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Amelioration of Salt Stress in Grapevines Based on Use of Both Resistant Rootstocks and Exogenous Silicon

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Abstract

Salt stress was induced on grape vines with 100 mM NaCl and amelioration was evaluated by irrigating with 100 mM NaCl plus 2.0 mM $K_2SiO_3 \cdot 9H_2O$ for 30 days. The three cultivars tested included 'Cabernet Sauvignon', the rootstock '5BB', and 'Cabernet Sauvignon' grafted onto '5BB'. Plant daily height increment and the dry weight of the whole plant were significantly suppressed by NaCl. Na^+ and Cl^- accumulation (AIR) was significantly increased by salt stress, with an AIR order: leaves > stems > roots. The total N and P concentrations were significantly reduced by NaCl stress. The K^+ concentration in the roots and the stems was also significantly decreased by salt stress, however it was significantly increased in the leaves. For all cultivars, *Tr*, *Gs*, *Ci* and *Pn* were significantly reduced by NaCl, except ***Ci*** for '5BB'. The palisade and spongy leaf tissue thickness was significantly reduced by salt treatments. The order of salt stress tolerance was '5BB' rootstock > grafted plants > 'Cabernet Sauvignon'. Exogenously applied silicon significantly restored plant growth by 15.4 to 37.2% and 7.9 to 14.0% for the plant daily increment and the dry weight of the whole plants, respectively. Na^+ and Cl^- accumulation was also suppressed by 8.0 to 53.8% and 20.1 to 47.5%, respectively. In most cases the N and P concentrations were significantly increased, and leaf K^+ concentration was significantly decreased in the NaCl + Si treatment compared with the NaCl treatment, suggesting that both potassium and silicon are involved in ameliorating the adverse saline effects. Amelioration by exogenous silicon was further evidenced by enhanced photosynthetic indexes and leaf anatomy. Considering the extent of salt stress injury and restoration by exogenous silicon, the effective silicon restoration for the three cultivars was: '5BB' rootstock > grafted plants > 'Cabernet Sauvignon'. Ten of the 16 studied variables, including the daily height increment, dry weight, Na^+, Cl^-, N, K^+, Na^+/K^+, *Tr*, *Gs*, and the leaf spongy tissue thickness, responded positively to both NaCl stress and exogenously applied silicon, suggesting that these parameters may be reliably used in future studies.

Soil salinity is a major abiotic factor that severely limits crop growth and productivity in certain regions (Zhu and Gong, 2014). Soil salinization is increasing owing to irrigation with salty water or ignoring the principles of soil drainage (Pisinaras et al., 2010). Poor plant growth induced by salt stress is a consequence of two factors. First, the relatively high osmotic potential of soil solution results in a water deficit in plants (Zhu and Gong, 2014). Second, the high concentration of certain ions (Na^+ and Cl^-) causes ion toxicity and ion imbalance (Pisinaras et al., 2010; Yue et al., 2012). For example, the competition between Na^+ and K^+ absorption alters the K^+/Na^+ ratio, as more Na^+ is taken up through the K^+ absorption pathways under salt stress conditions (Zheng et al., 2008).

Silicon (Si) is the second most abundant

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element following oxygen in the earth's crust (Zhu and Gong, 2014). Silicon-deficient plants tend to grow abnormally, whereas plants with sufficient silicon grow well (Epstein and Bloom, 2005). Moreover, if supplied in excess, silicon does not detrimentaly effect plants (Ma et al., 2006). Silicon increased tolerance to salinity in some important crops, such as rice (Gong et al., 2006), barley (Liang 1999), wheat (Tuna et al., 2008), sugarcane (*Saccharum officinarum* L.) (Ashraf et al., 2010), soybean (*Glycine max* L.) (Lee et al., 2010), tomato (Romero-Aranda et al., 2006), and zucchini (*Cucurbita pepo* L.) (Savvas et al., 2009).

Grape (*Vitis vinifera*) is the most widely grown and economically important fruit crop worldwide (Aradhya et al., 2003). Grapes are also one of the major crops in China. The grape production of China is the highest among grape growing countries worldwide (Wan et al., 2016; FAO, 2017). However, over 50% of the grape production in China comes from the arid and potential salinization areas, such as provinces of Xinjiang, Gansu, Ningxia, and the coastal regions of Shandong, Niaoning and Jiangsu provinces (Wan et al, 2008; Zhu and Gong, 2014). High evaporation (annual evaporation> 1600 mm) and low precipitation (annual precipitation < 200 mm) in the arid areas of Xinjiang, Gansu, and Ningxia usually leads to soil salinization in these areas (Zhu and Gong, 2014).

The '5BB' grape rootstock is one of the most important genotypes widely used in the United States, with tolerance to limestone and dry soils (Main et al., 2002). Several studies reported salt stress and drought resistance in grapevines and rootstocks (Fisarakis et al., 2001; Walker et al., 2010). However, effects of exogenous silicon on amelioration of salt stress in grapevine rootstocks were rarely investigated. This study is the first to comprehensively evaluate responses of three rootstock/scion combinations of grape including 'Cabernet Sauvignon' (*V. vinifera* L.), the rootstock '5BB' (*V. berlandieri* × *V. riparia*), and grafted plants 'Cabernet Sauvignon/'5BB' to salt stress and the effects of exogenous silicon to ameliorate salt stress in these grapevines. This study provides information for utilization of both grapevine rootstocks and exogenous silicon to improve productivity in the saline regions in China and other grape regions with similar conditions.

