_2SiO_3. KNO_3 was reduced, in the preparation of the Hoagland solution, proportionally to the K supply provided by K_2SiO_3, and additional K introduced by K_2SiO_3 was subtracted from KNO_3 and the resultant nitrate loss was supplemented with dilute nitric acid. Notably, in a preliminary experiment, adding different concentrations of Si (0, 0.5, 1.0, 1.5 and 2.0 mM) partially alleviated the negative impacts of salt stress on growth in white clover, but 1.5 mM Si was found to be the most effective. Therefore, 1.5 mM was used in all subsequent experiments. A randomized block design consisting of a control and three treatments (1.5 mM Si, 120 mM NaCl and 1.5 mM Si plus120 mM NaCl) was used. NaCl concentrations were incrementally increased with 30 mM NaCl day^{-1} increments until final concentrations (120 mM NaCl) were achieved. Four-week-old white clover seedlings were harvested after 7 days of receiving the silicon and salinity treatments. Three plants for white clover were pooled in each replicate. The experiment was repeated five times.

The relative growth rate (RGR) of whole plants was calculated using the formula $RGR = (\ln W_j - \ln W_i)/\Delta t$, where W_j and W_i are final (after 7 days of treatments) and initial (before treatments) dry weights (DW), respectively, and Δt is the time elapsed (days) between the two measurements; initial dry weight was determined before treatments (Martínez et al. 2005).

At the end of each treatment, plant roots were washed twice for 8 min in ice-cold 20 mM $CaCl_2$ to exchange cell wall-bound Na^+ and shoots rinsed in deionized water to remove surface salts (Wang et al. 2007). Harvested plants were washed thoroughly with running distilled water, separated into shoots and roots; fresh weights were determined immediately and then oven dried at 80°C for 3 days to obtain dry weights. Na^+ and K^+ were extracted from dried plant tissue in 100 mM acetic acid at 90°C for 2 h and ion analysis was performed using an atomic absorption spectrophotometer (AA-6300C, Shimadza, Kyoto, Japan).

According to Wang et al. (2009), the net Na^+, K^+ uptake rate was calculated for each time interval, as net Na^+, K^+ uptake rate (nmol g RFW^{-1} min^{-1}) = $(C_2 - C_1)/R_2/(t_2 - t_1)$, where C is Na^+, K^+ content in whole plant, R_2 is the root fresh weight (RFW), and t is the time at two harvests, respectively. Sub-indexes 1 and 2 are indicated before treatments and after 7 days of treatments, respectively.

Selective transport capacity for K^+ over Na^+ (ST) value indicates the net capacity of selection for transport of K^+ over Na^+ from roots to shoots (Guo et al. 2012). ST values were estimated according to the following equation as described by Guo et al. (2012) where ST = (K^+/Na^+ in shoots)/(K^+/Na^+ in roots).

All the data are presented as means with standard errors (SE). Statistical analyses, one-way ANOVA, and Duncan's

Table 1 Effects of Si on DW of shoots and roots and RGR in white clover under salt stress

NaCl (mM)	Si (mM)	Shoots DW (g $plant^{-1}$)	Roots DW (g $plant^{-1}$)	RGR (mg g^{-1} d^{-1})
0	0	1.23 ± 0.061a	0.58 ± 0.026a	152.26 ± 8.36a
0	1.5	1.26 ± 0.042a	0.61 ± 0.031a	157.71 ± 9.23a
120	0	0.56 ± 0.053c	0.23 ± 0.028c	34.62 ± 3.62c
120	1.5	0.92 ± 0.042b	0.46 ± 0.016b	114.30 ± 5.72b

Four-week-old white clover seedlings were exposed to 0 or 120 mM NaCl with or without 1.5 mM Si for 7 days. Values are mean ± SE. Each value is a mean of five replicates (n = 5). Mean values (±SE) with different letters are significantly different at $p < 0.05$ (Duncan's test)

multiple range tests were performed by statistical software (Ver.13.0, SPSS Inc., Chicago, IL, USA).

Results and Discussion

As shown in Table 1, application of Si alone did not influence the DW and RGR in plants compared to the control. Added NaCl alone significantly reduced shoots and roots DW by 54.47 % and 60.34 %, respectively, compared to the control. However, when plants were treated with NaCl plus Si, the reductions of shoots and roots DW were only 26.98 % and 24.59 % relative to the additional Si alone, respectively. Based on these results, in the presence of NaCl, the addition of Si significantly increased RGR by 69.71 % in *T. repens* plants. Furthermore, in the presence of NaCl, added Si decreased roots Na^+ concentrations by 48.19 % and increased shoots K^+ concentration by 48.40 % in *T. repens* plants (Fig. 1). Meanwhile, NaCl plus Si caused a 114.11 nmol g RFW^{-1} min^{-1} reduction in net Na^+ uptake rate and 61.71 nmol g RFW^{-1} min^{-1} increase net K^+ uptake rate compared to NaCl treatments alone, leading to enhanced whole plant K^+/Na^+ ratios in *T. repens* (Table 2). Similar results were also reported in other plants exposed to salt stress (Liang 1999; Ashraf et al. 2010). The ameliorative effect of added Si in alleviating deleterious effects of NaCl could be related to Si being irreversibly precipitated as amorphous silica ($SiO_2 \cdot nH_2O$) in cell walls and lumens, leading to Si inducing a reduction of Na^+ in transpiration rate (Matoh et al. 1986) and to the partial blockage of the transpirational bypass flow (Yeo et al. 1999).

