Silicon Alleviates Temperature Stresses in Poinsettia by Regulating Stomata, Photosynthesis, and Oxidative Damages

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Received: 21 August 2020; Accepted: 16 September 2020; Published: 18 September 2020

Abstract: The effects of silicon (Si) on temperature stresses were investigated in poinsettia. Well-rooted cuttings supplemented with and without Si were exposed to 40 °C, and plants treated with or without Si during cutting propagation and cultivation were subjected to 4 °C. The results showed that almost all the stomata of cuttings without Si supplementation were closed, while some of them were still open in cuttings supplemented with Si under a high temperature stress. However, Si was not able to alleviate stomatal closure of poinsettia under low temperature stress. The increased epicuticular wax might contribute to enhanced resistance of poinsettia to low temperature stresses. In addition, poinsettia maintained a higher photosynthetic rate and lower malonaldehyde and hydrogen sulfide concentrations when supplemented with Si under high and low temperature stresses, which might contribute to lower APX activities. Overall, temperature stresses had negative impacts on the physiological characteristics of poinsettia, while Si could alleviate some effects of temperature stresses.

Keywords: antioxidant enzyme; chlorophyll fluorescence; hydrogen sulfide; malonaldehyde; stomata

1. Introduction

Silicon (Si) is a beneficial element that helps plants overcome temperature stresses. The mechanisms involved include stimulation of antioxidant systems [1,2], maintenance of photosynthetic proteins [3], retention of water content [4], fortification of cell wall [4], restoration of hormonal balances [5], and regulation of stress-related genes [6,7]. Although Si is largely present in soil, the plant-available form (monosilicic acid) is relatively limited, especially in Korea which has low available Si in soil (averaged 72 mg·kg⁻¹) [8,9]. Moreover, Si is not available in most of hydroponic systems when it is not supplemented since horticultural growing media usually do not contain any natural soil. Deficiency of Si usually leads to abnormal plant development such as reduced number of brown epidermal cells, yellow leaves, and malformed fruits [10,11], while excessive Si uptake has negative effects on growth of Phragmites australis [12].

Poinsettias (Euphorbia pulcherrima Willd.) are propagated in summer and they bloom in winter, which therefore could be exposed to high and low temperature stresses, respectively. Temperature stresses lead to numerous changes in plants. The initial response should be stomatal closure [13,14]. The stomata regulate gas exchange, which contribute to physiological processes including photosynthesis and transpiration. Most importantly, their roles in transpiration help plants control leaf temperature. Results of studies on stomatal responses to temperature are somewhat contradictory. Some studies revealed that stomata closed with increasing temperature [15–17], whereas some found
that stomata opened with increasing temperature [18,19]. On the other hand, studies have suggested that stomata close at temperatures lower than that at which plants are grown [20,21]. However, it depends on the sensitivity of plants. It was found that a sudden low temperature led to stomatal closure in cold-tolerant plants but not in cold-sensitive plants [22].

High temperatures not only cause photosynthesis of plants, but also damage the photosynthetic apparatus [23,24]. It was reported that chlorophyll biosynthesis is one of the most affected plant functions damaged by high temperatures [25]. Numerous studies have also reported that low temperatures stresses have negative impacts on photosynthesis in maize [26,27], rice [28,29], bean [27,30], potato [31,32], barley [33,34], wheat [35], and tomato [36,37]. The light absorbed by plants is either used for production of carbohydrates, dissipated as heat, or reemitted as fluorescence [38]. Thus, chlorophyll fluorescence-based techniques, such as JIP test, have been frequently used as a tool for early diagnosis of temperature stresses [39–41].

