

Effect of Water Stress and Sodium Silicate on Antioxidative Response in Different Grapevine (*Vitis vinifera* L.) Cultivars

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ABSTRACT

Drought is still accounted as a serious agronomic problem and also one of the most important factors contributing to crop yield loss. The effect of sodium silicate (Na_2SiO_3 , abbreviated as Si) was investigated on the four major antioxidant enzyme activities and five other relevant components in two different grapevine cultivars (*Vitis vinifera* L., cvs Mish Pestan and Sahebi, as tolerant and sensitive cultivars) under drought stress. The experiment was performed in a completely randomized design including three treatments i.e. the control, drought with no Si, and Si-drought (0.004M sodium silicate kg^{-1} soil), with three replications in a greenhouse. The results indicated that Si partially offset the negative impacts of drought stress by increasing the tolerance of grapevine by rising antioxidant enzyme activities and soluble protein content. Si treatment significantly affected the enzyme activities in both cultivars. Water stress induced a decrease in total Chlorophyll (Chl) and total protein contents, which was much larger in no-Si stress than in Si treatment. The results indicated that sodium silicate might decrease drought stress damages by raising the antioxidant enzymes activity.

Keywords: Antioxidant enzymes, Drought stress, Grapevine, Silicon.

INTRODUCTION

Drought stress is an important threat to plant growth and sustainable agriculture worldwide (Lipiec *et al.*, 2013). Reactive Oxygen Species (ROS) cause oxidative damage and are generated by various factors in plants. During the period of normal metabolism, ROS are induced by photosynthesis; and photo-oxidative alteration can happen when ROS production exceeds antioxidant content (Molassiotis *et al.*, 2006). Plants have evolved chains of enzymatic and non-enzymatic antioxidant systems to tackle with drought stress and to avoid photo-oxidative injury, either by stress elusion or by stress tolerance (Jung, 2004). The capability of tissues to cope with drought stress might be related to their strength to scavenge ROS by raising the

activities of the antioxidant enzymes during water loss (Mittler, 2002). In environmental stresses conditions such as drought, high activities of antioxidant enzymes are significant for plants to bear stresses. SOD is accounted as the most important enzymatic antioxidant and catalyzes dismutation of the superoxide anion ($\text{O}_2^{\cdot-}$) into hydrogen peroxide (H_2O_2), which is then decomposed to water by CAT and/or glutathione peroxidase. Silicon (Si) application exhibits beneficial effects on plant development; however, its effects on the phytohormone and enzymatic antioxidant regulation have not been fully comprehended (Kim *et al.*, 2014). Si plays an important role in mitigating both biotic (pests, insects, pathogens) and abiotic (drought, salinity, metal, chilling, freezing, radiation) stresses. However, in spite of its various beneficial

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roles in plants, it is not yet considered as a necessary mineral element for plants (Tripathi *et al.*, 2014). Si is never found in a free form, it is always combined with other elements, usually making oxides or silicates. Plants absorbed Si in the form of uncharged silicic acid. Si is ultimately irreversibly precipitated throughout the plant as amorphous silica. Results on the beneficial effects of Si in raising the tolerance to both biotic and abiotic stresses have been reported in several plants. Si causes to tolerate abiotic stress in sorghum (Hattori *et al.*, 2005), cucumber (Jiao-jing *et al.*, 2009), grapevine (Soylemezoglu *et al.*, 2009), and wheat (Tale-Ahmad and Haddad, 2011). It has also been related to affect the antioxidant enzyme activity. Soylemezoglu *et al.* (2009) reported that activity of CAT is significantly increased by salinity and boron+salinity treatments. Moreover, in the presence of Si, the CAT activity is decreased in such treatments and Si supply has been caused to reduce SOD activity in boron stressed in grapevine, while this activity is increased in boron+salinity treatment. Si ameliorated the decrease in dry weight under drought stress conditions in sorghum. Such treated plants illustrated a lower shoot to root ratio, indicating the simplification of root growth and the maintenance of the photosynthetic rate and stomatal guidance at a higher level compared with plants grown without Si application (Hattori *et al.*, 2005). Many plant species naturally accumulate Pro and GB as major organic osmolytes, when subjected to different abiotic stresses. These materials are imagined to play adaptive roles in interceding osmotic adjustment and protecting subcellular structures in plants under stress. GB is abundant basically in chloroplasts where it plays a vital role in arrangement and protection of thylakoid membrane, therewith maintaining photosynthetic efficiency (Genard *et al.*, 1991).

Si effects have been reported to reduce the drought stress damages in grasses. On the other hand, Si investigation affecting free radicals and osmolytes has not been

previously reported in grapevine to evaluate ROS. The objective of the present research project was to investigate the effects of Si on two different grapevine cultivars (named Mish Pestan and Sahebi) under drought stress.

