Research Report

# Influence of Silicon Supplementation on the Growth and Tolerance to High Temperature in *Salvia splendens*

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**Abstract.** In this study, effects of silicon (Si) on the growth and activities of major antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX), and protein profile under high temperature stress were investigated. *Salvia splendens* 'Vista Red' and 'Sizzler Red' were grown in a glasshouse and potassium silicate (0 or 100 mg·L$^{-1}$) was supplemented hydroponically. Overall important growth parameters, chlorophyll content, and biomass were significantly increased by Si application. In both cultivars, more Si deposition occurred in the root, followed by leaf and flower. In both normal and high temperature conditions, supplemented Si increased the activity of SOD, APX, and GPX, while it decreased the CAT activity. In the SDS-PAGE protein profile, three bands responding differentially to the Si treatment were observed. Especially in 'Vista Red' treated with Si, a protein band, approximately 46 kDa, was expressed strongly under the temperature stress. Results of this experiment showed that Si not only promoted the growth of salvia, but also played a vital role against temperature stress.

***Additional key words***: antioxidant enzymes, SDS-PAGE, silicate, temperature stress

## Introduction

Silicon (Si) is the second most abundant element in the earth crust next to oxygen. The beneficial effects of Si to monocots such as rice (Ma and Takahashi, 2002; Pereira et al., 2004) and sugarcane (Savant et al., 1999) created an interest to study the role of Si in horticultural plants as well. In greenhouses, many of the floricultural crops are grown in soilless substrates and therefore have only limited plant available Si as compared to the plant grown in mineral soils (Voogt and Sonneveld, 2001).

Double layer formation by amorphous Si with the cuticle layer is assumed to decrease the evapotranspiration, increase photosynthetic rate, and also gives rigidity and erectness to the plants. Supplemented Si has been proven to improve plant growth, and its effects are distinct during abiotic and biotic stresses (Lina et al., 2013; Ma, 2004). Though Si effects are apparent under stress conditions, yet the Si-mediated mechanisms for stress response are poorly understood. During stress conditions, higher amount of reactive oxygen species (ROS) generation leads to the oxidative damage of cell organelles. So, the major antioxidant enzymes, such as superoxide dismutase (SOD), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), and catalase (CAT), are actively involved to scavenge ROS groups (Krieger-Liszkay, 2005). It has been reported that Si enhanced the enzymatic activity under salt stress in cotton (Gossett et al., 1994), wheat (Dionisio and Tobita, 1999), barley (Liang et al., 2003), tomato (Al-Aghabary et al., 2005) and cucumber (Zhu et al., 2004), and drought stress in sorghum (Lux et al., 2002). However, Si-mediated tolerance to high temperature stress remains largely unexplored in most crops except in rice (Agarie et al., 1998) and St. Augustinegrass (Brecht et al., 2004). Up to our knowledge this is the first report explaining the influence of Si on antioxidant enzymes activity, and total protein and protein profile under temperature stress.

*Salvia splendens* is one of the commonly grown ornamental species in the *Salvia* genus. Mostly, it is cultivated worldwide

as a bedding plant. High temperature stress impacts the plant growth in warm temperate continental climates. For most ornamental plants, tolerance to high temperatures remains largely unstudied. Despite both the natural and the garden diversity of *Salvia* taxa, their relations with Si are also not well documented. This study was conducted to study the beneficial effect(s) of Si on the physiological development of *S. splendens* 'Vista Red' and 'Sizzler Red', and also resistance against high temperature stress.

## Materials and Methods

### Plant Materials, Cultivation, and Silicon Supplementation

Seeds of *S. splendens* 'Vista Red' and 'Sizzler Red' were sown on 26 January 2012 in 128-cell plug trays containing a commercial medium (Tosilee Medium, Shinan Precision Co., Jinju, Korea). Trays were maintained in the growth chamber at 24/18ºC under a dark condition with 75-80% relative humidity (RH) for germination. After germination, seedlings were grown under a 14 h photoperiod with 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux density (PPFD). Germination percentage of 'Vista Red' and 'Sizzler Red' was 81 and 53%, respectively. Two week-old uniform seedlings were transferred to the glasshouse and a nutrient solution with 0 or 100 $mg \cdot L^{-1}$ Si was supplied by an ebb-and-flow subirrigation system. The chemicals used in the nutrient solution (in $mg \cdot L^{-1}$) were 708 $Ca(NO_3)_2 \cdot 4H_2O$, 246 $MgSO_4 \cdot 7H_2O$, 505 $KNO_3$, 230 $NH_4H_2PO_4$, 1.24 $H_3BO_3$, 0.124 $CuSO_4 \cdot 5H_2O$, 4 Fe-EDTA, 2.2 $MnSO_4 \cdot 4H_2O$, 0.08 $H_2MoO_4$, and 1.15 $ZnSO_4 \cdot 7H_2O$. Potassium silicate ($K_2SiO_3$) was used as the Si source, and addition of K was subtracted from $KNO_3$ and the nitrate loss was balanced by the supplementation of nitric acid (Sivanesan et al., 2010).