Moreover, malonaldehyde (MDA) accumulation is often used as an indicator of lipid peroxidation or maker of oxidative disruption of lipids [42–45] which is caused by reactive oxygen species such as hydrogen peroxide (H$_2$O$_2$), superoxide, and hydroxyl radical [46,47]. Among these reactive oxygen species, H$_2$O$_2$ has relatively a long-life span and plays central roles in stress signal transduction pathways [48,49]. Plants generate enzymatic and non-enzymatic antioxidant systems to remove the damage of excessive H$_2$O$_2$. In enzymatic antioxidant systems, antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) are involved. Studies revealed that their activities are elevated under high temperature stresses [50–52], providing a protective role for plants.

Therefore, in this study, stomata, chlorophyll fluorescence, and antioxidant enzyme activities were determined, hoping to obtain a better understanding of how Si alleviates temperature stresses in poinsettia.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

Poinsettia cultivar ‘Flame’ was used in this study. Terminal cuttings were harvested and stuck in foam wedge substrate in trays (Smithers Oasis Korea, Seoul, Korea). Then, they were kept on a fogged (for 10 min at every 15-min interval) propagation bench for 4 weeks. The mean day/night air temperature during the whole propagation period is 30/25 °C and average relative humidity is 80%. The well-rooted cuttings were then transplanted to 15-cm pots and cultivated for 18 weeks.

2.2. Supplementary Si and Temperature Stress Treatments

The cuttings, in trays, were treated with either 0 (Si$_0$) or 75 (Si$_{75}$) mg·L$^{-1}$ of Si from potassium silicate ($K_2$SiO$_3$) every 3 days for 4 weeks during propagation. The cuttings from each treatment were then separated into two groups and supplied with either 0 or 75 mg·L$^{-1}$ of Si during cultivation. The treatments were designated as CP$_0$→C$_0$ (supply 0 mg·L$^{-1}$ Si during cutting propagation and cultivation), CP$_{75}$→C$_0$ (supply 75 mg·L$^{-1}$ Si during cutting propagation and 0 mg·L$^{-1}$ Si during cultivation), CP$_0$→C$_{75}$ (supply 0 mg·L$^{-1}$ Si during cutting propagation and 75 mg·L$^{-1}$ Si during cultivation), and CP$_{75}$→C$_{75}$ (supply 75 mg·L$^{-1}$ Si during cutting propagation and 75 mg·L$^{-1}$ Si during cultivation). The composition of nutrient solutions was described in our previous report [53]. The temperature stress responses in cuttings and plants were evaluated by treating them with high (40 °C) or low (4 °C) temperatures in plant growth chambers for 3 or 9 days, respectively. Each treatment consisted of 3 replicates, and each replicate contained 15 cuttings or 3 plants. Samples were collected at the end of treatments.
2.3. Scanning Electron Microscopy

Leaf samples were excised and fixed with 2.5% glutaraldehyde for 4 h. After 3 washes with a 0.1 M phosphate buffer (pH 7.0), samples were slowly dried at room temperature in a desiccator. The dried samples were coated with gold and then observed using a DS-130 ISI (Oxford, UK) scanning electron microscope.

2.4. Chlorophyll Fluorescence

The chlorophyll fluorescence parameters were measured every 24 h with a portable fluorometer (FluorPen FP110, Photon Systems Instruments, Drásov, Czech Republic).

2.5. H$_2$O$_2$ and MDA Concentrations

About 0.1 g of leaf material was homogenized in ice with 0.1% (w/v) TCA. The homogenate was centrifuged at 13,000 rpm for 15 min at 4 °C and 0.5 mL of the supernatant was added to 0.5 mL of a potassium phosphate buffer (10 mM, pH 7.0) and 1 mL of KI (1 M). The absorbance of the assay mixture was read at 390 nm and H$_2$O$_2$ concentration was calculated based on a standard curve of known concentrations of H$_2$O$_2$. For MDA concentration quantification, 0.1 g leaf samples were homogenized with 2 mL of a sodium phosphate buffer (pH 7.0). The homogenates were centrifuged at 13,000 rpm and 4 °C for 20 min. The supernatants were added into 2 mL of 20% trichloroacetic acid containing 0.5% thiobarbituric acid. The reaction solutions were boiled for 10 min, and then cooled to room temperature and centrifuged at 3000 rpm for 10 min. The absorbance was measured at 450 nm, 532 nm, and 600 nm. The MDA concentration was calculated according to the following formula:

$$\text{MDA (µmol·g}^{-1} \text{FW)} = [6.452 \times (A_{532} - A_{600}) - 0.56 \times A_{450}] \times V_T/(V_0 \times W)$$

where FW indicates the fresh weight of leaf sample, $V_T$ indicates total volume of the extraction solution, $V_0$ indicates the measured volume, and W indicates the weight of leaf sample.

2.6. Activities of Antioxidant Enzymes

The activities of antioxidant enzymes SOD, POD, CAT, and APX were determined by the method introduced by Manivannan et al. [54]. The specific enzyme activity was represented as unit·µg$^{-1}$ protein. One unit of SOD activity was defined as the amount causing 50% inhibition of initial rate of NBT reduction. One unit of POD activity was defined as the amount of enzyme that caused an increase of 0.01 per minute at 470 nm. One unit of CAT activity was defined as the amount of enzyme needed to reduce 1 µM of H$_2$O$_2$ per minute. One unit of APX activity was defined as the enzyme activity catalyzing oxidation of 1 µM ascorbic acid per minute.

2.7. Statistical Analysis

The SAS statistical software Release 8.2 (SAS Inst., Cary, NC, USA) was used. The differences between the control and plants treated with supplementary Si were tested by Student’s t-test ($p \leq 0.05$), while multiple comparisons were analyzed by Duncan’s test ($p \leq 0.05$).

3. Results

3.1. Silicon Affected Stomata and Epicuticular Wax of Poinsettia

With a high temperature stress, almost all the stomata of cuttings not supplemented with Si were closed, while some of them were still open in cuttings that were supplemented with Si (Figure 1). However, all the stomata of cuttings, with and without Si supplementation, were closed under a low temperature stress (Figure 2).
It was also found that leaves of Si-treated cuttings and plants had more epicuticular wax, compared to those of the control, especially on the guard cells (Figures 1 and 2). To prove that the white spots were epicuticular wax, wax was removed with chloroform and leaves were observed (Figure S1).
3.2. Silicon Maintained Photosynthesis in Poinsettia

With a high temperature stress, fluorescence quantum yields and OJIP transient curves showed significant differences after 2 days of treatment (Figure 3A,B). The photochemical efficiency (Fv/Fm) derived from JIP test was higher in the Si-treated cuttings (Figure 4A).

With a low temperature stress, the fluorescence quantum yield and OJIP transient curves showed significant differences after 6 days of treatment (Figure 3C,D). The Fv/Fm (maximum quantum efficiency of photosystem II) was higher in CP0→C75 and CP75→C75 than in CP0→C0 (Figure 4B).
Moreover, MDA concentration was lower in the Si 75-treated seedlings under a high temperature stress and in the CP 0 (Figure 5). Si 0, 0 mg·L⁻¹ Si; Si 75, 75 mg·L⁻¹ Si; CP 0→C 0, grown with 0 mg·L⁻¹ Si during cutting propagation and cultivation; CP 75→C 0, grown with 75 mg·L⁻¹ Si during cutting propagation and 0 mg·L⁻¹ Si during cultivation; CP 0→C 75, grown with 0 mg·L⁻¹ Si during cutting propagation and 75 mg·L⁻¹ Si during cultivation; CP 75→C 75, grown with 75 mg·L⁻¹ Si during cutting propagation and 75 mg·L⁻¹ Si during cultivation.

