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ARTICLE

Effects of soil- and foliar-applied silicon on the resistance of grapevine plants to freezing stress

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ABSTRACT Grapes are frequently injured by freezing stress. Silicon (Si) is reported to reduce the effects of freezing on various crops. The main objective of this study was to elucidate the role of foliar- and soil-applied Si in enhancing grape (*Vitis vinifera* L.) tolerance to cold stress. The results indicated that the freezing stress dramatically decreased leaf fresh mass, relative water content, and caused an increased necrotic leaf area, but these effects were alleviated by both soil and foliar-applied Si. Foliar-applied Si reduced significantly damaging effects of freezing stress on maximum quantum yield of PSII after 2 and 96 h recovery after freezing treatment, while soil application of Si could not. This may be attributed to the enhancement of non-photochemical quenching, because of its effect on elevation of protective pigments; carotenoids, and more protection of PSII from photodamage following a foliar spray of Si. In addition, freezing stress increased membrane damage, as estimated by malondialdehyde content, while foliar Si application significantly decreased the membrane damage, because of an efficient scavenging by peroxidase, but soil application of Si could not. We conclude that foliar-applied Si can effectively alleviate adverse effects of freezing via maintenance of membrane integrity and alleviating photoinhibition during recovery. **Acta Biol Szeged 59(2):109-117 (2015)**

KEY WORDS

cold stress,
malondialdehyde,
non-photochemical quenching,
potassium metasilicate,
Vitis vinifera

Introduction

Cold stress includes chilling (<20 °C) and freezing (<0 °C) temperatures, and adversely influences the growth and development of plants (Waśkiewicz et al. 2014). Exposure to freezing increases the production of reactive oxygen species (ROS), which leads to damages to proteins, lipids, nucleic acids and carbohydrates (Suzuki et al. 2012). The plant cells respond to elevation in ROS levels by increasing the expression and activity of ROS-scavenging enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione peroxidase (GPX) and non-enzymatic antioxidants such as ascorbic acid (AsA) and glutathione (GSH) in order to maintain redox homeostasis (Miller et al. 2010). In addition, some plants are tolerant to cold stress, although most are not so tolerant to freezing, but can increase their freezing tolerance by being exposed to low temperatures, a process called "cold acclimatization" (Takahashi et al. 2013). Acclimatization engages the synthesis and accumulation of low-molecular-weight cryoprotective molecules and the alterations in the membrane lipid composition that contributes to an increase in freezing tolerance (Easlon et al. 2013). In contrast, the plants of tropical and subtropical regions, exhibit sensitivity to cold stress and usually lack the ability for cold acclimatization (Zhu et al. 2007). Exposure of plants to freezing stress increases leaf expansion and growth, and may lead to necrosis in sensitive plant species (Kumar et al. 2011). Plants possess a multitude of physiological and metabolic processes such as the production of osmolytes, and phytohormones to decrease cold-induced damage (Kaur et al. 2011).

Although Si has not been considered among the essential elements for higher plants, its uptake has been widely found to be beneficial in improving the biotic and abiotic stress tolerance (Ma and Yamaji 2006). The improvement of plant tolerance to cold stress by Si supplementation is achieved by maintenance of photochemical reactions and photosynthetic gas exchange as well as activation of antioxidant defense capacity (Liang et al. 2008; Waraich et al. 2011; Habibi 2014).

Grape (*Vitis vinifera* L.) is one of the most important and temperate fruit crops in the Mediterranean climate. It is most frequently damaged by freezing temperatures in spring, fall, or winter in many of the grape growing regions (Fennell 2004). Because of the fact that the freezing injury can result in decreased yield and substantial economic losses to grape

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growers, an understanding of the mechanisms involved in freezing tolerance of this species is very important. Silicon application to crops has been reported to enhance their tolerance of freezing stress; however, the underlying mechanisms have not been well identified (Liang et al. 2008). The main objective of this work was to investigate whether foliar- and root-applied Si are involved in freezing resistance in grapes. To the authors' knowledge, this is the first report to prove that Si is involved in grape resistance to low temperature stress. Furthermore, our results can contribute to research related to diminishing cold damage in agriculture applications.

Materials and Methods

Plant growth and Si treatments

Vitis vinifera, cultivar Bidaneh Sefid, was used as plant material in the current experiment. This cultivar is extensively grown in Iran. During dormancy, cuttings (middle parts of 2 year old shoot, with 35-40 cm length and four nodes) were rooted in humid sand crates in a controlled greenhouse with day/night temperature of 25 ºC /18 ºC, relative humidity of 45-55% and daily photon flux density (PFD) of about 1100-1200 µmol/m^2/s throughout the experimental period. In spring, cuttings of unique size were planted in the cylindrical plastic pots, two cuttings per pots. Pots were 14 cm in diameter and 105 cm in depth, filled with 15 kg sandy loam soil. For the basal fertilization, 200 mg nitrogen/kg soil as NH_4NO_3 and 50 and 62.5 mg phosphorus and potassium/kg soil as KH_2PO_4 were applied.