Materials and Methods

Plant materials and growth conditions. Three grape cultivars [self-rooted 'Cabernet Sauvignon' (*Vitis vinifera* L.) (referred to as 'Cabernet Sauvignon' hereafter), the rootstock '5BB' (*Vitis berlandieri* × *Vitis riparia*) (referred to as '5BB rootstock' hereafter), and grafted pants 'Cabernet Sauvignon'/'5BB' (referred to as 'the grafted plants' hereafter)] were used in this study. In early April, young grapevines were planted in plastic pots (52 cm × 43 cm × 33 cm) containing a mixture of sand, perlite and vermiculite (1:1:1, v/v/v) and grown in a greenhouse with natural light. Temperature was maintained at 26 °C during the day (6:30 am to 6:30 pm) and 18 °C at night and relative humidity was 55% during the day and 85% at night. Plants were watered daily with 70 ml of a half-strength Hoagland nutrient solution (Zheng et al., 2008) for 50 days from the start of the experiment.

The experiment was carried out at the Hebei Normal University of Science and Technology, Qinhuangdao, China in 2015-2017. Salinity and silicon treatments consisted of application of sodium chloride (NaCl) and potassium silicate ($K_2SiO_3 \cdot 9H_2O$) when the grapevine shoots were 25-30 cm in height and the treatments were sustained for 30 days (Zheng et al., 2008; Walker et al., 2010). The additional K introduced with K_2SiO_3 was subtracted from KNO_3, and the loss of nitrate in the resulting solution was added with dilute nitric acid (Moussa, 2006). To avoid shock, the level of salinity was progressively raised by 25 mM (70 ml liquid added) per day until the final concentration was achieved in a week (Zheng et al., 2008). The treatments

were (1) control (CK), dd H_2O without addition of NaCl or Si; (2) 100 mM NaCl ; (3) NaCl + Si, with 100 mM NaCl plus 2.0 mM $K_2SiO_3 \cdot 9H_2O$ (Wang and Han, 2007). Each genotype was considered a separate experiment with three treatments in a randomized complete block design. The experimental unit was a group of five plants per replicate and three replicates per treatment (Verma et al., 2010; Wang and Han, 2007). Data were collected from the five-plant unit and average values used for statistical analyses. The plants were harvested and dried at 70 °C in an oven for the dry weight measurement, then finely ground and sieved through a 1mm mesh for determination of mineral ion concentration (Seemann and Critchley, 1985).

Assay of plant growth. Plant growth was quantified as the daily height increment of the whole plant (the length after 30 days minus the initial length divided by 30 days, referred to as 'DHI' hereafter) and the dry weights of roots, stems and leaves measured 30 days after treatment (DAT) (referred to as 'DW' hereafter) (Seemann and Critchley, 1985).

The growth decrease index caused by the salt stress (GDI) was calculated as: [(DHI or DW in CK) - (DHI or DW in the NaCl treatment)] × 100/ the index in CK.

The growth restoration index by the exogenous silicon (GRI) was calculated as: [(DHI or DW in the NaCl +Si treatment) – (DHI or DW in the NaCl treatment)] × 100/ the index in the NaCl treatment.

Sodium (Na^+) and chloride (Cl^-) ion accumulation. Sodium concentration in grapevine tissues was determined using an atomic absorption spectrophotometry (Thermo, iCE3300, America) (Rowan et al., 1982). Chloride was estimated by silver nitrate ($AgNO_3$) titration (Xu et al., 2006).

The ion accumulation increase index by the salt stress (IAII) was calculated as: (the ion concentration in the NaCl treatment - the ion concentration in CK) × 100/ the ion concentration in CK.

The ion accumulation restoration index by the exogenous silicon (IARI) was calculated as: (the ion concentration in the NaCl treatment - the ion concentration in the NaCl+Si treatment) × 100/ the ion concentration in the NaCl treatment.

Concentration of nitrogen (N), phosphorus (P) and potassium (K^+). Total N concentration was determined by the Kjeldahl method (Walinga et al., 1995), P by the Watanabe and Olsen's colorimetric method (Watanabe, 1965), and K by atomic absorption spectrophotometry (Rowan et al., 1982). All the analyses are expressed on a dry weight basis.

The nutrient decrease index by the salt stress (NDI) was calculated as: [the nutrient (N, P or K^+) concentration in CK - the nutrient (N, P or K^+) concentration in the NaCl treatment] × 100/ the nutrient (N, P or K^+) concentration in CK.

The nutrient restoration index by the exogenous silicon (NRI) was calculated as: [the nutrient (N, P or K^+) concentration in the NaCl+Si treatment - the nutrient (N, P or K^+) concentration in the NaCl treatment] × 100/ the nutrient concentration in the NaCl treatment.

Analysis of photosynthetic parameters. Net photosynthesis (*Pn*), stomatal conductance (*Gs*), transpiration (*Tr*) and intercellular CO_2 concentration (*Ci*) were measured using a portable photosynthetic system GFS-3000. (Walz, Effeltrich, Germany). The system was equipped with a clamp-on leaf cuvette covering 3 cm^2 of leaf area. During photosynthetic measurements, the conditions were set at: photosynthetically active radiation 1000 $\mu mol.m^{-2}.s^{-1}$, and chamber temperature at 30 °C. The concentration of CO_2 was kept constant at 400 $\mu mol.l^{-1}$ and was measured using a LI-6400-01 CO_2 injector provided with a high pressure liquid CO_2 cartridge source (Walz, Effeltrich, Germany). Measurements were repeated three times for each replicate.