3.3. Concentration of H₂O₂ and MDA Was Low in Silicon-Treated Cuttings

The H₂O₂ concentration was significantly lower in the Si 75-treated seedlings under a high temperature stress and in the CP 75→C 75-treated seedlings under a low temperature stress (Figure 5A,C). Moreover, MDA concentration was lower in the Si 75-treated seedlings under a high temperature stress and in the CP 0→C 75 and CP 75→C 75-treated seedlings under a low temperature stress (Figure 5B,D).

Figure 4. The Fv/Fm of poinsettia subjected to high (A) and low (B) temperature stresses. The asterisk and different letters besides error bars indicate significant differences according to Student’s t-test and Duncan’s test, respectively (p ≤ 0.05). Si 0, 0 mg·L⁻¹ Si; Si 75, 75 mg·L⁻¹ Si; CP 0→C 0, grown with 0 mg·L⁻¹ Si during cutting propagation and cultivation; CP 75→C 0, grown with 75 mg·L⁻¹ Si during cutting propagation and 0 mg·L⁻¹ Si during cultivation; CP 0→C 75, grown with 0 mg·L⁻¹ Si during cutting propagation and 75 mg·L⁻¹ Si during cultivation; CP 75→C 75, grown with 75 mg·L⁻¹ Si during cutting propagation and 75 mg·L⁻¹ Si during cultivation.

Figure 5. The MDA and H₂O₂ concentrations in leaves of poinsettia subjected to high (A,B) and low (C,D) temperature stresses. The asterisk and different letters besides error bars indicate significant differences according to Student’s t-test and Duncan’s test, respectively (p ≤ 0.05). Si 0, 0 mg·L⁻¹ Si; Si 75, 75 mg·L⁻¹ Si; CP 0→C 0, grown with 0 mg·L⁻¹ Si during cutting propagation and cultivation; CP 75→C 0, grown with 75 mg·L⁻¹ Si during cutting propagation and 0 mg·L⁻¹ Si during cultivation; CP 0→C 75, grown with 0 mg·L⁻¹ Si during cutting propagation and 75 mg·L⁻¹ Si during cultivation; CP 75→C 75, grown with 75 mg·L⁻¹ Si during cutting propagation and 75 mg·L⁻¹ Si during cultivation.
3.4. APX Activity Was Low in Silicon-Treated Seedlings

With a high temperature stress, the SOD and APX activities were lower in the Si$_{75}$-treated seedlings, while POD and CAT activities showed no significant differences (Figure 6A). With a low temperature stress, APX activity was lower in the CP$_{0}$→C$_{75}$- and CP$_{75}$→C$_{75}$-treated seedlings, while the POD activity was higher in the CP$_{0}$→C$_{75}$-treated seedlings (Figure 6B). There were no significant differences in SOD and CAT activities.

![Figure 6. Antioxidant enzyme activities in leaves of poinsettia subjected to high (A) and low (B) temperature stresses. The asterisk and different letters besides error bars indicate significant differences according to Student’s t-test and Duncan’s test, respectively (p ≤ 0.05). NS, non-significant; Si$_{0}$, 0 mg L$^{-1}$ Si; Si$_{75}$, 75 mg L$^{-1}$ Si; C$_{0}$, 0 mg L$^{-1}$ Si during cutting propagation and cultivation; C$_{75}$, 75 mg L$^{-1}$ Si during cutting propagation and cultivation; CP$_{0}$→C$_{0}$, 0 mg L$^{-1}$ Si during cutting propagation and cultivation; CP$_{0}$→C$_{75}$, 0 mg L$^{-1}$ Si during cutting propagation and cultivation; CP$_{75}$→C$_{75}$, 75 mg L$^{-1}$ Si during cutting propagation and cultivation; SOD, superoxide dismutase; POD, peroxidase; CAT, catalase; APX, ascorbate peroxidase.](image)

4. Discussion

Silicon is abundant in tissues around the stomata such as guard cells, spongy mesophyll, and veins [55,56]. The presence of Si modulates ion fluxes, which contributes to stomatal movement [57]. Under drought stresses, Si reduced transpiration in maize through stomatal movements and improved water use efficiency as a result [58,59]. In this study, we found that Si maintained stomatal opening in poinsettia under a high temperature stress. Based on previous reports, stomatal opening is believed to be associated with an enhanced evaporative cooling of leaves, reducing the likelihood of thermal damage to tissues [60]. Poinsettia is a sub-tropical plant, which might be sensitive to low temperature stresses. All stomata were closed under a low temperature stress, and Si was not able to alleviate stomatal closure.