Soil Si-application

Before filling the pots, soils of Si treatments were fertilized with 0.43 g potassium metasilicate (K_2SiO_3)/kg soil (3.44 mmol/dm^3 soil; 2.73 mmol/kg soil). Control pots were treated with equimolar concentrations of KCl for balancing K amounts.

Foliar Si-application

The seven weeks after planting, half of the plants were sprayed with 10 mM K_2SiO_3 (pH adjusted to 5.8 with phosphoric acid). A drop of Tween 20 (0.05%, v/v) as surfactant was added to 500 ml of the spray solutions. Control plants were sprayed with Tween 20 and equimolar concentrations of KCl for balancing K amounts.

Freezing treatment

Ten days after the foliar-applied Si treatments, half of the control (untreated with Si) and half of the Si-treated plants were placed to a controlled environment chamber under a 12 h (1±1 °C) light (at 300 µmol/m^2/s photosynthetic photon flux)/12 h (–2±1 °C) dark cycle at 85% relative humid for 2 days. After the freezing treatment, all plants were returned to normal conditions as described above, to allow leaves to recover from freezing stress. Samples were taken 2 and 96 h after recovery after cold treatment.

Analysis of growth parameters

Leaves were harvested and washed with distilled water, blotted dry on filter paper and after determination of leaf fresh weight (LFW) they were dried at 70 °C for 48 h for determination of leaf dry weight (LDW). Relative water content (RWC) was measured and calculated according to Lara and co-workers (2003). Before harvest leaf chlorophyll fluorescence parameters were determined in the second or third youngest, fully developed leaves.

Measurements of chlorophyll fluorescence parameters

Chlorophyll fluorescence parameters were recorded using a portable fluorometer (OSF1, ADC Bioscientific, UK) for both dark adapted and light adapted leaves. Leaves were acclimated to dark for 30 min using leaf clips before measurements were taken. Initial (F_0), maximum (F_m), variable ($F_v = F_m - F_0$) fluorescence as well as maximum quantum yield of PSII (F_v/F_m) were recorded. Light adapted leaves were used for measurement of steady-state (F_s) and maximum (F'_m) fluorescence. Calculations were made for F'_0 ($F'_0 = F_0/[(F_v/F_m) + (F_0/F'_m)]$), photochemical quenching, qP $[(F'_m - F_s)/(F'_m - F'_0)]$ and non-photochemical quenching, NPQ $(1 - [(F'_m - F'_0)/(F_m - F_0)])$ (Krall and Edwards 1992).

Determination of total chlorophyll, carotenoids, anthocyanin, total free amino acids, carbohydrates and Si concentrations

Leaf concentrations of total Chl and carotenoids were determined after extraction of pigments in the cold acetone and allowing the samples to stand for 24 h in the dark at 4 ºC (Lichtenthaler and Wellburn 1985). Determination of anthocyanin contents was carried out using the method of Wagner (1979). To calculate the amount of anthocyanins, the extinction coefficient 33,000/mol/cm) was used and anthocyanin content were expressed as µmol/g FM. Content of total free α-amino acids was assayed using ninhydrin colorimetric method. Glycine was used for production of standard curve (Hwang and Ederer 1975). Soluble protein was estimated spectrophotometrically by the Bradford method (1976).

For determination of carbohydrates, leaves were homog-

Table 1. Effects of soil-applied Si on the leaf fresh weight (LFW), leaf dry weight (LDW), relative water content (RWC), necrotic leaf area, and the concentration of chlorophyll *a* and *b*, carotenoid, anthocyanin, soluble sugars, starch and the leaf Si in grape plants after 96 h recovery after freezing treatment. Data of each row indicated by the same letters are not significantly different (*Tukey's test*, $P < 0.05$). Data are the mean ± SD ($n = 6$).