After two weeks, uniform plants were transplanted into an individual 10 cm pots and grown in a glasshouse (February-April, 2012) at Gyeongsang National University, Jinju, Korea. The set RH and the temperature of the glasshouse was 65% and 17-25°C, respectively. In the ebb and flow system bed, both 'Vista Red' and 'Sizzler Red' were grown together for each treatment such as the control and Si treatment, respectively. Plants were placed at a density of 20 cm x 20 cm during both the vegetative stage and the flowering stage on the ebb and flow bed of 100 cm × 200 cm in size. Totally 25 plants per cultivar for each treatment were used.

### Growth Parameter Measurement

Shoot length, number of nodes, internode length, stem diameter, pedicel length, total leaf area, content of chlorophyll a, b and total chlorophyll, root length, and fresh and dry

weights of the shoot, root and flower were measured on 10 April, 2012 for 'Vista Red' and on 16 April, 2012 for 'Sizzler Red'. Shoot, root, and flowers were kept in a dry oven (FO-450M, Jeio Tech Co., Seoul, Korea) at 70°C for 48 h for dry weight measurement.

For chlorophyll estimation, 10 mg fresh leaf material without veins/ribs were taken from the young fully developed leaf, and extracted using 80% of ice cold acetone. After centrifuge at 3,000 rpm, absorbance of supernatant was measured with a spectrophotometer (Biochrom Libra S22, Young-Wha Scientific Co., Seoul, Korea) at 663 and 645 nm. Calculations were done according to the method of Dere et al. (1998).

Uptake of Si by the plants was estimated by a wet-autoclave induced digestion method (Elliott and Snyder, 1991) using 100 mg dry mass. Sample preparation and reading have been done according to Sivanesan et al. (2010).

### Estimation of Activity of Antioxidant Enzymes

To study the role of Si on temperature stress responses, 45 day-old plants were kept under $35 \pm 1$°C for two days in the growth chamber. At the same time, another set of plants were maintained in the glasshouse as the comparative control. In both greenhouse and growth chamber set RH was 65%. For enzyme extraction, 100 mg fresh leaf sample was ground in 1 mL of 100 mM sodium phosphate buffer (pH 7.0) for GPX assay and 50 mM sodium phosphate buffer for SOD, CAT, and APX assays. Homogenized samples were centrifuged at 13,000 rpm at 4°C for 20 min and the supernatant was transferred to a 1.5 mL Eppendorf tube. The SOD activity was assayed by the nitroblue tetrazolium (NBT) method of Giannopolitis and Ries (1977). The GPX activity was measured by following the procedure of Egley et al. (1983). The APX activity was estimated according to the protocol described by Nakano and Asada (1981). The CAT activity was determined based on the Cakmak and Marschner (1992) method.

### Protein Extraction and SDS-PAGE

To study the effect of Si on short-term temperature stress responses, plants were subjected to 25 or 35°C for 0 and 4 h. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was conducted to analyze the protein pattern. For protein extraction, 100 mg of leaf ground in liquid nitrogen was homogenized in a 500 µL of extraction buffer containing 50 mM tris-HCl, 10 mM NaCl, 1% sodium dodecyl sulphate, 5% 2-mercaptocthanol, 0.1 mM phenylmethanesulfonylfluoride, and 0.1 mM dithiotheritol (Rehm, 2006). After vortexing vigorously, samples were centrifuged at 13,000 rpm at 4°C for 20 min and the supernatant was carefully transferred to a tube. Total protein content was estimated by

Hort. Environ. Biotechnol. 55(4):271-279. 2014.

273

Bio-Rad Bradford assay (Bradford, 1976). For protein profiling, 40 μg of protein was taken from each sample and mixed with 1X dye at 1:1 ratio. After heating at 95°C for 3 min in a hot water bath incubator [T100 heated circulating baths, Grant Instruments (Cambridge) Ltd., Cambridgeshire, UK], the samples were immediately kept in ice for 5 min and loaded in 10% polyacrylamide gel. Gels were run at 40 V for 15 min and 110 V for 45 min. Finally, silver staining was carried out by following the procedure of Merril et al. (1981), and the bands were visualized under white light.