MATERIALS AND METHODS

Growth Conditions and Treatments

Grape saplings were grown as potted vine in a greenhouse of Agricultural Biotechnology Department, Imam Khomeini International University, with three treatments including Control (C), Drought (D), and Sodium Silicate+Drought (D-Si) treatments. The soil structure was a mixture of peat/sand/clay (1/2/3). The soil was mixed sufficiently, divided into several parts, each of 10 kg weight, and then sodium silicate (0.004M of sodium silicate kg^{-1} soil) was added to D-Si treatment (Soylemezoglu *et al.*, 2009). Drought stress was imposed by withholding water application in treated plants at the developmental stages of 10 to 12 leaves, coinciding with unripe grapes developmental stage. This growth stage was adjusted by soil water potential of -1.0 MPa as measured by gypsum block. The experiment was carried out based on a completely randomized design with three replications. Leaf samples were collected 24 hours after the soil water potential to -1.0 MPa (almost between the 4th and 5th day after irrigation), and were frozen in liquid N₂ immediately until analysis.

Measurements of Leaf Water Content Ratio (LWCR) and Chl

To evaluate LWCR, the leaves were placed in distilled water for 24 hours and weighed to obtain the turgid weight. The LWCR of leaves was calculated by the following equation:

$$LWCR = \frac{a-b}{b} \times 100$$

a: Turgid weight, *b*: Fresh weight.

Leaf Chl was extracted in 80% acetone and the absorbance was read spectrophotometrically at 663 and 645 nm. The content of Chl was evaluated using the formula proposed by Arnon (1949).

Measurement of Total Soluble Protein Contents

In order to extract total soluble protein, 1 gr leaf tissue was homogenized in 3 ml of extraction buffer including 0.5 M Tris-HCl buffer (pH 7.6) and 0.001M sodium dimethyldithiocarbamate. The homogenate was centrifuged (Beckman Culter, Allegra-64R) at 18,000 rpm for 20 minutes to collect the supernatant as the source of enzyme assays. All the extraction steps were carried out at 4°C. Enzyme activity was estimated spectrophotometrically in laboratory conditions at 25°C. Total soluble protein was used for determination of protein content by the method of Bradford (1976). Bovine Serum Albumin (BSA) was employed in different concentrations to draw a standard curve. Native-PAGE was prepared to analyze the antioxidant enzyme activity according to Laemmli (1970).

Enzyme Activity Assay

SOD (EC 1.15.1.1) activity was determined by measuring the inhibition in the photochemical reduction of nitroblue tetrazolium at 560 nm as described by Beauchamp and Fridovich (1971). The enzyme activity was expressed as units/mg protein. CAT (EC 1.11.1.6) activity was determined by measuring H₂O₂ consumption at 240 nm for 3 min according to Aebi (1984) method and the enzyme activity was expressed as $\Delta 240 \text{ mg}^{-1} \text{ protein min}^{-1}$. POD (EC 1.11.1.7) activity was determined by measuring peroxidation of H₂O₂ with guaiacol as an electron donor (Chance and Maehly, 1955). GPX (EC 1.11.1.7) activity

was determined by measuring the reduction of guaiacol at 470 nm as described by Urbanek *et al.* (1991). SOD activities were analyzed by Native-PAGE (10% separating and 4% stacking gels). Specified staining of SOD was done according to the method of Beauchamp and Fridovich (1971). CAT and POD activity were estimated on Native-PAGE using 6% separating and 4% stacking gels. POD isoforms were detected according to the method of Hart *et al.* (1971), and finally Robertson *et al.* (1987) description was employed to illustrate specified staining of CAT.

H₂O₂, Pro and GB Determination

H₂O₂ content was determined using the methodology described by Nakano and Asada (1987). 0.5 gr of fresh plant material was homogenized in 5 ml of 1% trichloroacetic acid. The homogenate was centrifuged at 12,000 rpm for 15 minutes to use supernatant for H₂O₂ content determination. Pro was analyzed according to the method of Bates *et al.* (1973). Approximately 0.5 gr of fresh plant material was homogenized in 10 ml of 3% aqueous sulphosalicylic acid and filtered. Two ml of filtrate was mixed with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid in a test tube. The mixture was placed in a water bath for 1 hour at 100°C. The reaction mixture was then extracted with 4 ml toluene and the chromophore containing toluene was aspirated, cooled to room temperature, and the absorbance was measured at 520 nm with spectrometer. The amount of GB was estimated according to the method of Grieve and Grattan (1983). The dried plant material was finely ground, mechanically shaken with 20 ml deionized water for 48 hours at 25°C. The samples were then filtered and filtrates were diluted 1:1 with 2M H₂SO₄. Aliquots were kept in centrifuge tubes and cooled in ice water for 1 hour. Cold KI-I₂ reagent was added and the reactants were gently stirred with a vortex mixer. The tubes were stored at 4°C for 16 hours and then centrifuged at



15,000 rpm for 20 minutes at 0°C. The supernatant was carefully aspirated. The remaining crystals were dissolved in 9 ml of 1,2 dichloroethane. After 2 hours, the absorbance was measured at 365 nm using GB as standard.