	Control		96 h recovery	
	–Si	+Si	–Si	+Si
LFW (g/plant)	5.35±0.42 ^{ab}	5.79±0.75 ^{a}	3.56±0.38 ^{c}	4.71±0.12 ^{b}
LDW (g/plant)	0.72±0.11 ^{a}	0.74±0.09 ^{a}	0.63±0.12 ^{a}	0.66±0.13 ^{a}
RWC (%)	74.7±4.78 ^{ab}	75.4±3.57 ^{a}	58.0±3.20 ^{c}	67.9±1.07 ^{b}
Necrotic leaf area (%)	00.0±00.0 ^{c}	00.0±00.0 ^{c}	5.00±1.30 ^{a}	2.50±0.72 ^{b}
Chl *a* (mg/g FW)	6.15±0.66 ^{a}	5.90±0.97 ^{a}	4.04±0.36 ^{b}	4.27±0.22 ^{b}
Chl *b* (mg/g FW)	2.18±0.12 ^{a}	2.16±0.52 ^{a}	2.06±0.43 ^{a}	2.23±0.19 ^{a}
Carotenoid (mg/g FW)	1.92±0.24 ^{a}	1.72±0.37 ^{a}	1.68±0.51 ^{a}	1.75±0.21 ^{a}
Anthocyanin (μmol/g FW)	2.38±0.68 ^{a}	3.06±0.94 ^{a}	3.22±1.00 ^{a}	2.98±0.13 ^{a}
Soluble sugars (mg/g FW)	17.3±3.2 ^{b}	15.7±2.15 ^{b}	24.1±3.05 ^{a}	25.8±4.02 ^{a}
Starch (mg/g FW)	122±14.0 ^{ab}	132±14.3 ^{a}	101±10.2 ^{b}	98±11.3 ^{b}
Amino acids (μmol/g FW)	5.15±0.35 ^{c}	5.02±0.74 ^{c}	6.80±0.29 ^{b}	8.80±1.07 ^{a}
Leaf Si (mg/g DW)	1.09±0.22 ^{b}	3.03±0.25 ^{a}	0.96±0.23 ^{b}	3.18±0.36 ^{a}

enized in 100 mM phosphate buffer (pH 7.5) at 4 ºC, after centrifugation at 12 000 *g* for 15 min, supernatant was used for determination of total soluble sugars whereas the pellets were kept for starch analysis (Magné et al. 2006). The dried powdered leaf samples were ashed in a muffle oven at 500 ºC for 5 h. The ashes were dissolved in diluted HCl at about 100 ºC. Diluted samples were prepared for determination of Si (Jaiswal 2004) using Inductively-Coupled Plasma-Atomic Emission Spectrometry (ICP-AES, INTEGRA XL2, GBC, Australia).

Assay of antioxidant enzyme activities and related metabolites

The activities of superoxide dismutase (SOD, EC 1.15.1.1) and peroxidase (POD, EC 1.11.1.7) were determined in leaves harvested in the middle of the day according to the methods described elsewhere (Habibi and Hajiboland 2012). Lipid peroxidation was estimated from the amount of malondialdehyde (MDA) formed in a reaction mixture containing thiobarbituric acid. The hydrogen peroxide (H_2O_2) contents in the leaves were assayed according to the method of Velikova and co-workers (2000). Leaves were homogenized in ice bath with 0.1% (w/v) TCA. The extract was centrifuged at 12 000 g for 15 min, after which to 0.5 ml of the supernatant was added 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) and 1 ml of 1 M KI, the reaction was performed for 1 h in the dark and measured spectrophotometrically at 390 nm. The content of H_2O_2 was given on a standard curve.

Measurement of leaf necrotic area

The percentage of necrotic area was calculated by measuring separately green and necrotic leaf area according to Irigoyen et al. (1996).

Statistical analysis

Experiment was performed according to a factorial design on the basis of Completely Randomize Design (CRD) with 4 pots as 4 independent replications. Statistical analyses were carried out using Sigma stat (3.5) with Tukey's test ($P < 0.05$).

Results

Plant biomass

Soil Si-application

A significant loss of leaf fresh weight (LFW) was observed when the plants were exposed to freezing shock; however, the decrease extent in the silicon treatments was less than that in the non-silicon treatments. As shown in Table 1, cold stress did not influence the leaf dry weight (LDW) in the grape plants under both silicon and no silicon supplementation. Soil-applied Si decreased significantly damaging effects of cold on relative water content (RWC), accompanied by an increase in LFW (Table 1). Cold alone increased necrotic leaf area by 5% after treatment for 96 h recovery, but the increase was only 2.5% when silicon was applied.

Foliar Si-application

Similar results were achieved for the foliar-applied silicon

Table 2. Effects of foliar-applied Si on the leaf fresh weight (LFW), leaf dry weight (LDW), relative water content (RWC), necrotic leaf area, and the concentration of chlorophyll *a* and *b*, carotenoid, anthocyanin, soluble sugars, starch and the leaf Si in grape plants after 96 h recovery after freezing treatment. Data of each row indicated by the same letters are not significantly different (Tukey's test, $P < 0.05$). Data are the mean ± SD ($n = 6$).