Assay of leaf anatomical structures. Leaf anatomical structure was observed with a light microscope (Olympus, CZX16, Japan) (Longstreth and Nobel, 1979). Sections of mature leaves (2 mm × 2 mm) were collected at the fourth to fifth node from the shoot tip

Table 1. Average daily height increment and dry weights of three organs of three grape cultivars as affected by three salinity treatments measured 30 days after initiation of treatment.

Cultivar [z]	Treatment [y]	Daily height increment (cm/day)	Dry weight (g)			
			Roots	Stems	Leaves	Whole plant
CS	CK	2.58 a [x]	11.90 a	10.96 a	11.32 a	34.17 a
	NaCl	0.78 c	7.01 b	5.50 b	4.33 b	16.84 c
	NaCl+Si	1.07 b	6.94 b	6.70 b	5.55 b	19.19 b
5BB	CK	1.90 a	14.74 a	11.03 a	10.49 a	36.27 a
	NaCl	1.04 b	8.90 c	7.38 b	7.94 b	24.22 c
	NaCl+Si	1.20 b	10.76 b	8.60 b	8.60 b	27.96 b
CS/5BB	CK	2.95 a	17.54 a	10.68 a	12.90 a	41.11 a
	NaCl	1.29 c	12.10 c	7.18 b	5.65 c	24.94 c
	NaCl+Si	1.57 b	13.65 b	5.96 c	7.31 b	26.92 b

[z] Cultivar abbreviations: CS, own rooted 'Cabernet Sauvignon'; own rooted 5BB; and CS grafted onto 5BB rootstock.
[y] Treatment abbreviation: CK, watered with water; NaCl, watered with 100 mM NaCl; and NaCl+Si, watered with 100 mM NaCl plus 2.0 mM $K_2SiO_3 \cdot 9H_2O$.
[x] Means within columns and cultivars followed by common letters do not differ at the 5% level by Duncan's multiple range test.

and were promptly fixed in FAA. Specimens were progressively dehydrated in series of ethanol and xylol and embedded in Spurr's resin (Longstreth and Nobel, 1979). Then 8-µm-thick sections were produced with an ultramicrotome (Leica, RM2235, Germany) and stained with safranin O-fast greening. Leaf sections were photographed with an Olympus camera (Olympus, Japan).

Statistical analyses. Average values from each replicate were subjected to analysis of variance SPSS for Windows Version 17.0 (SPSS Inc., Chicago, USA) based on the randomized block design. Average of three replicates was used as the mean for the Duncan's multiple range test for the three treatments within a cultivar in Tables 1, 3, 5 and 7 according to Li (2017).

Results

Plant growth. Compared to the control group, both of the daily height increment and the whole-plant dry weight of three grape cultivars were significantly ($P < 0.05$) lower

Table 2. The growth decrease index (GDI) and growth restoration index (GRI) of various organs of three grape cultivars following 30 days of salt stress with and without silicon amelioration.

Indexes	Cultivars [z]	Daily height increment (%)	Dry weight (%)			
			Roots	Stems	Leaves	The whole plant
GDI	CS	69.8 c [y]	41.1 b	49.8 b	61.8 c	50.7 c
	5BB	45.3 a	39.6 b	33.1 a	24.3 a	33.2 a
	CS/5BB	56.3 b	31.0 a	32.8 a	56.2 b	39.3 b
GRI	CS	37.2 c	-1.0 a	21.8 c	28.2 b	14.0 b
	5BB	15.4 a	20.9 c	16.5 b	8.3 a	15.4 b
	CS/5BB	21.7 b	12.8 b	-17.0 a	29.4 b	7.9 a

[z] Cultivar abbreviations: CS, own rooted 'Cabernet Sauvignon'; own rooted 5BB; and CS grafted onto 5BB rootstock.
[y] Means within columns and indexes followed by common letters do not differ at the 5% level of significance, by Duncan's multiple range test.

for the 100 mM NaCl treatment (Table 1), with a growth decease index (GDI) of 45.3 to 69.8% and 33.2 to 50.7% for the daily height increment and the whole-plant dry weight measured 30 DAT, respectively (Table 2), indicating that the grapevine growth was severely inhibited by the 100 mM NaCl stress. The plant daily height increment for the NaCl+Si treatment was significantly ($P < 0.05$) higher than that for the NaCl treatment (Table 1), with a growth restoration index (GRI) of 15.4 to 37.2% (Table 2). Effects of the NaCl+Si treatment on the plant daily height increment were differed for the three cultivars (Table 2). This suggests that exogenous Si may alleviate adverse effects caused by the salt stress.

Exogenous Si (NaCl+Si) significantly ($P < 0.05$) increased the dry weight of the whole plant in the three grape cultivars compared with the NaCl treatment with a restoration index of 7.9 to 14.0% (Table 2). However, the amelioration effect on the dry weight varied for the roots, stems and leaves (Table1). In the grafted and NaCl+Si treated plants, the dry weight of three organs were all significantly higher ($P < 0.05$) compared with the NaCl treated plants (Table 1). In most cases, the amelioration effect was not significant ($P > 0.05$) for the three organs in the 'Cabernet Sauvignon' plants or the '5BB' rootstocks (Table1).