Statistical Analysis

The experimental data were analyzed in a completely randomized design with three replications using software package of SPSS, version 16. The GLM procedure was employed and the output treatment means were compared by Duncan's multiple range test. The correlation of experimental characteristics was also analyzed by the aforementioned SPSS software.

RESULTS

LWCR of Sahebi cultivar leaves was significantly modified by different treatments in the present research project. The *LWCR* of C, D-Si, and D treatments were 19.5, 22.1 and 18.7% in Mish Pestan and 27.4, 13.1 and 9.2% in Sahebi cultivars, respectively (Figure 1). Drought stress

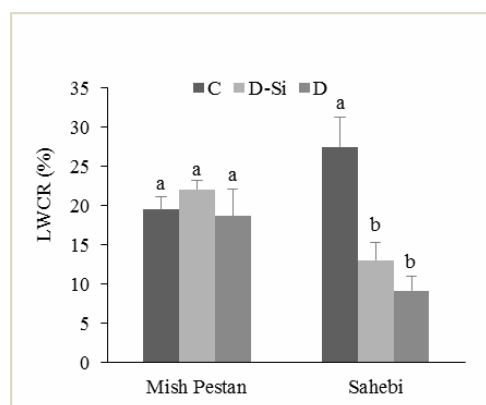


Figure 1. Effect of Si on Leaf Water Content Ratio (*LWCR*) of two grapevine cultivars under drought stress; data are mean \pm standard error of three replicates; bars with different letters are significantly different at the $P < 0.01$ level. (C: Control; D-Si: Si-Drought, D: Drought treatments).

caused reduction in *LWCR* to 4.1 % in Mish Pestan and 66.4 % in Sahebi cultivars in D compared to C treatment. The water content of grape leaves decreased under drought stress. Under drought stress, the Si applied plants maintained higher *LWCR* compared to those without application of Si, indicating that application of Si might be caused to improve the water status of stressed grape plants. The correlation was significantly different between *LWCR* and H_2O_2 , total soluble protein and prolin content (Table 1). Drought stress did not significantly decline the pigment content. The contents of Chl a, b, and total Chl were respectively 5.56, 2.7, and 11.61 $mg\ gr^{-1}$ FW in Mish Pestan cultivar, and 6.1, 3.47, and 13 $mg\ gr^{-1}$ FW in Sahebi cultivar in the D treatment, while Si treatment caused an increase in these contents under drought stress. Significant changes were observed between both cultivars in Chl a under D treatment, but no significant changes were observed between Chl b and total Chl content. As it has been shown in Table 1, positive correlation was illustrated for Chl a, b and total Chl. Moreover, total soluble protein content was significantly decreased in the D treatment (Figure 2). The contents of total soluble protein were 89.6 $\mu g\ g^{-1}$ FW and 68.8 $\mu g\ g^{-1}$ FW in Mish Pestan and Sahebi cultivars, respectively in D treatment, while Si

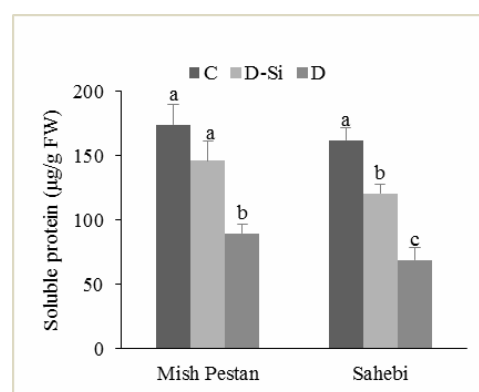


Figure 2. Effect of Si on total soluble protein content under drought stress in two grapevine cultivars; bars with different letters are significantly different at the $P < 0.01$ level. (C: Control; D-Si: Si-Drought, D: Drought treatments).

Table 1. Correlation coefficients between different characters.^a

	GB ^a	Pro ^b	H ₂ O ₂	GPX ^c	POD ^d	CAT ^e	SOD ^f	Soluble protein	Total Chl ^g	Chl b	Chl a	LWCR ^h
LWCR	-0.282	-0.414*	-0.685**	-0.295	-0.170	0.084	0.325	0.694**	-0.185	-0.165	-0.002	1
Chl a	0.218	0.180	-0.262	0.297	0.255	0.003	0.393	0.206	0.848**	0.684**	1	
Chl b	-0.126	-0.082	-0.008	0.043	0.039	-0.327	0.056	0.004	0.930**	1		
Total Chl	-0.010	-0.009	-0.040	0.103	0.061	-0.270	0.141	0.023	1			
Soluble protein	-0.261	-0.548**	-0.833**	-0.118	-0.109	0.075	0.323	1				
SOD	0.879**	0.900**	0.062	0.820**	0.623**	0.428*	1					
CAT	0.558**	0.554**	0.016	0.650**	0.822**	1						
POD	0.622**	0.738**	0.209	0.732**	1							
GPX	0.866**	0.783**	-0.009	1								
H ₂ O ₂	0.097	0.349	1									
Pro	0.829**	1										
GB	1											