	Control		96 h recovery	
	–Si	+Si	–Si	+Si
LFW (g/plant)	5.07±0.62 ^{a}	5.12±0.35 ^{a}	4.02±0.31 ^{b}	4.94±0.13 ^{a}
LDW (g/plant)	0.70±0.08 ^{a}	0.68±0.08 ^{a}	0.62±0.13 ^{a}	0.60±0.10 ^{a}
RWC (%)	70.1±2.70 ^{ab}	71.6±4.30 ^{a}	55.0±4.58 ^{c}	63.4±1.99 ^{b}
Necrotic leaf area (%)	00.0±00.0 ^{c}	00.0±00.0 ^{c}	4.50±1.10 ^{a}	1.30±0.42 ^{b}
Chl *a* (mg/g FW)	5.80±0.52 ^{a}	6.07±0.40 ^{a}	3.74±0.12 ^{b}	4.02±0.26 ^{b}
Chl *b* (mg/g FW)	2.11±0.44 ^{a}	2.30±0.24 ^{a}	2.36±0.40 ^{a}	2.21±0.31 ^{a}
Carotenoid (mg/g FW)	2.02±0.41 ^{c}	2.78±0.32 ^{b}	3.12±0.31 ^{b}	3.77±0.11 ^{a}
Anthocyanin (µmol/g FW)	2.11±0.48 ^{b}	2.75±0.34 ^{b}	2.28±0.60 ^{b}	3.85±0.40 ^{a}
Soluble sugars (mg/g FW)	16.0±2.2 ^{b}	15.8±3.2 ^{b}	26.2±3.65 ^{a}	27.5±4.80 ^{a}
Starch (mg/g FW)	114±17.5 ^{ab}	120±19.1 ^{a}	79±15.3 ^{c}	82±13.6 ^{bc}
Amino acids (µmol/g FW)	6.03±0.61 ^{b}	5.92±0.70 ^{b}	6.80±0.19 ^{b}	7.93±0.47 ^{a}
Leaf Si (mg/g DW)	0.89±0.30 ^{b}	2.76±0.37 ^{a}	0.92±0.13 ^{b}	3.28±0.58 ^{a}

treatments (Table 2), except that the necrotic leaf area percentage was much lower than in the soil-applied Si treatments.

Effect of silicon on cold-induced changes of pigments, solutes, starch and Si concentrations

Soil Si-application

Grape plants that had been exposed to the freezing shock had significantly lower Chl *a* concentration, and soil-applied Si did not affect the Chl *a* concentration in the grape seedling under both freezing and control conditions. Soil-applied Si had no effect on Chl *b*, carotenoid and anthocyanin concentrations, regardless of temperature treatment (Table 1). Concentrations of soluble sugars in the leaves were increased by freezing stress, accompanied by a decrease in the concentration of starch (Table 1). No significant increases of soluble sugars and starch concentrations were found by soil application of Si under both freezing and normal temperatures. Cold stress significantly increased the amino acids concentrations. Silicon-supplied plants exhibited the higher amino acids concentrations as compared with those without application of silicon under cold conditions. Silicon content was increased by soil application of Si, but it was not affected by freezing treatment during all treatment periods.

Foliar Si-application

The concentration of Chl *a* was significantly reduced when the plants were exposed to freezing treatment in comparison with the control. Silicon-supplied plants showed the higher carotenoid and anthocyanin concentration as compared with those without application of silicon under cold stress conditions (Table 2). Foliar-applied Si had no effect on Chl *b* and anthocyanin concentrations under both freezing and control conditions.

Compared to the soil-applied Si treatments, similar results were obtained for soluble sugars, starch, amino acids and Si in the foliar-applied Si treatments (Table 2).

Effect of silicon on freezing-induced changes of chlorophyll fluorescence parameters

Soil Si-application

The results showed that freezing treatment decreased the leaf F_v/F_m ratio (Fig. 1). However, an increase in F_v/F_m was observed in cold-stressed plants upon Si application but this change was negligible. Soil-supplied Si had no effect on photochemical quenching (qP) and non-photochemical quenching (NPQ) parameters in both Si and non-Si treated leaves during recovery after freezing treatment (Fig. 1).