Sodium and chloride accumulation. As shown in Table 3, the salt stress (NaCl) treatments significantly ($P < 0.05$) increased the sodium and chloride concentrations in the roots, stems and leaves of the three grape cultivars compared with the control group. The Na^+ and Cl^- accumulation increase index was 164.1 to 521.1% and 89.9 to 373.7%, respectively (Table 4), indicating that the accumulation of both Na^+ and Cl^- ions were extensively increased in the three grapevine organs under salt stress (Table 3 and 4). The accumulation index of sodium and chloride was the highest in the 'Cabernet Sauvignon' plants among three cultivars (Table 4).

In most cases, application of the exogenous silicon (NaCl+Si) significantly ($P < 0.05$) decreased accumulation of the sodium and chloride ions in grape plants compared with the NaCl treatment, with a restoration index of 8.0 to 53.8% and 20.1 to 47.5% for the Na^+ and Cl^- accumulation, respectively (Table 4). For Na+ and Cl^- the IAII was highest in all plant tissues of CS, except for leaf Cl^- which was not affected by cultivar. The influence of cultivar on IARI for both elements varied

Table 3. The Na^+ and Cl^- concentration (g/kg) in roots, stems and leaves of three grape cultivars 30 days after initiation of three salinity treatments.

Cultivar [z]	Treatment [y]	Roots		Stems		Leaves	
		Na^+	Cl^-	Na^+	Cl^-	Na^+	Cl^-
CS	CK	1.36 b [x]	0.65 c	0.94 c	0.38 a	0.57 c	0.71 c
	NaCl	5.54 a	2.80 a	4.93 a	1.80 c	3.54 a	2.22 a
	NaCl+Si	4.65 a	2.12 b	3.62 b	1.31 b	1.85 b	1.48 b
5BB	CK	2.23 c	0.81 c	1.28 c	0.69 c	0.68 c	0.58 c
	NaCl	5.89 a	2.17 a	4.21 a	1.31 a	2.32 a	1.89 a
	NaCl+Si	5.07 b	1.14 b	3.23 b	0.87 b	1.51 b	1.42 b
CS/5BB	CK	1.93 c	0.84 b	0.96 c	0.40 b	0.55 c	0.51 b
	NaCl	5.26 a	2.09 a	4.50 a	1.20 a	2.88 a	1.57 a
	NaCl+Si	4.84 b	1.67 a	3.83 a	0.65 b	1.33 b	1.25 a

[z] Cultivar abbreviations: CS, own rooted 'Cabernet Sauvignon'; own rooted 5BB; and CS grafted onto 5BB rootstock.
[y] Treatment abbreviation: CK, watered with water; NaCl, watered with 100 mM NaCl; and NaCl+Si, watered with 100 mM NaCl plus 2.0 mM $K_2SiO_3 \cdot 9H_2O$.
[x] Means within columns and cultivars followed by common letters do not differ at the 5% level by Duncan's multiple range test

Table 4. Ion accumulation increase index (IAII) and ion accumulation restoration index (IARI) of three grape cultivars as affected by 30 days of salinity treatments.

Index	Cultivar [z]	Roots		Stems		Leaves	
		Na^+	Cl^-	Na^+	Cl^-	Na^+	Cl^-
IAII	CS	307.4 b [y]	330.8 c	424.5 c	373.7 c	521.1 c	212.7 a
	5BB	164.1 a	167.9 b	228.9 a	89.9 a	241.2 a	225.9 a
	CS/5BB	172.5 a	148.8 a	368.8 b	200.0 b	423.6 b	207.8 a
IARI	CS	16.1 c	24.3 b	26.6 b	27.2 a	47.7 b	33.3 c
	5BB	13.9 b	47.5 c	23.3 b	33.6 b	34.9 a	24.9 b
	CS/5BB	8.0 a	20.1 a	14.9 a	45.8 c	53.8 c	20.4 a

[y] Cultivar abbreviations: CS, own rooted 'Cabernet Sauvignon'; own rooted 5BB; and CS grafted onto 5BB rootstock.
[z] Means within columns and index followed by common letters do not differat the 5% level by Duncan's multiple range test.

with plant tissue.

Nitrogen, phosphorus and potassium concentration. In most cases (except the stem of '5BB'), the salt treatment significantly ($P < 0.05$) reduced the nitrogen concentration in the three organs of all cultivars compared with the control. The reduction extent in the 'Cabernet Sauvignon' plants was significantly ($P < 0.05$) higher than that in the grafted plants and the '5BB' rootstocks (Table 6). In most cases, the nitrogen concentration in the NaCl+Si treatment was not significantly ($P > 0.05$) different from that in the NaCl treatment (Table 5).

In most cases, the phosphorus concentration of the roots, stems and leaves in NaCl treatments was significantly ($P < 0.05$) lower in the three grape cultivars compared with the control group (Table 5). In general, the reduction of P was greatest for roots, followed by the leaves and stems (Table 5). The reduction of phosphorus was highest for 'Cabernet

Table 5. N, P and K^+ concentration (g/kg) in roots, stems and leaves of three grape cultivars 30 days after initiation of three salinity treatments.