On the other hand, Si reduced Na^+ uptake by roots due to Si-induced stimulation of the root PM H^+-ATPase under salt stress (Liang et al. 2005, 2006), which contributed to increased K^+ uptake and -transport in plants (Liang 1999). It is known that the proton motive force created by PM H^+-ATPases drives Na^+ effluxes from plant cells through Na^+/H^+ antiporters in the PM (Blumwald et al. 2000). The most likely candidate is a putative PM Na^+/H^+ antiporter (SOS1), and SOS1 also is the major component of selective transport capacity for K^+ over Na^+ (ST) in plants (Guo et al. 2012). Interestingly, we observed the ST values in white clover plants treated with NaCl plus Si were 1.60 times higher than that of NaCl treatment alone (Table 2). As for higher plants, cation/H^+ antiporters are the main transport systems involved in Na^+ and K^+ homeostasis through PM associated transport processes (Olías et al. 2009), thereby contributing to maintaining higher K^+/Na^+ selectivity in plants (Hasegawa et al. 2000). Therefore, Si might stimulate activity of PM H^+-ATPase which would provide additional energy needed for PM Na^+/H^+

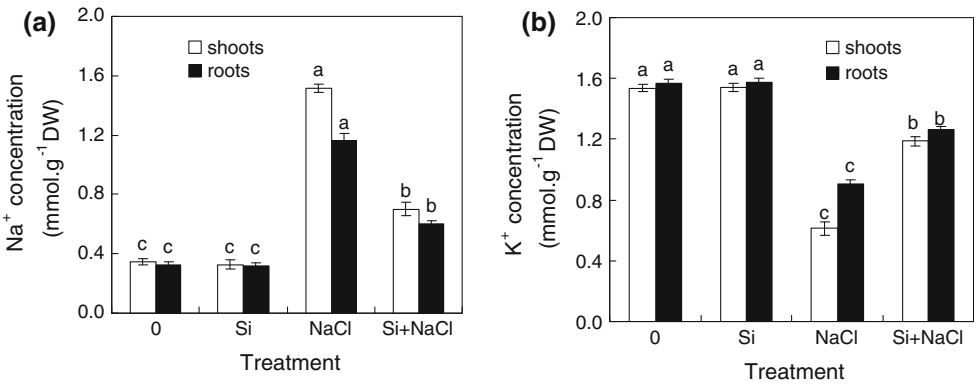

Fig. 1 Effects of Si on Na^+ and K^+ concentration in white clover under salt stress. Four-week-old white clover seedlings were exposed to 0 or 120 mM NaCl with or without 1.5 mM Si for 7 days. Values are mean ± SE. Each value is a mean of five replicates (n = 5). *Bars* indicate ±SE. Mean values (±SE) with *different letters* are significantly different at $p < 0.05$ (Duncan's test)

Table 2 Effects of Si on whole plant K^+/Na^+ ratio, net Na^+, K^+ uptake and ST value in white clover under salt stress

NaCl (mM)	Si (mM)	Whole plant K^+/Na^+ ratio	Net Na^+ uptake (nmol g RFW^{-1} min^{-1})	Net K^+ uptake (nmol g RFW^{-1} min^{-1})	ST value
0	0	4.57 ± 0.212a	–	–	0.92 ± 0.024a
0	1.5	4.75 ± 0.305a	–	–	0.95 ± 0.028a
120	0	0.53 ± 0.082c	145.39 ± 5.25a	−85.98 ± 3.36b	0.52 ± 0.038c
120	1.5	1.58 ± 0.125b	31.28 ± 4.62b	−24.27 ± 4.32a	0.83 ± 0.018b

Four-week-old white clover seedlings were exposed to 0 or 120 mM NaCl with or without 1.5 mM Si for 7 days. Values are mean ± SE. Each value is a mean of five replicates (n = 5). Mean values (±SE) with different letters are significantly different at $p < 0.05$ (Duncan's test)

antiporter and exclude more Na^+ from the plant cell. This would further enhance the selective transport capacity for K^+ over Na^+, hence regulating K^+/Na^+ homeostasis in plants under salt stress.

In conclusion, Si could alleviate Na toxicity in white clover plants subjected to salt stress due to enhance the selective transport capacity for K^+ over Na^+, hence regulating K^+/Na^+ homeostasis and improving the plant growth.

