Effect of foliar-applied silicon on photochemistry, antioxidant capacity and growth in maize plants subjected to chilling stress

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ABSTRACT

Low temperature is one of the major adverse climatic factors that suppress plant growth and sustainable agricultural development. In these climate conditions, silicon (Si) can mitigate various abiotic stresses including low temperature. In this study, the roles of foliar-applied silicon (10 mM potassium metasilicate) in enhancing tolerance to chilling stress were investigated in maize (Zea mays ‘Fajr’) plants. The low temperature stress caused significant reduction of plant growth and relative water content; however, Si ameliorated these effects. Si supply in maize exhibited a significantly positive effect on accumulation of free amino acids, and reduced the necrotic leaf area. The decrease in maximum quantum yield of PSII (Fv/Fm) was reversible during recovery, but not in the non-Si-treated leaves. This can be explained by enhancement of protective pigments; carotenoid and anthocyanin leading to the protection of PSII from damage. Additionally, analysis of OJIP transients revealed that Si reduced cold damaging effect on performance index (PIabs) and Fv/Fm through improvement of excitation energy trapping (TR0/CS) and electron transport (ET0/CS) per excited cross-section of leaf. The malondialdehyde (MDA) concentration, which was significantly increased under chilling stress, was decreased by Si. The reduced glutathione and ascorbate concentrations were higher in Si-treated plants as compared to those without application of Si under chilling stress. These results indicated that Si could enhance the chilling stress tolerance of maize plants through improving the biomass accumulation, maintaining a high level of glutathione, ascorbic acid, protein, protective pigments, and enhancing the photochemical reactions. This study also suggests that the foliar-applied Si increases recovery ability from chilling injury.

Key words: chilling stress, lipid peroxidation, non-photochemical quenching, silicon, Zea mays

IZVLEČEK

UČINEK FOLIARNEGA DODAJANJA SILICIA NA FOTOKEMIČNO IN ANTIOKSIDACIJSKO UČINKOVITOST TER RAST KORUZE V RAZMERAH HLADNEGA STRESA

Nizka temperatura je eden izmed glavnih neugodnih klimatskih dejavnikov, ki zavira rast rastlin in trajnostni razvoj kmetijstva. V takšnih klimatskih razmerah lahko silicij oblaži abiotični stress vključno z učinki nizke temperature. V tej raziskavi je bila preučevana vloga foliarnega dodajanja Si (10 mM kalijevega metasilikata) pri povečevanju odpornosti koruze (Zea mays ‘Fajr’) na hladni stres. Stres zaradi nizkih temperature je značilno zmanjšal rast in vsebnost vode v rastlinah, kar je dodajanje Si oblažilo. Dodatek silicija je sprožil v koruzi značilne pozitivne učinke v kopčenju prostih amino kislin in v zmanjšanju nekrotičnosti listov. Zmanjšanje v fotokemični učinkovitosti PS II (Fv/Fm) je bilo povratno med okrevanjem, vendar ne pri rastlinah, ki niso bile tretirane s silicijem. To bi lahko razložili s povečanjem vsebnosti zaščitnih pigmentov karotenoïdov in antocianov, kar vodi v zaščito PSII pred poškodbami. Dodatno so analizirali prehodne fluorescencne klorofila a (OJIP) odkrile, da je dodatek Si zmanjšal učinek poškodb zaradi hlada na fotosintetski elektronski transport (PIabs in Fv/Fm) preko boljšega prestrezanja ekscitacijske energije (TR0/CS) in boljšega elektronskega transporta (ET0/CS) na presek ekscitiranega lista. Hladni stres je povzročil poškodbe membran, kar je odrazilo v povečani koncentraciji malondialdehida. V rastlinah tretiranih s Koncentracije reducirane glutationa so bile večje v rastlinah, tretiranih s Si v razmerah hladnega stresa v primerjavi s tistimi, ki s silicijem niso bile tretirane. Ti rezultati nakazujejo, da bi lahko silicij povečal odpornost na hladni stres pri koruzi z izboljšanjem pričakovanja koruze, vzdrževanjem visoke ravnine glutationa, skozi konservacijske kisline, beljakovina, zaščitnih pigmentov in v povečevanju fotokemičnih reakcij. Raziskava nakazuje, da foliarno dodajanje silicija povečuje sposobnost okrevanja iz od hlada nastalih poškodb.