Cultivar [z]	Treatment [y]	Roots			Stems			Leaves		
		N	P	K^+	N	P	K^+	N	P	K^+
CS	CK	0.52 a [x]	0.43 a	0.89 a	0.36 a	0.38 a	1.19 a	1.08 a	0.35 a	1.09 b
	NaCl	0.31 b	0.30 b	0.47 c	0.24 b	0.29 b	0.83 c	0.70 c	0.28 b	1.24 a
	NaCl+Si	0.30 b	0.34 b	0.65 b	0.26 b	0.32 b	0.90 b	0.84 b	0.31 b	1.04 b
5BB	CK	0.35 a	0.36 ab	0.66 a	0.30 a	0.52 a	1.11 a	0.85 a	0.39 a	0.80 c
	NaCl	0.28 b	0.32 b	0.48 c	0.27 a	0.49 a	0.89 b	0.68 b	0.35 b	1.39 a
	NaCl+Si	0.27 b	0.41 a	0.59 b	0.29 a	0.51 a	0.96 b	0.70 b	0.40 a	1.16 b
CS/5BB	CK	0.43 a	0.45 a	0.74 a	0.36 a	0.49 a	1.06 a	1.15 a	0.38 a	1.11 b
	NaCl	0.33 b	0.36 b	0.49 c	0.28 b	0.42 a	0.85 c	0.89 b	0.34 b	1.52 a
	NaCl+Si	0.34 b	0.44 a	0.61 b	0.32 ab	0.47 a	0.93 b	0.96 b	0.40 a	1.20 b

[z] Cultivar abbreviations: CS, own rooted 'Cabernet Sauvignon'; own rooted 5BB; and CS grafted onto 5BB rootstock.
[y] Treatment abbreviation: CK, watered with water; NaCl, watered with 100 mM NaCl; and NaCl+Si, watered with 100 mM NaCl plus 2.0 mM $K_2SiO_3 \cdot 9H_2O$.
[x] Means within columns and cultivars followed by common letters do not differ at the 5% level by Duncan's multiple range test

Table 6. The nutrient decrease index (NDI) and nutrient restoration index (NRI) of various organs of three grape cultivars following 30 days of salt stress with and without silicon amelioration

Index	Cultivar [z]	Roots			Stems			Leaves		
		N	P	K^+	N	P	K^+	N	P	K^+
NDI	CS	40.38 c [y]	30.23 c	47.19 c	33.33 c	23.68 c	30.25 b	35.19 c	20.00 b	-13.76 a
	5BB	20.00 a	11.11 a	27.27 a	10.00 a	5.77 a	19.82 a	20.00 a	10.26 a	-73.75 c
	CS/5BB	23.26 b	20.00 b	33.78 b	22.22 b	14.29 b	19.81 a	22.61 b	10.53 a	-36.94 b
NRI	CS	-3.23 a [x]	13.33 a	38.30 b	8.33 b	10.34 b	8.43 a	20.00 c	10.71 b	-16.13 a
	5BB	-3.57 a [w]	28.13 c	22.92 a	7.41 a	4.08 a	7.87 a	2.94 a	14.29 a	-16.55 a
	CS/5BB	3.03 b	22.22 b	24.49 a	14.29 c	11.90 b	9.41 b	7.87 b	17.65 c	-21.05 b

[z] Cultivar abbreviations: CS, own rooted 'Cabernet Sauvignon'; own rooted 5BB; and CS grafted onto 5BB rootstock.
[y] Means within columns and cultivars followed by common letters do not differ at the 5% level by Duncan's multiple range test.
[x] The minus value suggests the N concentration in the NaCl treatment is higher than that in CK, however, the total N of the whole plant in the NaCl treatment is much lower than that in CK considering the plant growth was severely suppressed by NaCl stress.
[w] The minus value suggests the N concentration in the NaCl treatment is higher than that in the NaCl+Si treatment, however, the total N of the whole plant in the NaCl treatment is lower than that in the NaCl+Si treatment considering the plant growth was restored by Exogenously applied silicon.

Sauvignon', followed by grafted plants and '5BB' (Table 6). In most cases, the addition of the exogenous silicon significantly ($P < 0.05$) increased the phosphorus concentration of the roots and leaves compared with the NaCl treatments in all three cultivars, suggesting that exogenous silicon alleviated salt stress concerning the plant P concentration restoration in the roots and leaves. However, addition of the exogenous silicon did not significantly ($P < 0.05$) increase the phosphorus concentration of the stems compared with the NaCl treatments (Table 5).

The root and stem potassium concentrations were lower ($P < 0.05$) in the salt stress treatments. However, leaf potassium concentrations were significantly ($P < 0.05$) higher in the salt stress treatments compared with the other two treatments. In most cases, addition of exogenous silicon significantly ($P < 0.05$) increased the potassium concentration in the roots and stems, however, it significantly ($P < 0.05$) decreased the potassium concentration in the leaves compared to the NaCl treatment in all three grape cultivars (Table 5).

Ratio of Na^+ to K^+ in the NaCl treatment was significantly ($P < 0.05$) (over four times on average) higher than that in CK (Table 7), indicating that Na^+ extensively accumulated in the grapevines under the 100 mM NaCl stress (Table 3). Ratio of Na^+/K^+ in the NaCl +Si treatment was significantly ($P < 0.05$) lower than that in NaCl treatment (Table 7) for all plant tissues and all cultivars.

Photosynthetic parameters. As shown in Fig. 1, the photosynthetic parameters including *Tr*, *Gs*, *Pn* and *Ci* were significantly (*P*

Table 7. Ratio of Na^+ to K^+ in three grape cultivars following 30 days of salinity treatments.