Epicuticular wax plays an important role in modifying stomatal conductance, reflecting irradiation, and reducing water loss [61]. It has been reported that Si supplementation can increase epicuticular wax deposition in banana [62] and strawberry [63]. Moreover, Si-induced epicuticular wax deposition on leaves of wheat improved its ability to alleviate drought stresses [64]. Thus, the increased wax load on leaves of poinsettia, especially on guard cells, might help poinsettia overcome temperature stresses.

The value of fluorescence quantum yield and shape of OJIP transient curve has been found to be very sensitive to environmental conditions and thus are used as early diagnoses of stresses [37,65]. Researchers may find that it is difficult to determine optimal timing for sampling during experiment, especially when the plant materials are very limited. Thus, the fluorescence quantum yield and OJIP transient curve can provide important information for researchers. In this study, chlorophyll fluorescence was measured at intervals of 1 and 3 days for high and low temperature stresses, respectively. Samples were collected at one interval after significant differences were found. At that
time point, fluorescence quantum yield and OJIP transient curve showed no significant differences, which might result in no differences in chlorophyll contents among differently treated plants (data not shown). At the time when fluorescence quantum yields were significantly different, Fv/Fm was higher in Si-treated plants than in control plants. Studies have revealed that Si supply mitigates high temperature stresses by maintaining photosynthetic proteins [3], which is essential for photosynthesis in plants. These results indicated that Si maintained photosynthetic proteins and/or pigments in poinsettia under temperature stresses.

The accumulation of MDA reflects oxidative cellular and tissue damages [66]. The Si-treated poinsettia had a significantly lower MDA concentration than control plants did. The same trends were found with H2O2 concentration. These results suggested that Si alleviated temperature stresses of poinsettia evidenced by lower production of H2O2 and MDA.

The accumulation of H2O2 was reported to increase under temperature stresses [67]. Generally, a decrease in concentration of H2O2 was due to high activity of peroxide scavenging antioxidative enzymes [68]. However, we found that both H2O2 concentration and APX activity were lower in Si-treated poinsettia under temperature stresses. There is a possibility that low H2O2 concentration contributed to lower APX activity. On the other hand, a previous study reported that generation of H2O2 and activity of antioxidant enzymes increased to a certain degree and then decreased with the duration of stress exposure [69], which suggested that H2O2 concentration and antioxidant enzyme activities should be measured at more time points to observe the changes.

5. Conclusions

It can be concluded in this study that Si alleviated temperature stresses in poinsettia by maintaining photosynthetic rate, preventing lipid peroxidation, and producing less H2O2. Moreover, SOD and APX activities were lower in Si-treated poinsettia under a high temperature stress, and APX activity was lower in the Si-treated poinsettia under a low temperature stress. Further studies could focus on the regulation of epicuticular wax by Si.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/9/1419/s1, Figure S1: Observation of leaf surface of poinsettia after (A) and before (B) removal of epicuticular wax.

Author Contributions: Conceptualization, B.R.J.; Methodology, B.R.J.; Formal Analysis, J.H., Y.L.; Resources, B.R.J.; Data Curation, J.H., Y.L.; Writing—Original Draft Preparation, J.H.; Writing—Review and Editing, B.R.J.; Project Administration, B.R.J. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: Jiangtao Hu and Yali Li were supported by BK21 Plus Program, Ministry of Education, Korea.

Conflicts of Interest: The authors declare no conflict of interest.

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