Ključne besede: hladni stres, peroksidacija lipidov, nefotokemična pretvorba svetlobe, silicij, Zea mays

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1 INTRODUCTION

Because of global climate change, we can expect increased damage to plants, such as increased spring frost. Cold stress includes chilling (< 20 °C) and/or freezing (<0 °C) temperatures, and negatively affects the growth and development of plants (Waśkiewicz et al., 2014). Exposure to cold increases the production of reactive oxygen species (ROS) (Zhang et al., 2011), resulting in cytotoxic conditions that affects plant metabolism by stimulating oxidative damage to lipid, proteins, and nucleic acid (Suzuki et al., 2012).

To alleviate the effect of cold stress, plants adapt various approaches for their survival. Proper plant nutrition is one of the strategies in mitigating the stress induced damage in plants (Habibi, 2014a). Si is the second most common element in the lithosphere after oxygen and has been proved to be beneficial for the healthy growth and development of many plant species; particularly Gramineae plants (Broadley et al., 2011). Si application to crops has been reported to enhance their tolerance of multiple stresses (Guntzer, 2011), including pests and pathogens (Dallagnol et al., 2012), salt and water stress (Liu et al., 2014). However, the effects of Si on plant resistance to cold stress and the underlying mechanisms have not been well identified (Liang et al., 2008). It has been reported that the protective role of Si in plants exposed to cold-stress conditions in most cases has been attributed to increase water use efficiency and antioxidant activity in winter wheat (Liang et al., 2008) and cucumber leaves (Liu et al., 2009). In previous work, we concluded that supplementation of water-deficient pistachio (Habibi and Hajiboland, 2013) and canola (Habibi, 2014b) plants with Si alleviates the adverse effects of drought due to its enhancement of photochemical efficiency and photosynthetic gas exchange, as well as an activation of the antioxidant defense capacity in these plants.

The maize (Zea mays) is one of the most important crops, and adaptation of this plant to early annual planting dates requires improvement of chilling tolerance (Battal et al. 2008). One of the major problems arising in some maize cultivation areas includes different levels of injuries caused by lower temperatures in early spring. Because of the fact that the yield of maize was reduced due to chilling damage, the understanding of the physiological and biochemical mechanisms improving chilling tolerance of this species is very significant. Alleviating this growth suppression requires a further improvement of the maize chilling stress tolerance. There is no information about the physiological responses of the maize to Si under chilling stress, which may increase cold tolerance. In this work, we investigated photosynthetic and chlorophyll fluorescence parameters in chilling shocked maize plants, in order to determine the mechanisms of chilling tolerance and survival ability after chilling exposure.

2 MATERIALS AND METHODS

2.1 Plant growth and treatments

Seeds of maize (Zea mays ‘Fajr’) were sown in top of the cylindrical plastic pots; four seeds were planted in each pot. Pots were 14 cm in diameter and 105 cm in depth, filled with 15 kg sandy loam soil (pH 7.6, EC 1.32 dS m⁻¹, field capacity (FC) 23 %, organic carbon (OC) 1.09 %). After emergence, the seedlings were thinned to one plant per pot and irrigated every 5 days to maintain at 90 % field capacity (FC). Plants were grown in a growth chamber located near the city of Miandoab, NW Iran (46°6'E and 36°46'N) with day/night temperature of 25-28 °C /17-19 °C, relative humidity of 45–55 % and daily photon flux density (PFD) of about 1100–1200 µmol m⁻² s⁻¹ throughout the experimental period. Four weeks after sowing, half of the plants were sprayed with 10 mM Si (as potassium metasilicate, pH adjusted to 5.8). The volume of the spray was 400 ml per pot. 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. Ten days after the treatment, 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 (3±1°C) light (at 300 µmol m⁻² s⁻¹ photosynthetic photon flux)/12 h (2±1°C) dark cycle at 65 % relative humidity for 2 days. After the chilling treatment, all plants were returned to normal conditions as described above, to allow leaves to recover from stress. Samples were taken 2, 48 and 96 h after recovery after cold treatment. Each measurement was done independently and experiments were repeated at least three times.

2.2 Analysis of growth parameters
Leaves and roots were harvested and washed with distilled water, blotted dry on filter paper and after determination of fresh mass (FM) they were dried for 48 h at 70 °C for determination of dry mass (DM). Relative water content (RWC) was measured and calculated according to Habibi and Hajiboland (2012). The percentage of necrotic area was determined by measuring separately green and necrotic leaf area according to Irigoyen et al. (1996).