^a Glycine Betaine; ^b Proline; ^c Guaiacol Peroxidase; ^d Peroxidase; ^e Catalase; ^f SuperOxide Dismutase; ^g Chlorophyll; ^h Leaf Water Content Ratio. ** Correlation is significant at the 0.01 level. * Correlation is significant at the 0.05 level.

treatment caused an increase in such contents under drought stress. Compared to the control, soluble protein content decreased, respectively, by 16 and 49% in Mish Pestan cultivar, and by 26 and 58% in Sahebi cultivar for D-Si and D treatments. Total soluble protein content revealed a negative correlation with H₂O₂ and Pro characters at the 0.01 level (Table 1). The results of this experiment indicated that the total soluble protein content was reduced considerably under drought stress, while only a minor decrease in this protein was observed in D-Si treatment.

Drought stress caused an increase in the activity of SOD, and it was higher in D-Si treatment than in the other treatments for both cultivars. Compared to the control, SOD activity was increased by 51 and 20% in Mish Pestan and Sahebi cultivars under D treatment, respectively; while under D-Si treatment, Si caused an increase in this activity by 86 and 70% in Mish Pestan and Sahebi cultivars, respectively (Figure 3). SOD activity illustrated a significant positive correlation at the 0.01 level with GPX, POD, Pro and GB, and at the 0.05 level with CAT activity (Table 1).

CAT activity was changed in the leaves of control and drought stress of both cultivars (Figure 4). Compared to the control, CAT activity was increased respectively by 9 and 7% in Mish Pestan and Sahebi cultivars in D treatment; while application of Si increased CAT enzyme activity by 53 and 22% in Mish Pestan and Sahebi cultivars, respectively, in D-Si treatment. The findings also indicated a significant positive correlation between CAT activity and GPX, POD, Pro and GB at the 0.01 level (Table 1).

Judging by the results, the enhanced activities of POD appeared under conditions of D and D-Si treatments. Compared to the control, POD activity increased by 9 and 7%, in Mish Pestan and Sahebi cultivars in D treatment, while Si increased POD activity in Mish Pestan and Sahebi cultivars by 34 and 122%, respectively (Figure 5). Significant positive correlation was

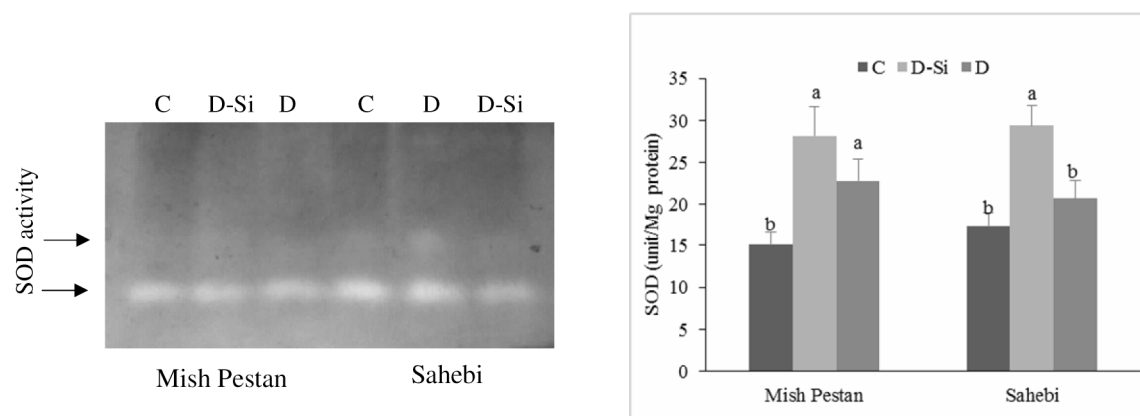


Figure 3. Effect of Si on SuperOxide Dismutase (SOD) activity (40 μg protein per well) subjected to Native-PAGE under drought stress in two grapevine cultivars; data are mean \pm standard error of three replicates; bars with different letters are significantly different at the $P < 0.01$ level.