Cultivar [z]	Treatment [y]	Roots	Stems	Leaves
CS	CK	1.53 a [x]	0.79 a	0.52 a
	NaCl	11.79 c	5.94 c	2.85 c
	NaCl+Si	7.15 b	4.02 b	1.78 b
5BB	CK	3.38 a	1.15 a	0.85 a
	NaCl	12.27 c	4.73 c	1.67 c
	NaCl+Si	8.59 b	3.36 b	1.30 b
CS/5BB	CK	2.61 a	0.91 a	0.50 a
	NaCl	10.73 c	5.29 c	1.89 c
	NaCl+Si	7.93 b	4.12 b	1.11 b

[z] Cultivar abbreviations: CS, own rooted 'Cabernet Sauvignon'; own rooted 5BB; and CS grafted onto 5BB rootstock.
[y] Treatment abbreviation: CK, watered with water; NaCl, watered with 100 mM NaCl; and NaCl+Si, watered with 100 mM NaCl plus 2.0 mM $K_2SiO_3 \cdot 9H_2O$.
[x] Means within columns and cultivars followed by common letters do not differ at the 5% level by Duncan's multiple range test.

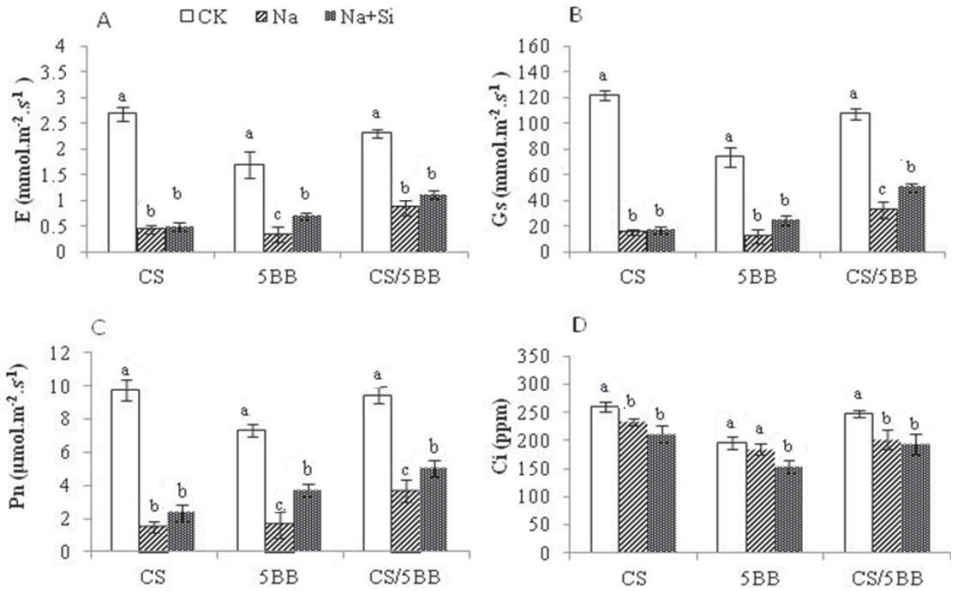

Figure 1. Transpiration (*Tr*), *stomatal conductance* (*Gs*), *net photosynthesis* (*Pn*) and interncellular CO_2 concentration (*Ci*) of three grape cultivars as affected by three salinity treatments.
Means within cultivars followed by common letters do not differ at the 5% level by Duncan's multiple range test. Cultivar abbreviations: CS, own rooted 'Cabernet Sauvignon'; own rooted 5BB; and CS grafted onto 5BB rootstock. Treatment abbreviation: CK, watered with water; NaCl, watered with 100 mM NaCl; and NaCl+Si, watered with 100 mM NaCl plus 2.0 mM $K_2SiO_3 \cdot 9H_2O$.

< 0.05) lower in the NaCl treatments for all three cultivars compared to control, except ***Ci*** in the '5BB'. Addition of the exogenous silicon did not significantly ($P > 0.05$) increase the four photosynthetic parameters in 'Cabernet Sauvignon' compared with the NaCl treatments (Fig 1). Addition of the exogenous silicon significantly ($P > 0.05$) increased *Tr* and *Pn* in '5BB', and *Gs* and *Pn* in the grafted plants compared to the NaCl treatments (Fig 1). For '5BB', *Ci* was lower for 'NaCl + Si' than for NaCl (Fig. 1).

Leaf anatomical structures. The leaf anatomical structures including the epidermis, the palisade tissue, the spongy tissue, and ratio of spongy tissue to palisade were significantly ($P < 0.05$) lower in the NaCl treatments for all three cultivars compared with the control, except that the epidermis thickness in 'Cabernet Sauvignon'(Table 8). The exogenous silicon significantly ($P < 0.05$) increased the epidermis, the palisade and the spongy tissue thickness in the grafted plants, and the palisade tissue thickness in 'Cabernet Sauvignon' compared with the NaCl treatments (Table 8).

Correlation coefficients (r). A correlation matrix for 16 variables is presented in Table 9. Of the 120 pairs of variables, 55 (45.8%) were significantly related in a linear manner. DHI was positively related to DW, N, K, *Tr*, *Gs*, and *Pn*, but negatively related to Na^+, Cl^-, ratio of Na^+ to K^+ and the leaf spongy tissue thickness. *Pn* was positively correlated with N, and negatively correlated with Na^+, Cl^- and ratio of Na^+ to K^+.