## Statistical Analysis

All data were subjected to analysis of variance (ANOVA) followed by Duncan's multiple range tests at $p \leq 0.05$, and F-test was carried out to find the significance between treatments and cultivars by using SAS software (Statistical Analysis System, V. 6.12, Cary, NC, USA). Totally, ten replications per treatment were carried out for all growth parameters, chlorophyll estimation, and Si concentration analysis. For enzyme assays and protein measurement, three replicates per treatment were carried out.

# Results

Increase in growth upon Si supplementation has been clearly observed in both cultivars of salvia (Figs. 1A and 1B). Significant increases by Si application in growth parameters, such as shoot length, number of nodes, total leaf area per plant, chlorophyll content, root length, and fresh and dry weights of shoot and root, except stem diameter, and flower fresh and dry weights, were observed in both cultivars

(Table 1). However, statistical interaction between the cultivar and the treatment (A × B) of 'Vista Red' and 'Sizzler Red' on most of the growth traits was not significant, except in internode length, total leaf area per plant, shoot fresh weight, and root dry weight. The positions of leaves were highly differed between the control and the Si treatment (Fig. 1). Especially, in 'Vista Red' (Fig. 1A) decreases in self-shading and erectness in the leaf on Si treatment were observed clearly. In detail, shoot length of Si treatment increased by 25% in 'Vista Red', but only 9.6% in 'Sizzler Red', as compared to the control. Though in 'Vista Red' the Si treatment efficiently increased number of nodes by 22.9%, it promoted only 2.6% of the internode length. On the other hand, in 'Sizzler Red' Si increased the internode length (12.7%) more than the number of nodes (9.0%). Stem diameter of 'Vista Red' increased by 2.4%, while that of 'Sizzler Red' increased only by 4.9%, by the Si treatment. Compared to the control, pedicel length was promoted in the Si treatment by 14.4 and 8.3% in 'Vista Red' and 'Sizzler Red', respectively. In addition, enhancement of total leaf area per plant by the Si treatment was 29.3% in 'Vista Red' and 10.9% in 'Sizzler Red'. Similarly, root length of 'Vista Red' and 'Sizzler Red' increased by 9.7 and 16.1%, respectively, by the Si treatment. Apparently, significant increase in fresh and dry weights was accomplished by Si in both the shoot and root, but not in the flower (Table 1).

Upon Si inclusion, contents of chlorophyll a and b increased by 83.3 and 80.0% in 'Vista Red', and 69.2 and 60.0% in 'Sizzler Red', respectively. Consequently, 82.3 and 72.2% of increase in total chlorophyll content was achieved in 'Vista Red' and 'Sizzler Red' by the Si treatment, respectively
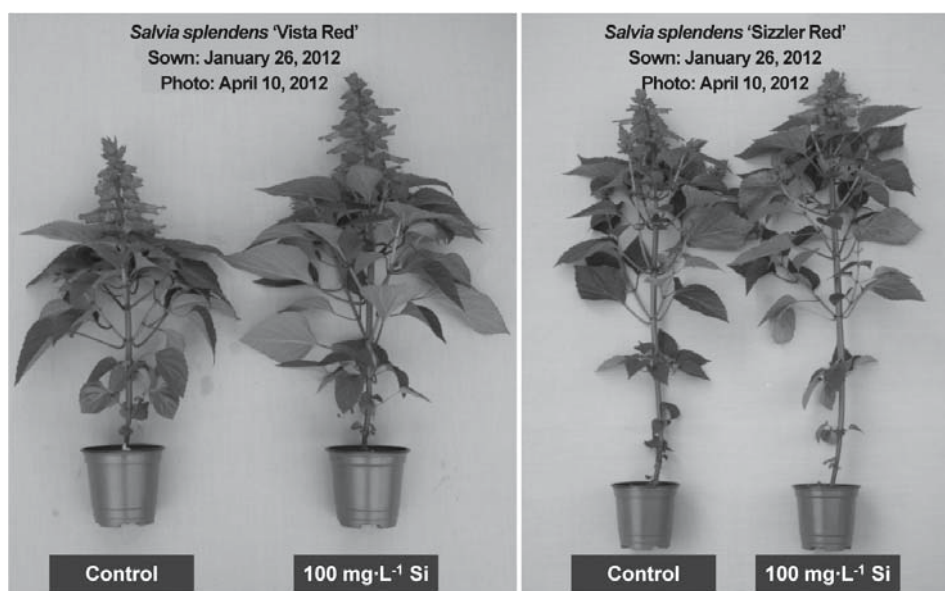


**Fig. 1.** Effect of Si supplementation on the growth of salvia 'Vista Red' (A) and 'Sizzler Red' (B). The photographs were taken after 15 days of flowering in both cultivars.