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References

Ashraf M, Rahmatullah M, Afzal R, Ahmed F, Mujeeb A, Sarwar LA (2010) Alleviation of detrimental effects of NaCl by silicon nutrition in salt-sensitive and salt-tolerant genotypes of sugarcane (*Saccharum officinarum* L.). Plant Soil 326:381–391

Blumwald E, Aharon GS, Apse MP (2000) Sodium transport in plant cells. Biochim Biophys Acta 1465:140–151

Chinnusamy V, Jagendorf A, Zhu JK (2005) Understanding and improving salt tolerance in plants. Crop Sci 45:437–448

Epstein E (1994) The anomaly of silicon in plant biology. Proc Natl Acad Sci USA 91:11–17

Flowers TJ, Yeo AR (1995) Breeding for salinity resistance in crop plants: where next? Aust J Plant Physiol 22:875–884

Guo Q, Wang P, Ma Q, Zhang JL, Bao AK, Wang SM (2012) Selective transport capacity for K^+ over Na^+ is linked to the expression levels of *PtSOS1* in halophyte *Puccinellia tenuiflora*. Funct Plant Biol 39:1047–1057

Hasegawa P, Bressan RA, Zhu JK, Bohnert HJ (2000) Plant cellular and molecular responses to high salinity. Annu Rev Plant Mol Biol 51:463–499

Liang YC (1999) Effects of silicon on enzyme activity, and sodium, potassium and calcium concentration in barley under salt stress. Plant Soil 209:217–224

Liang YC, Shen QR, Shen ZG, Ma TS (1996) Effects of silicon on salinity tolerance of two barley cultivars. J Plant Nutr 19:173–183

Liang YC, Zhang WH, Chen Q, Ding RX (2005) Effects of silicon on tonoplast H^+-ATPase and H^+-PPase activity, fatty acid composition and fluidity in roots of salt-stressed barley (*Hordeum vulgare* L.). Environ Exp Bot 53:29–37

Liang YC, Zhang WH, Chen Q, Liu YL, Ding RX (2006) Effect of exogenous silicon (Si) on H^+-ATPase activity, phospholipids and fluidity of plasma membrane in leaves of salt-stressed barley (*Hordeum vulgare* L.). Environ Exp Bot 57:212–219

Martínez JP, Kinet JM, Bajji M, Lutts S (2005) NaCl alleviates polyethylene glycol-induced water stress in the halophyte species *Atriplex halimus* L. J Exp Bot 419:2421–2431

Matoh T, Kairusmee P, Takahashi E (1986) Salt-induced damage to rice plants and alleviation effect of silicate. Soil Sci Plant Nutr 32:295–304

Mehanni AH, Repsys AP (1986) Perennial pasture production after irrigation with saline groundwater in the Goulburn Valley, Victoria. Aust J Exp Agric 26:319–324

Olías R, Eljakaoui Z, Li J, De-Morales PA, Marín-Manzano MC, Pardo JM, Belver A (2009) The plasma membrane Na^+/H^+ antiporter SOS1 is essential for salt tolerance in tomato and affects the partitioning of Na^+ between plant organs. Plant, Cell Environ 32:904–916

Rogers ME, Noble CL, Nicolas ME, Halloran GM (1993) Variation in yield potential and salt tolerance of selected cultivars and natural-populations of *Trifolium repens* L. Aust J Agric Res 44:785–798

Romero-Aranda MR, Jurado O, Cuartero J (2006) Silicon alleviates the deleterious salt effect on tomato plant growth by improving plant water status. J Plant Physiol 163:847–855

Tang R, Li C, Xu K, Du YH, Xia T (2010) Isolation, functional characterization, and expression pattern of a vacuolar Na^+/H^+ antiporter gene *TrNHX1* from *Trifolium repens* L. Plant Mol Biol Rep 28:102–111

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

Wang CM, Zhang JL, Liu XS, Li Z, Wu GQ, Cai JY, Flowers TJ, Wang SM (2009) *Puccinellia tenuiflora* maintains a low Na^+ level under salinity by limiting unidirectional Na^+ influx resulting in a high selectivity for K^+ over Na^+. Plant, Cell Environ 32:486–496

Wang JP, Drayton MC, George J, Cogan NOI, Baillie RC, Hand ML, Kearney GA, Erb S, Wilkinson T, Bannan NR, Forster JW, Smith KF (2010) Identification of genetic factors influencing salt stress tolerance in white clover (*Trifolium repens* L.) by QTL analysis. Theor Appl Genet 120:607–619

Yeo AR, Flowers SA, Rao G, Welfare K, Senanayake N, Flowers TJ (1999) Silicon reduces sodium uptake in rice (*Oryza sativa* L.) in saline conditions and this is accounted for by a reduction in the transpirational bypass flow. Plant, Cell Environ 22:559–565