Foliar Si-application

Maximum quantum yield of PSII (F_v/F_m) was decreased by freezing treatment after 2 h recovery in the freezing-treated leaves (Fig. 2). Although, Si application ameliorated this effect and decreased significantly damaging effects of cold on F_v/F_m. Therefore, compared to soil-applied silicon treatments, foliar-applied silicon significantly increased the F_v/F_m ratio after 2 and 96 h recovery after freezing treatment. The qP of leaves showed no change in response to both freezing and Si treatment. In contrast, the NPQ of the grape plants was significantly elevated after freezing, and the most marked increase in NPQ was observed for Si-supplemented leaves during 2 h after freezing (Fig. 2). During recovery, NPQ gradually decreased in the Si-supplemented leaves, but not

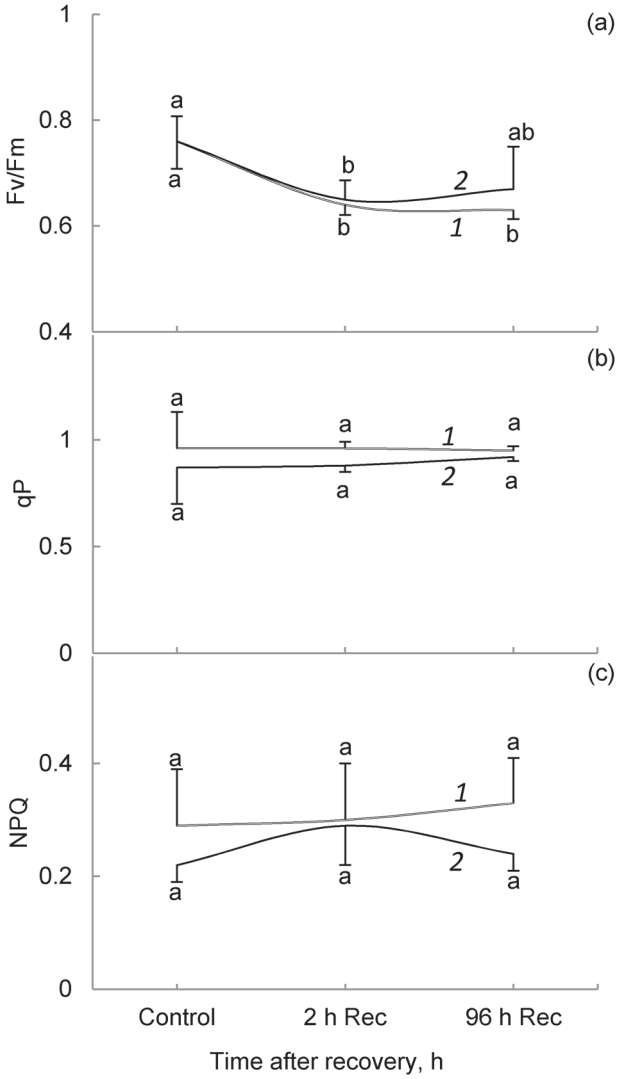

Figure 1. Changes in the maximum quantum yield of PSII (F_v/F_m), photochemical quenching (qP) and non-photochemical quenching (NPQ) in grape plants grown with soil-applied Si (a, b, c) after 2 and 96 h recovery after freezing treatment. Bars indicated with the same letter are not significantly different (Tukey's test, $P < 0.05$). Data are the mean ± SD ($n = 6$). 1 = –Si, 2 = +Si.