**Table 1.** Effect of Si supplementation on growth parameters of salvia 'Vista Red' and 'Sizzler Red'.

| Cultivar (A) | Si (mg·L⁻¹) (B) | Shoot length (cm) | No. of nodes | Internode length (cm) | Stem diameter (mm) | Pedicel length (cm) | Total leaf area/plant (cm²) | Root length (cm) | Shoot | | Root | | Flower | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | FW (g) | DW (g) | FW (g) | DW (g) | FW (g) | DW (g) |
| 'Vista Red' | 0 | 29.9 ± 0.62dᶻ | 6.1 ± 0.10d | 3.8 ± 0.07c | 7.1 ± 0.19b | 9.0 ± 0.52b | 764 ± 11.2d | 23.8 ± 0.57c | 33.2 ± 0.77c | 3.3 ± 0.08d | 11.2 ± 0.33c | 1.2 ± 0.01c | 2.5 ± 0.13c | 0.31 ± 0.03b |
| | 100 | 37.4 ± 0.87c | 7.5 ± 0.16c | 3.9 ± 0.08c | 7.2 ± 0.19b | 10.3 ± 0.53b | 988 ± 10.76c | 26.1 ± 0.39a | 40.8 ± 0.56b | 4.4 ± 4.66c | 13.2 ± 0.25a | 1.4 ± 0.08b | 2.7 ± 0.11c | 0.28 ± 0.01b |
| 'Sizzler Red' | 0 | 61.2 ± 0.75b | 8.8 ± 020b | 6.3 ± 0.22b | 8.1 ± 0.09a | 14.5 ± 0.43a | 1,328 ± 5.25b | 22.3 ± 0.57c | 70.6 ± 1.60a | 8.3 ± 0.17b | 16.3 ± 0.62a | 1.2 ± 0.03c | 7.0 ± 0.37b | 0.71 ± 0.04a |
| | 100 | 67.1 ± 0.82a | 9.6 ± 0.16a | 7.1 ± 0.24a | 8.5 ± 0.08a | 15.7 ± 0.44a | 1,473 ± 4.05a | 25.9 ± 0.40a | 72.7 ± 0.71a | 9.2 ± 0.03a | 17.1 ± 0.55a | 1.7 ± 0.07a | 8.1 ± 0.46a | 0.78 ± 0.05a |
| F-test | A | *** | *** | *** | *** | *** | *** | NS | *** | *** | *** | ** | *** | *** |
| | B | *** | *** | ** | NS | * | *** | *** | *** | *** | ** | ** | NS | NS |
| | A x B | * | NS | * | NS | NS | *** | NS | ** | NS | NS | * | NS | NS |

ᶻMean separation within columns by Duncan's multiple range test at $p \leq 0.05$.
NS,*,**,***Nonsignificant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively. Values are mean ± SE from ten replicates.
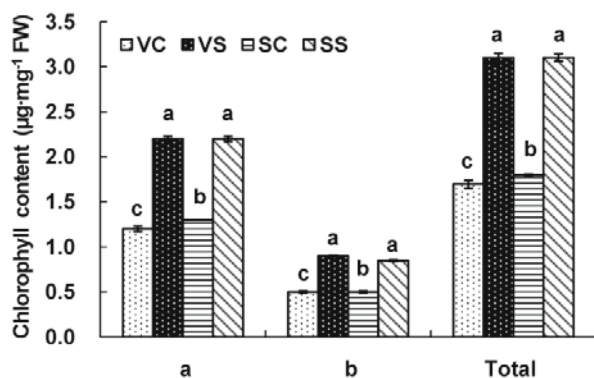


**Fig. 2.** Effect of Si supplementation on contents of chlorophyll a, b, and total chlorophyll of salvia 'Vista Red' and 'Sizzler Red': VC, 'Vista Red' control; VS, 'Vista Red' with 100 mg·L⁻¹ Si; SC, 'Sizzler Red' control; and SS, 'Sizzler Red' with 100 mg·L⁻¹ Si. Data are the mean ± SE from three replicates. Different letters in one measurement indicate treatments are significantly different at $p \leq 0.05$.

(Fig. 2).