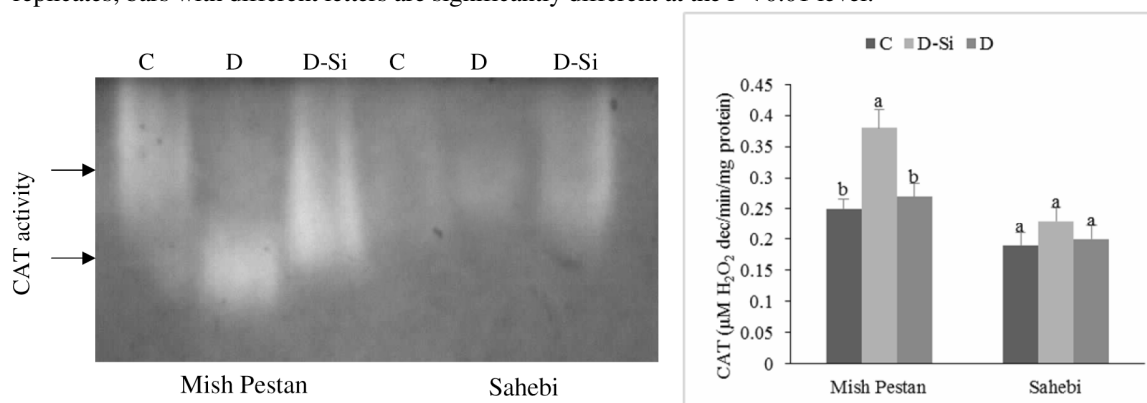


Figure 4. Effect of Si on Catalase (CAT) activity (20 μg protein per well) subjected to Native-PAGE under drought stress in two grapevine cultivars; data are mean \pm standard error of three replicates; bars with different letters are significantly different at the $P < 0.01$ level.

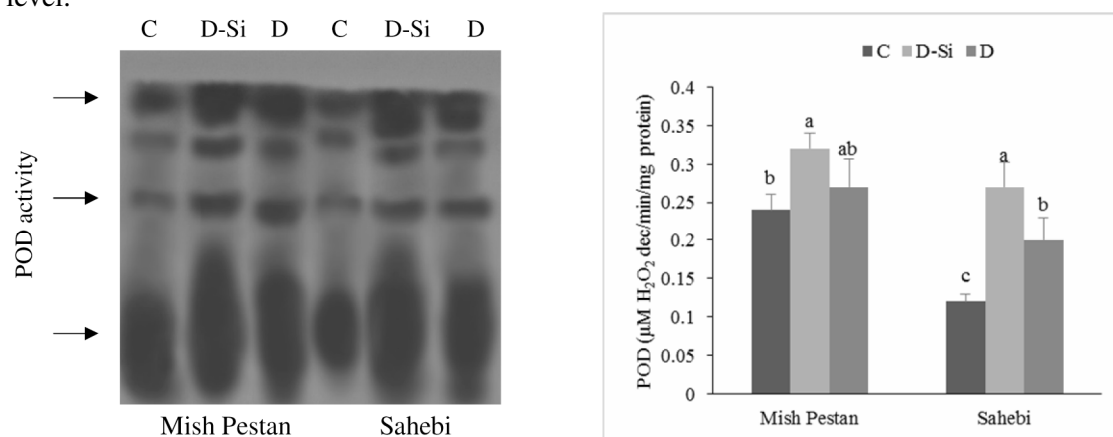


Figure 5. Effect of Si on Peroxidase (POD) activity (30 μg protein per well) subjected to Native-PAGE under drought stress in two grapevine cultivars; data are mean \pm standard error of three replicates; bars with different letters are significantly different at the $P < 0.05$ level. (C: Control; D-Si: Si-Drought, D: Drought treatments).

observed between POD and GPX, Pro and GB activities at the 0.01 level (Table 1).

Compared to the control plants, GPX activity was increased by approximately 5 and 7% in Mish Pestan and Sahebi cultivars, respectively, in D treatment. In D-Si treatment, the corresponding increase was about 20% in both cultivars (Figure 6). Results indicate that all correlations were positive and significant between GPX, Pro and GB at the 0.01 level (Table 1).

The H_2O_2 content of Mish Pestan and Sahebi cultivars increased in D treatment. Compared to the control, H_2O_2 content increased, respectively, by 11.19 and 29.1% in Mish Pestan and 72.05 and 210.29% in Sahebi cultivar under D-Si and D treatment (Figure 7).