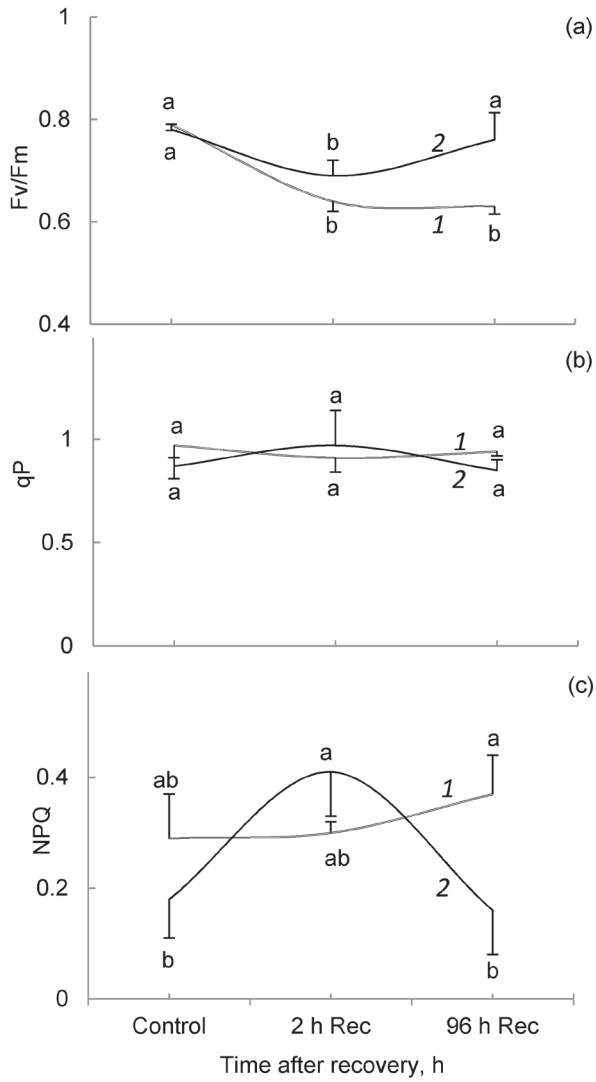

Figure 2. Changes in the maximum quantum yield of PSII (F_v/F_m), photochemical quenching (qP) and non-photochemical quenching (NPQ) in grape plants grown with foliar-applied Si (a, b, c) after 2 and 96 h recovery after freezing treatment. Bars indicated with the same letter are not significantly different (Tukey's test, $P < 0.05$). Data are the mean ± SD ($n = 6$). 1 = –Si, 2 = +Si.

in the non-Si-treated ones. There was a significant correlation between the concentration of carotenoids and the F_v/F_m ratio ($r = 0.87$, $P < 0.01$ in cold+Si treatment; Fig. 4).

Effect of silicon on cold-induced changes of antioxidants and membrane stability

Soil Si-application

Freezing dramatically increased the superoxide dismutase (SOD) and peroxidase (POD) activities. Compared with cold treatment alone, these enzymes activities were not affected after 2 and 96 h recovery after freezing treatment, by supplementary silicon (Fig. 3). Freezing significantly raised the leaf hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents, but the contents of these metabolites were not changed by Si during all treatment periods.

Foliar Si-application

The activities of SOD and POD enzymes increased under freezing stress and foliar-applied Si caused an additional