The Si estimation result showed that highest amount of Si was present in the root followed by the leaf and flower. The Si content of the leaf, flower, and root was 132.3, 73.1, and 214.5 µg·g⁻¹ DW in 'Vista Red' and 116.8, 100.7, and 289.2 µg·g⁻¹ DW in 'Sizzler Red', respectively.

Under temperature stress (35℃) for 2 days Si promoted the SOD activity of 'Sizzler Red' more than 'Vista Red', which was the other way on day 0 (Fig. 3A). Despite the higher GPX activity in the control 'Vista Red' than the control 'Sizzler Red', the Si treatment showed almost similar activity in both cultivars during normal and temperature stress conditions (Fig. 3B). Consistently, the Si treatment promoted the APX activity of 'Sizzler Red' more than 'Vista Red' (Fig. 3C). However, CAT activity limited by Si was found to be in a similar fashion as in 'Sizzler Red' on days 0 and 2 (Fig. 3D).

In a normal condition, uptake of Si by 'Vista Red' and 'Sizzler Red' was only 130 and 107 µg·g⁻¹ DW, respectively.

However, under high temperature stress for 2 days, Si uptake arte increased by approximately 2.5 folds in both cultivars, i.e., 307 µg·g⁻¹ DW in 'Vista Red' and 255 µg·g⁻¹ DW in 'Sizzler Red' (Table 3). For seed germination and seedling growth, only normal tap water was used until the seedlings were moved to the glasshouse. Normally, tap water will have a little amount of Si. This could be the reason for the presence of Si in the control treatment of both cultivars.

Significant influence of Si on total protein content was observed under the high temperature condition (Fig. 4). Even though in a normal condition Si reduced the protein content, during the temperature stress it consistently increased the protein content in both cultivars. Supplementation of Si significantly influenced the protein pattern at 0 or 4 h under 35℃ (Fig. 5). From the examination of the SDS-PAGE, it was observed that three bands appeared to be responded differentially to the Si treatment. Particularly, one of the bands around 46 kDa was more strongly expressed in the Si-treated 'Vista Red' under a temperature stress condition. Interestingly, the protein pattern of Si-treated 'Sizzler Red' showed slightly higher expression in the control than the temperature stress condition. In the future the identification and characterization of the proteins can be performed to investigate the molecular mechanism of Si-mediated temperature stress tolerance. Without knowing the underlying molecular mechanism yet, our results indicated Si supplementation could increase the growth and resistance to temperature stress in salvia.

## Discussion

Previously, addition of Si has been proven to be useful for monocots belonging to the Poaceae family such as rice (Durgesh Kumar et al., 2012), barley (Liang et al., 2003), sugarcane (Savant et al., 1999), and wheat (Dionisio and Tobita, 1999). Only a few studies have reported the positive effects of Si in horticultural plants. Therefore, the current
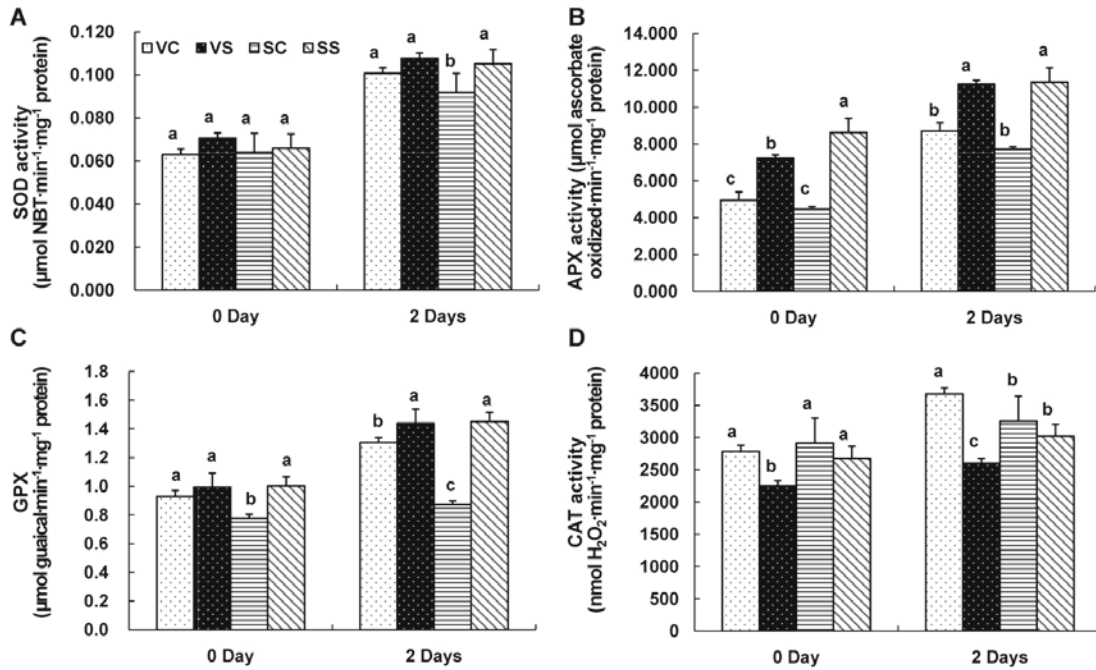
**Fig. 3.** Effect of Si supplementation on activities of antioxidant enzymes in the leaves of salvia under high temperature stress (exposed to 35°C for 0 or 2 days): A, superoxide dismutase (SOD); B, ascorbate peroxidase (APX); C, guaiacol peroxidase (GPX) and D, catalase (CAT); VC, 'Vista Red' control; VS, 'Vista Red' with 100 mg·L⁻¹ Si; SC, 'Sizzler Red' control; and SS, 'Sizzler Red' with 100 mg·L⁻¹ Si. Data are the mean ± SE from three replicates. Different letters on the bars indicate treatments are significantly different at $p \le 0.05$.