Pro and GB contents are shown in Figures 8 and 9. With respect to these results, both Pro and GB contents significantly increased in D-Si and D treatments compared to the control. However, drought stress frequently caused a significant increase in osmolyte content; therefore remarkably higher Pro and GB concentrations were observed in D-Si treatment than in other treatments. The concentration of Pro increased from 102.67 in C to 369.29 and 413.01 $\mu M g^{-1} FW$ in Mish Pestan cultivar, and from 113 in C to 296.67 and 385.33 $\mu M g^{-1} FW$ in Sahebi cultivar, for D and D-Si treatments, respectively. GB content increased from

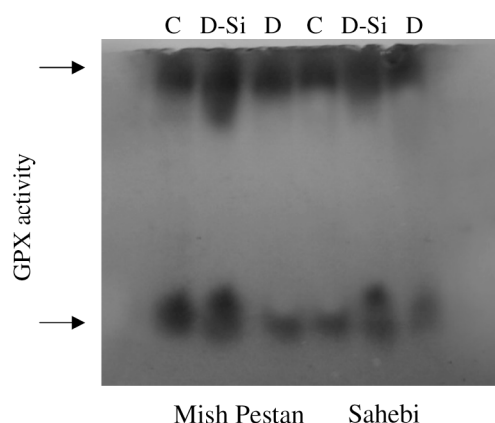


Figure 6. Effect of Si on Guaiacol Peroxidase (GPX) activity (40 μg protein per well) subjected to Native-PAGE under drought stress in two grapevine cultivars; data are mean \pm standard error of three replicates; bars with different letters are significantly different at the $P < 0.05$ level. (C: Control; D-Si: Si-Drought, D: Drought treatments).

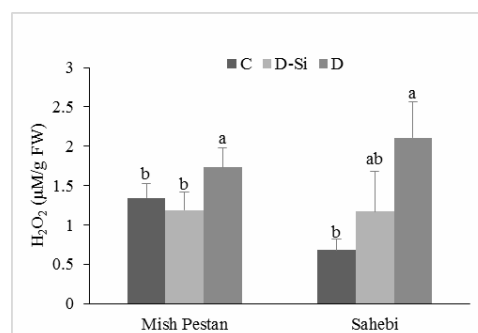
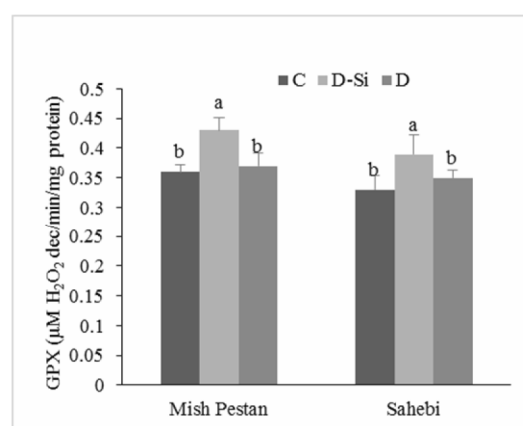


Figure 7. Effect of Si on Hydrogen peroxide (H_2O_2) content under drought stress in two grapevine cultivars; data are mean \pm standard error of three replicates; bars with different letters are significantly different at the $P < 0.01$ level. (C: Control; D-Si: Si-Drought, D: Drought treatments).

95.33 in C to 144.67 and 282.67 $\mu M g^{-1} DW$ in Mish Pestan cultivar, and from 114.6 in C to 198.35 and 233.41 $\mu M g^{-1} DW$ in Sahebi cultivar for D and D-Si treatments, respectively. The findings indicated a significant positive correlation between Pro and GB contents at the 0.01 level (Table 1).

DISCUSSION

In this study, the soil mingled sufficiently and then divided into several parts to cover all treatments. In such balanced condition of



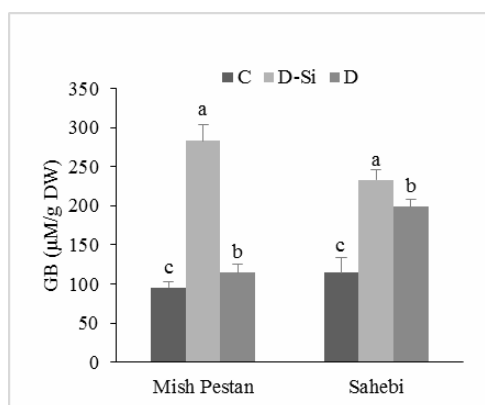


Figure 8. Effect of Si on Prolin (Pro) content under drought stress in two grapevine cultivars; data are mean±standard error of three replicates; bars with different letters are significantly different at the $P < 0.01$ level. (C: Control; D-Si: Si-Drought, D: Drought treatments).

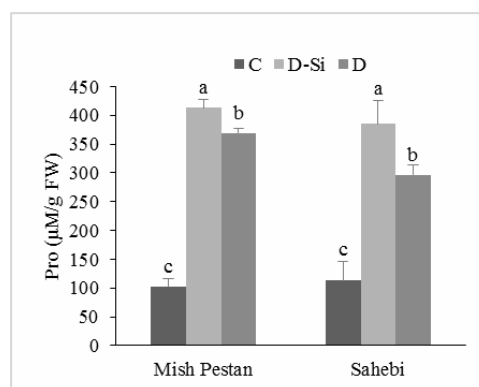


Figure 9. Effect of Si on Glycine Betaine (GB) content under drought stress in two grapevine cultivars; data are mean±standard error of three replicates; bars with different letters are significantly different at the $P < 0.01$ level. (C: Control; D-Si: Si-Drought, D: Drought treatments).