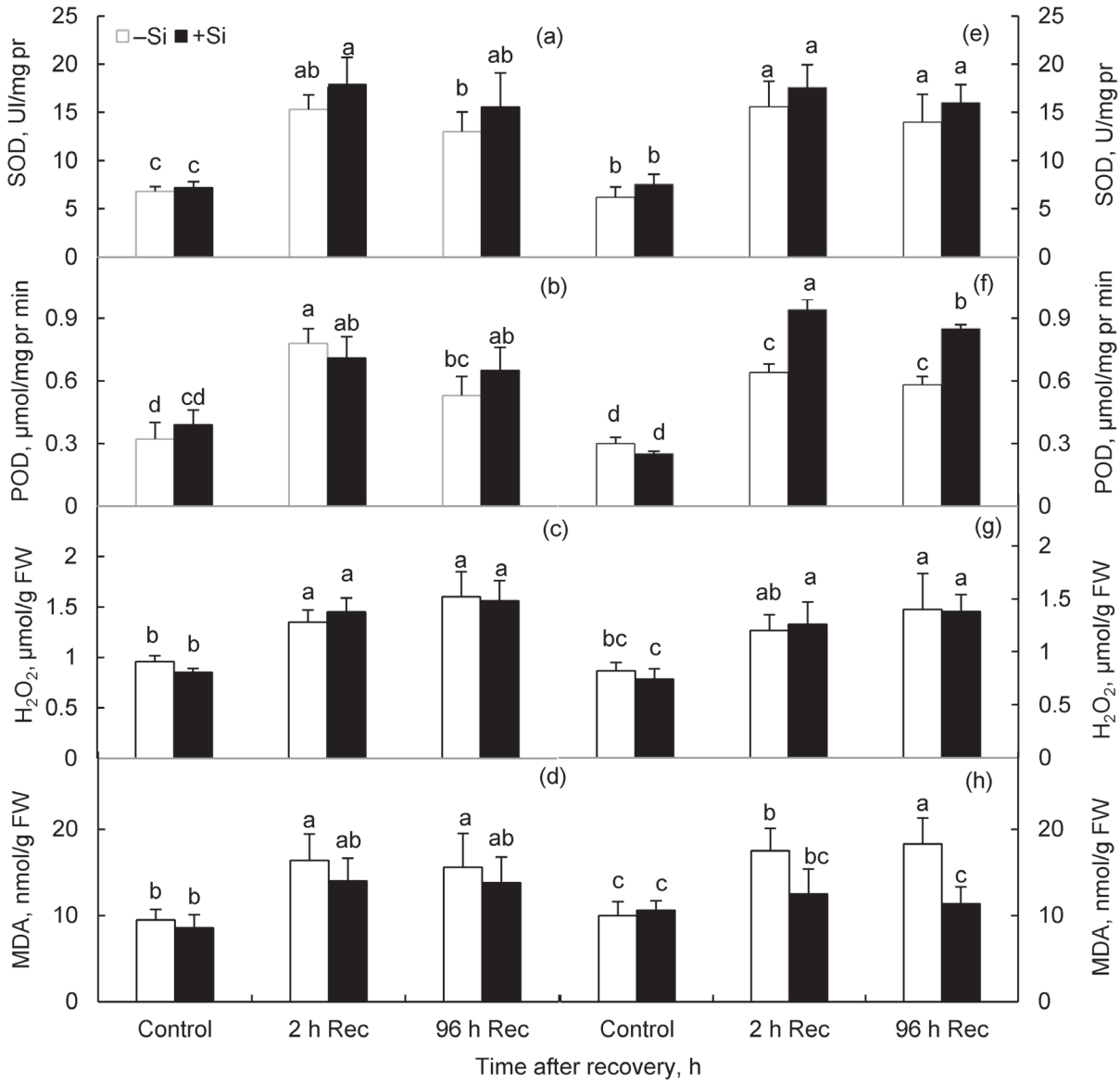

Figure 3. Changes in the specific activity of superoxide dismutase (SOD), peroxidase (POD), and the concentration of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) in grape plants grown with soil-applied Si (a, b, c, d) and foliar-applied Si (e, f, g, h) after 2 and 96 h recovery after freezing treatment. Bars indicated with the same letter are not significantly different (Tukey's test, $P < 0.05$). Data are the mean ± SD ($n = 6$).

significant increase of POD. In addition, H_2O_2 content was increased 2 and 96 h recovery after freezing. Cold stress induced membrane damage as shown by higher MDA content. However, the foliar-sprayed Si to the cold-stressed leaves significantly reduced MDA content compared with the corresponding freezing-treatment with no Si added. A positive correlation was found between the concentration of MDA and the percentage of necrotic leaf area ($r = 0.66$, $P < 0.05$ in cold treatment; $r = 0.81$, $P < 0.01$ in cold+Si treatment; Fig. 4).

Discussion

In this study, reduction of RWC under freezing stress was alleviated by both leaf- and root-applied Si, accompanied by an increase in LFM. In support of this, Yin et al. (2013) and Liang et al. (2008) showed the beneficial effects of silicon on the growth of *Sorghum bicolor* under salt stress and wheat cultivars under freezing stress conditions, respectively. Pos-

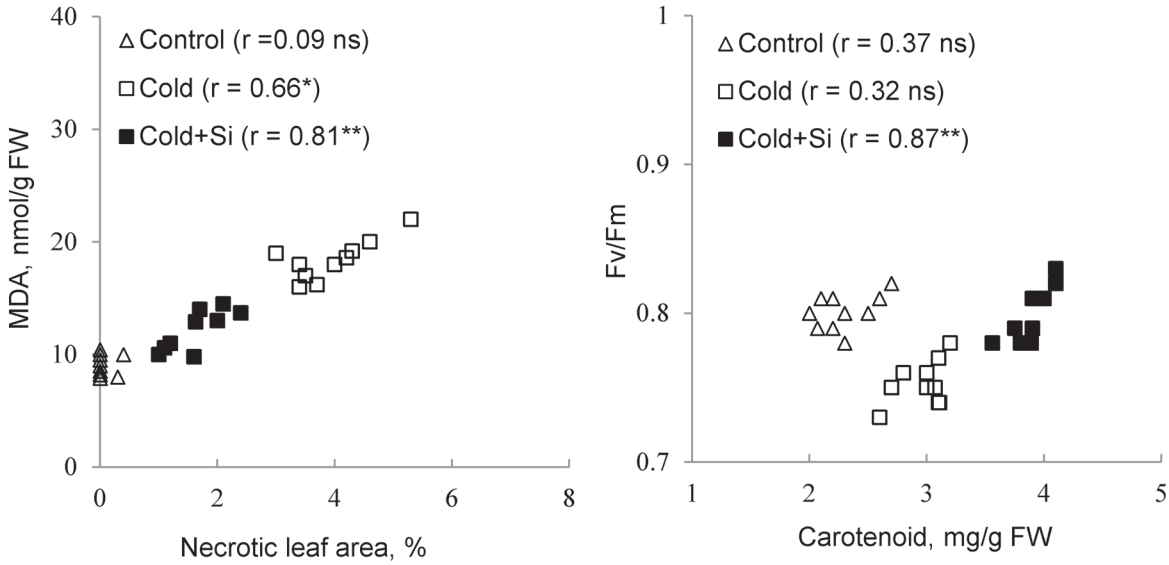

Figure 4. Correlations between the concentration of malondialdehyde (MDA) and the percentage of necrotic leaf area and between the concentration of carotenoid and the maximum quantum yield of PSII (F_v/F_m) in grape plants grown with or without foliar-applied Si after 96 h recovery after freezing treatment. ns, *, and **: non-significant, significant at the 5% and 1% levels of probability, respectively.