**Table 2.** Effect of Si supplementation on Si contents in the leaf, flower, and root of salvia 'Vista Red' and 'Sizzler Red'.

| Cultivar (A) | Si (mg·L⁻¹) (B) | Si content (µg·g⁻¹ DW) | | |
|---|---|---|---|---|
| | | Leaf | Flower | Root |
| 'Vista Red' | 0 | 44.4 ± 1.15 b[z] | 38.6 ± 1.99 b | 58.2 ± 11.66 b |
| | 100 | 132.3 ± 0.34 a | 73.1 ± 6.90 a | 214.5 ± 17.0 a |
| 'Sizzler Red' | 0 | 53.6 ± 5.01 b | 55.9 ± 1.99 b | 70.8 ± 3.04 b |
| | 100 | 116.8 ± 4.59 a | 100.7 ± 5.97 a | 289.2 ± 10.96 a |
| F-test | A | *** | ** | ** |
| | B | *** | *** | *** |
| | A x B | * | NS | * |

[z]Mean separation within columns by Duncan's multiple range test at $p \le 0.05$.
NS,*,**,***Nonsignificant or significant at $p \le 0.05$, 0.01, and 0.001, respectively. Values are mean ± SE from ten replicates.

**Table 3.** Effect of Si supplementation on Si contents in the leaf of salvia 'Vista Red' and 'Sizzler Red' under temperature stress.

| Cultivar (A) | Si (mg·L⁻¹) (B) | Si content under temperature stress (µg·g⁻¹ DW) | |
|---|---|---|---|
| | | 0 day | 2 days |
| 'Vista Red' | 0 | 23 ± 2.30 c[z] | 35 ± 1.15 c |
| | 100 | 130 ± 0.58 a | 307 ± 1.20 a |
| 'Sizzler Red' | 0 | 35 ± 1.76 c | 49 ± 1.86 c |
| | 100 | 107 ± 0.88 b | 255 ± 1.85 b |
| F-test | A | ** | ** |
| | B | *** | *** |
| | A × B | *** | *** |

[z]Mean separation within columns by Duncan's multiple range test at $p \le 0.05$.
NS,*,**,***Nonsignificant or significant at $p \le 0.05$, 0.01, and 0.001, respectively Values are mean ± SE from three replicates.
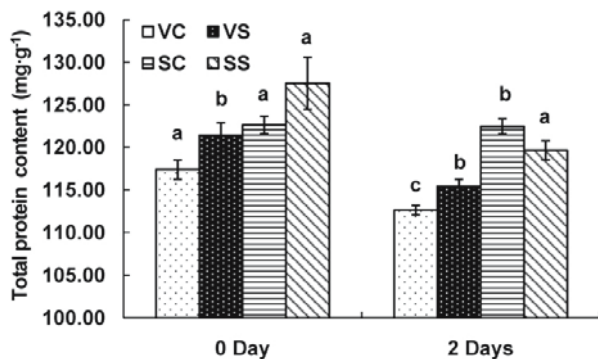
Fig. 4. Effect of Si supplementation on total protein content in the leaves of salvia exposed to 35°C for 0 or 2 days: VC, 'Vista Red' control; VS, 'Vista Red' with 100 mg·L$^{-1}$ Si; SC, 'Sizzler Red' control; and SS, 'Sizzler Red' with 100 mg·L$^{-1}$ Si. Data are the mean ± SE from three replicates Different letters on the bars indicate treatments are significantly different at $p \leq 0.05$.
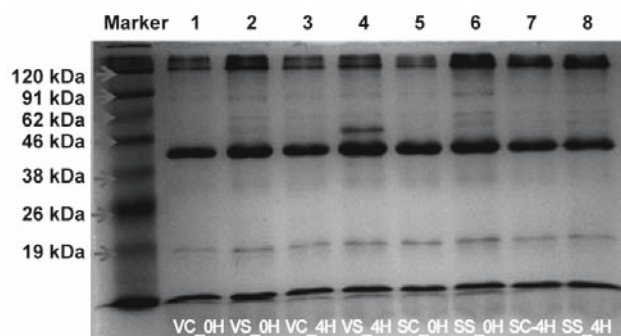