the minerals frequency, measurement of the Si combinations was ignored in the soil as reported in some of the other research projects (Soylemezoglu *et al.*, 2009; Tale-Ahmad and Haddad, 2011). Drought stress caused a decrease in *LWCR* as reported by Lipiec *et al.* (2013), but Si application could alleviate water stress. This result is in agreement with those of Sonobe *et al.* (2011) who concluded that Si application actively promoted water uptake that led to the development of highest water potential. The application of Si seems to be quite beneficial to plants grown under drought conditions by encouraging the development of a big root system and providing protection to roots against soil drying. Sacala (2009) reported that the possible role of Si in plant resistance mechanisms to water stress might be considered at different levels: molecular, cellular, and whole-plant. The improved ability to retain water by plants treated with Si may result from a lowered transpiration rate and a higher value of water use efficiency (Gao *et al.*, 2006). The Si in the hull is also deposited between the epidermal cell wall and the cuticle, forming a cuticle-Si double layer similar to the leaf blades. However, in contrast to the leaves, hull transpiration occurs only through the cuticle because the hull does not

have any stomata (Ma *et al.*, 2001). Si is effective in decreasing the hull transpiration. Therefore, Si plays an important role in keeping a high moisture condition within the hull by decreasing transpiration rate from the hull (Ma and Takahashi, 2002). The results indicated that drought stress has lower negative effects on *LWCR* in Mish Pestan cultivar than in Sahebi, similar to the advantage of previous researchers in other plants. Moreover, Si might cause a decrease in plant transcription to protect grape from damage by D stress.

Drought stress leads to the closure of stomata and subsequent decrease in the photosynthetic rate. The content of photosynthetic pigments was significantly decreased by drought stress. Such finding is in agreement with some of the reports (Tale-Ahmad and Haddad, 2011; Abbasi *et al.*, 2014; Nahar *et al.*, 2015; Saeidi and Abdoli, 2015), while application of Si led to a decrease in the decomposition of photosynthetic pigments. Si application significantly increased photosynthetic rate of rice plants (Chen *et al.*, 2011), and tomato (Cao *et al.*, 2015) under drought stress. Tale-Ahmad and Haddad (2011) reported that Si could increase photosynthesis of wheat plants under drought and this might be associated with the

enhancement in activities of photosynthetic enzymes including ribulose biphosphate carboxylase.

In the present study, the activity of SOD, CAT, POD, and GPX in grapevine which increased in the leaves under drought stress, were in agreement with Mirzaee *et al.* (2013) results, while the increase was significant (in SOD and CAT $P < 0.01$, in POD and GPX $P < 0.05$) and consistent in Si treatment compared to other treatments. The results related with responses of antioxidant enzymes under D-Si treatment were in agreement with the findings of some recent researchers (Kim *et al.*, 2014; Salekjalali *et al.*, 2012; Tale-Ahmad and Haddad, 2011; Jiao-jing *et al.*, 2009). Adaptation to drought may depend on different mechanisms, including the capacity to maintain high levels of antioxidants and/or through the induction of antioxidant enzymes. In metabolic processes, plants produce H_2O_2 , which causes damage to the cell oxidation function, while CAT can eliminate H_2O_2 and SOD plays a key role in the elimination of $O_2^{\cdot -}$ (Kesba and El-Beltagi, 2012). $O_2^{\cdot -}$ are toxic byproducts of oxidative metabolism and can interact with H_2O_2 to form highly reactive hydroxyl radicals ($OH^{\cdot -}$), (Beis and Patakas, 2012). The dismutation of $O_2^{\cdot -}$ into H_2O_2 and oxygen is an important step in protecting the cell (Azevedo *et al.*, 2005). Under drought conditions, the addition of Si increases SOD activity that is in agreement with Cao *et al.* (2015) results. In plants, a number of enzymes regulate H_2O_2 intracellular levels, but CAT is considered the most important (Noctor *et al.*, 2000). It was observed that a delicate regulation of H_2O_2 production via CAT activity modulation might contribute to rapid decrease in stomatal conductance preventing excessive water loss (Beis and Patakas, 2012). In this study, significant change in the activity of CAT was observed in plants subjected to drought stress when compared to the control. The increase in the activity of CAT in the D-Si treatment may indicate an adaptive response to changing conditions in the environment or else a compensatory mechanism developed to deal with increased generation of free radicals, in agreement with Tripathi *et al.* (2014) and

Akitha-Devi and Giridhar, (2015) results. POD and GPX, as well as CAT, play an essential role in scavenging the H_2O_2 toxicity, which is a major product produced by SOD (Noctor *et al.*, 2000), GPX is one of the defense enzymes that acts on peroxides to remove them.