Discussion and Conclusion

The dry biomass of the roots, stems and leaves in three grape varieties were significantly reduced by the salt stress (Table 1 and 2), similar to a previous study with

grapevines (Singh et al., 2000). Exogenous Si resulted in an increase of the dry matter of the grapevines grown at high NaCl (Table 1), and supports previous observations with rice (Yeo et al., 1999), maize (Moussa et al., 2006), barley (Liang et al., 2005) and alfalfa (Wang et al., 2007).

Generally, inhibition of plant growth by salinity may either be due to osmotic increase in soil solution or to accumulation of excessive ions, e.g., sodium and chloride, in plant tissues (Parida and Dan, 2005; Parvaiz, and Satyawati, 2008). Sodium and chloride accumulation in all three cultivars were significantly increased under salinized conditions (Table 3). The leaf sodium IAII in '5BB' rootstock and the grafted plants were significantly lower than those in the nongrafted 'Cabernet Sauvignon' (Table 4), suggesting that the '5BB' rootstocks may ameliorate the ion absorption in the roots as well as transportation of the ions to the aboveground parts resulting in reduced accumulation of ions in the leaves (Fisarakis et al., 2001; Zheng et al, 2008). Accumulation of the sodium and chloride ions was higher in leaves than the other two plant organs (Table 4). The vacuole volume to the cell size was higher in the leaves compared to others organs (Parvaiz and Satyawati, 2008) and the leaves produce the largest total volume of the vacuoles (occupying 60-90% vacuole volume of the whole plants) compared to other plant parts in the growing season. The vacuole is one of the most important organelles for storage of toxins in the cells (Parida and Das, 2005). Therefore, it is rational that the sodium and chloride ions gradually accumulate in the leaves when concentrations of these two ions reach levels higher than the plants normally need (Parida and Das, 2005).

The addition of Si significantly ($P < 0.05$) reduced accumulation of Na and Cl ions in the grapevines under salt stress (Table 3). Similar phenomenon was observed in alfalfa (Wang and Han, 2007) and barley (Liang, 1999). Therefore, the application of both resistant rootstocks and exogenous silicon may alleviate salt stress in grapevines by reducing accumulation of the sodium and chloride ions in the plants.

Considering that the plant growth was severely inhibited by the salt stress (Table 1), the total reduced quantity of N and P in the whole plant by the NaCl treatments is likely much greater than the data presented in Table 5. Similarly, considering that the plant growth under the salt stress was partially restored by application of exogenous silicon (Table 1), impact of the exogenous silicon on total restoration of these elements in the whole grapevines will be greater than presented in Table 5.

Interestingly, concentrations of K^+, Na^+ and Cl^- were higher in the NaCl treatments compared to the control (Table 1 and Table 5). Since NaCl plants had lower concentrations of K in the roots and stems, but higher concentrations in the leaves (Table 5), it seems that NaCl altered the partitioning of K within the plant. Potassium accumulation in the plants in the presence of NaCl salinity enhanced K^+ absorbance, and therefore alleviated the adverse saline effects (Zheng et al., 2008). Both of potassium and silicon alleviated the adverse saline effects in plants (Ashraf et al., 2010; Zhu and Gong, 2014). It is rational that the leaf potassium concentration was significantly decreased in the NaCl+Si treatments compared to the NaCl stress (Table 5), suggesting that silicon may replace potassium for amelioration of adverse saline effects in grapevines.

Several studies reported that appropriate application of silicon enhanced photosynthesis of plants subjected to salt stress by protecting the photosynthetic apparatus and ultrastructure of leaf cells (Liang, 1999). This study showed that salinity significantly changed the palisade and spongy tissue thickness, and photosynthetic parameters (Table 8), compatible with observations in soybean, cotton and quinoa (Ma and Yamaji, 2006).

Salt stress affected the three cultivars differently (Table 1 and 2). The 100 mM NaCl treatment suppressed whole-plant growth

Table 8. The leaf anatomical structures of three grape cultivars following 30 days of salinity treatments.

Cultivar [z]	Treat-Ment [y]	Leaf epidermis thickness (μm)	Palisade tissue thickness (μm)	Spongy tissue thickness (μm)	Spongy tissue/Palisade tissue
CS	CK	11.77 a [x]	27.94 b	38.73 c	1.39 b
	NaCl	13.73 a	31.37 a	49.90 a	1.59 a
	NaCl+Si	12.75 a	30.39 a	42.86 b	1.41 b
5BB	CK	8.92 b	26.57 b	47.55 b	1.79 a
	NaCl	12.75 a	30.06 a	68.14 a	2.27 a
	NaCl+Si	11.28 a	29.89 a	64.22 a	2.15 a
CS/5BB	CK	9.80 c	28.92 b	32.35 c	1.12 b
	NaCl	16.18 a	35.78 a	65.69 a	1.84 a
	NaCl+Si	12.84 b	29.57 b	47.55 b	1.61 a

[z] Cultivar abbreviations: CS, own rooted 'Cabernet Sauvignon'; own rooted 5BB; and CS grafted onto 5BB rootstock.
[y] Treatment abbreviation: CK, watered with water; NaCl, watered with 100 mM NaCl; and NaCl+Si, watered with 100 mM NaCl plus 2.0 mM $K_2SiO_3 \cdot 9H_2O$.
[x] Means within columns and cultivars followed by common letters do not differ at the 5% level by Duncan's multiple range test.

by more than 30% (Table 2), indicating that these plants were susceptible to salt stress (Verma et al., 2010). Comprehensively considering the data presented in Tables 2, 4 and 6, the order of salt stress tolerance was '5BB' rootstock > grafted plants > 'Cabernet Sauvignon'.