Fig. 5. Protein pattern of the control and the Si supplemented salvia 'Vista Red' and 'Sizzler Red' expressed after 0 (0H) or 4 h (4H) exposure to 35°C: VC, 'Vista Red' control; VS, 'Vista Red' with 100 mg·L$^{-1}$ Si; SC, 'Sizzler Red' control; and SS, 'Sizzler Red' with 100 mg·L$^{-1}$ Si.

study was conducted to investigate the possible role of Si on plant growth and tolerance to temperature stress in salvia, an important bedding plant worldwide. Even though not studied in this experiment, increase in shoot length and alteration of number of nodes and internodes length makes Si to be considered as a plant growth regulator-like compound. Growth regulation by means of controlling the cell enlargement and the cell division by the Si application might leads to the internode elongation and influence the plant height (Potts et al., 1985). Previously, levels of gibberellic acids (GAs) such as GA$_1$ and GA$_{20}$ enhanced with N and Si application was reported in rice (Hwang et al., 2008). McAvoy and Bernard (1996) found that Si application reduced the evapotranspiration which could have contributed to the increased turgor pressure within the leaves that led to cell swelling. Thus, larger total leaf area of salvia was obtained in the Si treatment. Maximum light interception by increased leaf area enables the plant to maintain an upright growth and increases CO$_2$ assimilation and the photosynthesis rate (Ma and Yamaji, 2006). Apparently, significant increase in a chlorophyll content was observed in the Si supplemented plants. Firstly, deposition of Si in the leaf promotes the chlorophyll synthesis because of alteration in the leaf position and enhancement of total leaf area by Si increases the light interception of the salvia. Secondly, degradation of chlorophyll a in the thylakoid membrane is directly proportional to the production of peroxidative enzymes (Gandul-Rojas et al., 2004). Under normal condition, Si reduced activities of both guaiacol peroxidase and ascorbate peroxidase in 'Vista Red' and 'Sizzler Red'. These could be the primary reason for enormous increase in the production of chlorophyll in the Si treatment as compared to the control. Similarly, in tomato chlorophyll content was increased significantly by Si supplementation (Silva et al., 2012). Moreover, in sugarcane breakdown of chloroplast and chlorophylls

during senescence was delayed by Si application (Cao et al., 2013). Hence, the photosynthetic rate and the crop yield were improved due to increase in chlorophyll content.

In salvia, additional supply of Si did not affect the stem diameter of both cultivars. On contrary, Si supplementation increased the stem thickness of marigold (Sivanesan et al., 2010) and sunflower (Kamenidou et al., 2008). In accordance with our result, Si addition increased the flower growth in zinnia (Kamenidou et al., 2009). Flower deformation in ornamental sunflower occurred due to Si-related mechanical strengthening effects on the cell wall plasticity of floral meristem (Kamenidou et al., 2008), but flowers of salvia with Si treatment remained unaffected/normal. Mali and Aery (2009) agreed on that higher levels of Si restricted shoot and root length. From the dry matter production of rice, Agarie et al. (1992) found the positive effects of Si were attributed to the maintenance of the photosynthesis and protection of chlorophyll. However, still more experiments are required to elucidate the exact mechanism of Si action on chlorophyll synthesis.

According to Epstein (1999), Si enhanced the biomass production of the plant by providing hardness to tissues. Increase in the shoot fresh and dry weights of 'Vista Red' and 'Sizzler Red' by Si may help to maintain an appropriate water status in leaves to prevent the photosynthetic depression and chlorophyll destruction. In this study enhancement on the growth was significantly correlated with the chlorophyll content and the biomass.