In the present study, the Pro and GB content in grapevine increased in the leaves under drought stress, while the increase was more significant and consistent in Si treatment than in other treatments. This finding is in agreement with Fariduddin *et al.* (2009) results that reported proline content in leaves exhibited an increase in response to drought stress in *Brassica juncea*. Tale-Ahmad and Haddad (2011) reported that Pro and GB contents were significantly increased in Si and drought treatments compared to the control. Moreover, Carlos *et al.* (2009) observed that concentrations of Pro in potato are increased under lower water availability and higher Si availability in the soil, which indicates that Si may be associated with plant osmotic adjustment. Pro and GB are two major organic osmolytes that accumulate in a variety of plant species in response to environmental stresses such as drought, salinity, extreme temperatures, UV radiation and heavy metals (Ashraf and Foolad, 2007).

Most of the studied characters illustrated significant positive correlation and caused to reduce drought stress (Table 1). It might be clear that all of the reactions coordinate together in order to protect the plant by stress decline. Negative correlation between H_2O_2 and both traits of LWCR and total soluble protein was significant at the 0.01 level (Table 1). Such result was expected as drought stress caused to decrease LWCR (Lipiec *et al.*, 2013) and then, this stress increased H_2O_2 production (Mittler, 2002). Therefore, such reaction decreases total soluble protein in the stressed plant cells (Molassiotis *et al.*, 2006).

CONCLUSIONS

Drought stress is an important threat to plant growth and sustainable agriculture worldwide. Although Si is the second most



abundant element in the Earth's crust, it has not yet been listed among the essential elements for higher plants. It is proved that Si has an effective influence on moderating and decreasing drought stress damages. The results of this study highlight the role of Si in regulating the drought stress responses of grapevine, and indicate that Si could protect plants against oxidative damage. This effect is achieved through increasing the antioxidant enzymes activity. The effects of Si on physiological aspects of the plant growth can therefore be seen to act in conjunction with the plants own endogenous stress responses. Si decreases H_2O_2 concentration and lipid peroxidation in grapevine, presumably through the observed increase in CAT, POD, SOD, and GPX. It was concluded that the grape cultivars were responsive to Si under drought stress condition. The Si treatment may cause overexpression of the antioxidant genes as an activator. In the present study, Si treatment revealed a positive effect on *Vitis vinifera* L. cvs. Mish Pestan and Sahebi under drought stress. According to the results, Mish Pestan cultivar was more tolerant than Sahebi cultivar as illustrated by molecular and biochemical analysis, and might be a candidate for drought area. Si application most probably possesses similar effects on the other grape cultivars. It might be suggested to investigate the antioxidant enzyme genes, introduce more sensitive Si promoters, and/or their overexpression to reduce the Si treatment effect. Hopefully, proper Si dosages to be applied in vineyards will be determined by further studies.

Abbreviations

C, Control; CAT, Catalase; Chl, Chlorophyll; D, Drought; D-Si, Drought+ Silicon; GB, Glycine Betaine; GPX, Guaiacol Peroxidase; LWCR, Leaf Water Content Ratio; POD, Peroxidase; Pro, Prolin; ROS, Reactive Oxygen Species; Si, Silicon; SOD, SuperOxide Dismutase; $O_2^{\cdot-}$, Superoxide anion.

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تأثیر تنش آبی و سیلیکات سدیم بر پاسخ آنزیم‌های ضد اکساینده در ارقام مختلف انگور

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چکیده

تأثیر سیلیکات سدیم بر روی فعالیت چهار آنزیم آنتی اکسیدان اصلی و پنج ترکیب در گیر در دو رقم انگور (میش پستان و صاحبی) تحت تنش خشکی بررسی شد. آزمایش در قالب طرح کامل تصادفی با سه تیمار شاهد، خشکی بدون سیلیکون و خشکی + سیلیکون (۰.۰۰۴ مولار سیلیکات سدیم بر کیلوگرم خاک)، با سه تکرار در گلخانه انجام شد. نتایج نشان داد که سیلیکون تأثیرات منفی تنش خشکی را با افزایش تحمل انگور از طریق افزایش فعالیت آنزیم‌های ضد اکسنده و مقدار پروتئین محلول کل کاهش می دهد. تیمار سیلیکون به طور معنی داری بر فعالیت آنزیم‌های ضد اکسنده در هر دو رقم تأثیر گذار بود. تنش کم آبی سبب کاهش در میزان کلروفیل و پروتئین محلول کل شد، که این کاهش در تیمار خشکی بدون سیلیکون بسیار بیشتر از تیمار خشکی + سیلیکون بود. نتایج نشان داد که سیلیکات سدیم می تواند خسارات ناشی از تنش خشکی را از طریق افزایش فعالیت آنزیم‌های ضد اکسنده کاهش دهد.