'Cabernet Sauvignon' responded to exogenous Si, but whole-plant growth was still only 56% that of non-stressed plants (Table 2), which was less than '5BB' rootstock and grafted plants. The photosynthetic parameters (Fig. 1) indicated that 'Cabernet Sauvignon' was affected more by salt than '5BB' and the grafted plants, but 'Cabernet Sauvignon' also responded less to Si treatment. Considering extent of the salt stress injury and restoration by exogenous silicon (Table 1 and Table 2), the effectiveness of silicon restoration for the three cultivars was: '5BB' rootstock > grafted plants > 'Cabernet Sauvignon'.

Sixteen variables were used to evaluate the extent of salt stress and restoration of stress with exogenous silicon (Table 9). The daily height increment and the dry weight were reported in earlier studies (Yeo et al., 1999; Wang and Han, 2007). Our data demonstrated that both daily height increment and dry weight were correlated with nine and eight variables of the 15 variables, respectively (Table 9), indicating that these two indexes are reliable and practical for studying salt stress in grapevines. Other variables such as Na^+, Cl^-, N, K^+, ratio of Na^+/K^+, *Tr*, *Gs*, *Pn*, and the leaf spongy tissue thickness were also correlated with other variables, suggesting that these variables may be useful in future studies. However, phosphorus concentration, *Ci*, the leaf epidermis thickness, the leaf palisade tissue thickness, and ratio of the leaf palisade tissue thickness to the leaf spongy tissue thickness were correlated with few of the 15 variables (Table 9), indicating that these variables may not respond to both NaCl stress and exogenously applied silicon.

Interestingly, K^+ and Na^+ were negatively correlated, suggesting that absorption of K^+ may reduce Na^+ accumulation, but may not impact Cl^- accumulation in grapevines during salt stress.

Currently, salt resistant rootstocks are rarely used by the grape industry in China. This study suggests that use of the grafted plants

Table 9. Correlation matrix showing correlation coefficients between 16 variables following 30 days of salinity treatments.

	DW	Na^+	Cl^-	N	P	K^+	RNaK	*Tr*	*Gs*	*Pn*	*Ci*	LE	Pa	Sp	**P/S**
DHI	0.9157* [z]	-0.9154*	-0.8892*	0.9370*	0.5569	0.6840*	-0.9041*	0.9684*	0.9643*	0.9741*	0.5762	-0.5810	-0.4991	-0.6998*	-0.6194
DW		-0.3185	-0.4632*	-0.0178	0.3799*	-0.6262*	-0.8692*	0.8564*	0.8612*	0.9200*	0.2606	-0.7197*	-0.5595	-0.4630	-0.3195
Na^+			0.72818*	-0.6430*	-0.2510	-0.6262*	0.9185 *	-0.9111*	-0.9205*	-0.9444*	-0.4323	0.7737*	0.7245*	0.6995*	0.5205
Cl^-				-0.1821	-0.6184*	-0.2962	0.6980 *	-0.8578*	-0.8688*	-0.9293*	-0.2055	0.7167*	0.6340	0.5299	0.3642
N					-0.2500	0.5864*	-0.5876 *	0.9418*	0.9340*	0.9132*	0.6852*	-0.3240	-0.3242	-0.7454*	-0.7442
P						0.0425	-0.2651	0.4392	0.4555	0.5709	-0.3187	-0.5454	-0.4046	-0.0292	0.1218
K^+							-0.7996*	0.7169*	0.7024*	0.6560	0.4837	0.0257	-0.0320	-0.2323	-0.2795
RNaK								-0.8980*	-0.9070*	-0.9351*	-0.4134	0.7534 *	0.7096 *	0.7219 *	0.5519
Tr									0.9984*	0.9837*	0.6582	-0.5126	-0.4915	-0.7004	-0.6231
Gs										0.9859 *	0.6406	-0.5358	-0.5278	-0.6986*	-0.6055
Pn											0.5255	-0.6012	-0.5439	-0.6677 *	-0.5628
Ci												-0.0606	-0.1485	-0.7347*	-0.7960*
LE													0.9058*	0.5028	0.2061
P														0.5347	0.2020
S															0.9353*

[z] *, a mark indicates a significant *r*-value at the 5% level.
DHI, daily height increment.
DW, dry weight.
RNaK, ratio of Na^+ to K^+.
LE, the leaf epidermis thickness.
Pa, the leaf palisade tissue thickness.
Sp, the leaf spongy tissue thickness.
Pa/Sp, ratio of the leaf palisade tissue thickness to the leaf spongy tissue thickness.
$n = 9$ when correlation coefficients (r) were calculated between pairs of DHI, DW (of the whole plants), *Tr, Gs, Pn, Ci,* LE, Pa, Sp, Pa/Sp, Na^+, Cl^-, N, P, and K^+. At this time, average values from the root, stem and leaf of each treatment was used as 'a variable' for Na^+, Cl^-, N, P, and K^+.
$n = 27$, when correlation coefficients (r) were calculated between pairs of DW, Na^+, Cl^-, N, P, and K^+.

on resistant rootstocks plus application of the exogenous silicon should be an alternative way to ameliorate the adverse saline effects and to improve the productivity in the saline regions.

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