sible mechanisms of Si-mediated improvement of RWC in cold-stressed grape may be attributed to the physical role of Si deposited on the leaf surfaces (Cooke and Leishman 2011) and/or accumulation of osmosis-regulated compounds such as soluble sugars and free amino acids. In the present study, though an expected enhancement in soluble sugars and free amino acids levels under freezing stress, Si application caused a significant stimulation in free amino acids levels. Accumulation of free amino acids can function as osmolytes to maintain cell turgor and have the ability to protect membranes from stress damage (Krasensky and Jonak 2012).

Leaf necrosis is considered as a typical external sign of cold injury in cold-sensitive plants. In this study, pre-Si treatment produced a significant reduction of the leaf area lost by freezing, the necrotic leaf area being lower in foliar-applied Si treatment than in soil-applied Si plants under cold treatment. This may be explained by enhancement of Si deposition in the cell walls of foliar-applied treatment, is immobilized and unavailable for redistribution (Samuels et al. 1991), and operates as a mere physical barrier in the cell walls.

Several monocots are considered silicon accumulators and display active absorption through their roots. However, many dicots are not accumulators of silicon and display passive absorption (Mitani and Ma 2005). The results from this study indicated that Si content was increased by both foliar and soil application of Si in grape plants. The present results agree with previous reports that the leaves from Si-sprayed or root-fed grape plants exhibits deposition of Si and generally higher than on untreated plants (Bowen et al. 1992).

In the present work, freezing stress significantly reduced F_v/F_m, but foliar-applied Si ameliorated this effect. The results from this study clearly suggest that foliar-applied Si can reduce significantly damaging effects of freezing stress on F_v/F_m, while soil application of Si cannot. This may be attributed to the enhancement of NPQ and more protection of PSII from photodamage. F_v/F_m is affected by both photochemical and non-photochemical factors, and the reduction of F_v/F_m may be due to a decrease in reaction centers capable of photochemistry or un-reversed NPQ (Baker and Rosenquist 2004). Based on the current results, the increase in NPQ was reversible in the foliar-applied treatment during recovery, but not in the soil-applied treatment. This can be explained by increasing of the protective pigments, such as carotenoid and anthocyanin leading to the protection of PSII from damage. In the foliar-Si-supplied leaves, the positive correlation between the concentration of carotenoids and the F_v/F_m ratio confirmed the idea that the synthesis of protective pigments, such as carotenoid have evolved against the stress induced damage to cellular components (Huang et al. 2010).

There is data supporting that positive correlation between higher activities of antioxidant enzymes and freezing tolerance (Zhang et al. 2011; Kishimoto et al. 2014). In the present work, freezing stress increased the membrane damage, as estimated by MDA (an end product of membrane lipid peroxidation), but foliar Si application significantly reduced the membrane damage, but not in the soil-Si-supplied leaves, because of an efficient scavenging by POD in the foliar-Si-supplied leaves (Fig. 2). In the foliar-Si-supplied leaves, the

significant correlation between the concentration of MDA and the percentage of necrotic leaf area confirmed the idea that, even if active oxygen formation was increased, the defense mechanisms had sufficient capacity, with the result that damage was not apparent. Similarly, it has been reported that Si increases the activity of antioxidant enzymes (Liu et al. 2009; Habibi and Hajiboland 2013), which in turn protect plants against ROS generation and lipid peroxidation. Therefore, results indicated that at least POD activity might be a factor that determines the higher tolerance of foliar-Si-supplied leaves to freezing injury.

In conclusion, under freezing conditions, plants showed an increase in soluble sugars and amino acids concentrations in the leaves, but these mechanisms involved in the acclimatization to cold stress, could not ameliorate the negative effect of freezing stress on RWC and FW. The study reported here provides novel information regarding the effects of Si on photosynthetic performance of grapevine plants. Foliar-applied Si decreased significantly damaging effects of cold on F_v/F_m, while soil application of Si could not. This can be explained by enhancement of efficiency for dissipation of excess photon energy in the PSII antenna, determined as non-photochemical quenching, and more protection of PSII from photodamage following a foliar spray of Si at a high concentration. The significant effect of foliar-applied Si on suppression of cold damage in grape may be attributed to the physical role of Si deposited on the leaf surfaces and/or activation of antioxidant enzymes reflected in the stable amount of lipid peroxidation while MDA content dramatically increased in cold-stressed leaves in the soil-applied Si treatments.

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