Sivanesan et al. (2013) mentioned the occurrence of marginal chlorosis in sub-irrigationaly Si-supplemented chrysanthemum. In contrast, none of the adverse effects of Si was observed in salvia throughout the experiment. Apart from internode length and stem diameter, leaf Si content had significant influence on growth characteristics than flower and root Si contents. Nevertheless the Si content was very

low as compared with the Si accumulators (Ma and Yamaji, 2006), but the accumulation rate in the plant tissues was high enough to render positive responses in growth and development of salvia.

In general, ROS generation is inevitable by vital metabolic processes such as photosynthesis, photorespiration, fatty acid oxidation, etc. However, under a stress condition increase in the rate of respiratory electron leakage lead to the release of more ROS groups which disturbs the plant metabolism (Krieger-Liszkay, 2005; Zhang et al., 2013). Major antioxidant enzymes, such as SOD, GPX, APX, and CAT, play vital roles in scavenging the free ion radicals and prevent the damage to cell membrane, pigments, lipids, proteins, and nucleic acids (Allen, 1995). Elevated SOD, GPX, and APX activities under the temperature stress would render to lower the $O_2^-$ and $H_2O_2$ accumulation rate. In contrast to our result, CAT activity increased by Si application under drought stress in wheat (Gong et al., 2005) and salt stress in barely (Liang et al., 2003). Especially, during a higher temperature period, Si influences and coordinates the activity of the above mentioned enzymes and aids in balancing ROS level. Such protection system plays a crucial role in preventing plants from oxidative stress damages. In the present study variation in the effect of Si on antioxidant enzymes between the cultivars might be due to species specificity and also difference in Si uptake level.

Previous study suggested that Si decreased the permeability of plasma membranes and membrane lipid peroxidation, and maintained the membrane integrity and its functions, and thus, mitigating against abiotic stress and improving the plants growth (Zhu et al., 2004). Beneficial effects are also associated with the silica gel deposition to overcome temperature stress by limiting the transpiration. So, passive uptake of Si in salvia deposited as amorphous form in the leaves might has assisted the plant to reduce transpiration and increase the water holding capacity. Under an abiotic stress condition, transpiration rate of the plant will be higher. To overcome the water loss, the plant will uptake more water (Álvarez et al., 2011). Normally, temperature stress induces many mechanisms for stress alleviation. One important factor is increased aggregation of aquaporin [formerly known as membrane intrinsic protein (MIP)] during the temperature stress. The low silicon 1 (Lsi1) protein also belongs to the NOD26-like intrinsic protein, a sub-family of aquaporin, and possesses silicic acid permeability (Ma and Yamaji, 2006). The proteins involved in the regulation of water and ion transportation from soil to plant are normally expressed more under the stress conditions (Johansson et al., 2000). Hence, abiotic stress increases the water flow, which is necessary for the internal microcirculatory system, and many stress-induced metabolic processes. Additionally, higher expressions of intrinsic transporter-like proteins increase the

passive flow of small polar molecules (Johansson et al., 2001). Hence, the Si content was higher under temperature stress and was directly proportional to the water uptake to overcome the higher transpiration rates.

Until now no report documented the involvement of Si in protein expression under a high temperature stress condition. Total protein content decreased by Si in a normal condition, while Si increased protein content under a temperature stress condition due to the association of Si in the thermal stability of the lipids in cell membranes (Agarie et al., 1998). Under cadmium (Cd) stress in rice Si regulated six categories of proteins such as $CO_2$ assimilation or photosynthesis-related proteins, redox homeostasis-related proteins, regulation or protein synthesis related proteins, and pathogen-response related proteins, chaperones, and energy/metabolism-related proteins (Nwugo and Herta, 2010). The differentially expressed protein band in Si-treated 'Vista Red' around 46 kDa in response to the temperature stress most probably belong to the heat shock protein (Hsps) family. Usually, Hsps function as molecular chaperones to prevent thermal aggregation and folding of proteins. (Ledesma et al., 2004). These mechanisms assist stabilizing or refolding and protect the conformation of denatured proteins (Lee et al., 1997). Clear insights on the effect of Si on protein expression are still poorly understood. Further proteomics studies would render knowledge on the Si effect on plant metabolism during temperature stress.

From this study, we strongly suggest that supplementation of Si with a nutrient solution can be beneficial for cultivation of salvia in soilless substrates. This is the first report dealing with the influence of Si on antioxidant activity, total protein content, and the protein pattern under high temperature stress. The way Si modulates the stress response in the plant remains unclear or at least not limited to a mechanical barrier and biochemical activity. Hence, further investigation has to be done to study the resistance mechanism provided by Si.

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