2.3 Measurements of chlorophyll fluorescence parameters
Chlorophyll fluorescence parameters were recorded using a portable fluorometer (OSF1, ADC Bioscientific Ltd., 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 (F₀), maximum (Fm), variable (Fv = Fm - F₀) fluorescence as well as maximum quantum yield of PSII (Fv/Fm) were recorded. Light adapted leaves were used for measurement of steady-state (Fₛ) and maximum (F’m) fluorescence. Calculations were made for F’₀ (F’₀ = F₀/[Fv/Fm] + [F₀/Fm])], photochemical quenching, qP [(Fm - Fd)/(Fm - F₀)] and non-photochemical quenching, NPQ (Fm - F’m)/F’m (Krall and Edwards, 1992).

2.4 Chlorophyll a fluorescence measurements
Chlorophyll a fluorescence transients (OJIP transients) were measured with a Plant Efficiency Analyser (PEA, Hansatech Instruments Ltd., King’s Lynn, Norfolk, PE 32 1JL, England) in dark-adapted (for at least 20 min) leaves. The OJIP transients were induced by a red light (peak at 627 nm) of 3500 µmol m⁻² s⁻¹ (sufficient excitation intensity to ensure closure of all PSII reaction centers to obtain a true fluorescence intensity of Fm) provided by the PEA through an array of six light-emitting diodes.

2.5 The JIP-test (The analysis of the fluorescence rise O-J-I-P)
The JIP-test (Strasser and Strasser, 1995; Strasser et al., 2004) was used to analyse each OJIP transient. The following data from the original fluorescence measurements were used: maximal fluorescence intensity (Fm); fluorescence intensity at 50 µs (considered as F₀); the specific energy fluxes (per reaction center) for absorption (ABS/RC), trapping (TR₀/RC), dissipation at the level of the antenna chlorophylls (DI₀/RC) and electron transport (ET₀/RC); the flux ratios or yields, i.e. the maximum quantum yield of primary photochemistry (φ_p=TR₀/ABS=Fᵥ/Fm), the efficiency (ψ₀=ET₀/TR₀) with which a trapped exciton can move an electron into the electron transport chain further than QA, the quantum yield of electron transport (φ_e=ET₀/ABS); the phenomenological energy fluxes (per excited cross-section of leaf, CS) for absorption (ABS/CS), trapping (TR₀/CS), dissipation (DI₀/CS) and electron transport (ET₀/CS). The fraction of active PSII reaction centers per excited cross-section (RC/CS) is also determined. In addition to above parameters, a multi-parametric expression, the performance index (PIabs), is also calculated (Strasser et al., 2000). The PIabs regards the three main steps that regulate photosynthetic activity by a PSII reaction centre (RC) complex, namely absorption of light energy (ABS), trapping of excitation energy (TR) and conversion of excitation energy to electron transport (ET).

2.6 Determination of total chlorophyll, anthocyanin, Si content and total free amino acids
Leaf concentration of total chlorophyll and carotenoid was determined after extraction of pigments in the cold acetone and allowing the samples to stand for 24 h in the dark at 4 °C (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⁻¹ cm⁻¹ was used and anthocyanin content were expressed as µmol g⁻¹ FM. Leaves were prepared for determination of Si (Jaiswal, 2004) using Inductively-Coupled Plasma-Atomic Emission
Spectrometry (ICP-AES, INTEGRA XL2, GBC Australia). 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).

2.7 Determination of antioxidants and malondialdehyde

Lipid peroxidation was estimated from the amount of malondialdehyde (MDA) formed in a reaction mixture containing thiobarbituric acid according to methods described elsewhere (Habibi and Hajiboland, 2012). The level of glutathione (GSH) was determined according to Singh et al. (2006) with few modifications. Samples of 0.5 g were homogenized in 6 % m-phosphoric acid (pH 2.8) containing 1 mM EDTA. Two solutions were then prepared. Solution A consisted of 110 mM Na$_2$PO$_4$.7H$_2$O, 40 mM NaH$_2$PO$_4$.H$_2$O, 15 mM EDTA, 0.3 mM 5, 5'-dithiobis (2-nitrobenzoic acid) and 0.4 ml l$^{-1}$ BSA (final pH 7). Solution B consisted of 1 mM EDTA, 50 mM imidazole, 0.2 ml l$^{-1}$ BSA and an equivalent of 1.5 units GR activity (Sigma). The absorbance at 412 nm was read after 2 min. The GSH concentration was determined from a standard curve by preparing solutions of 0.5–16 mM GSH. Levels of AsA followed the procedure described by Singh et al. (2006) with few modifications. Briefly, fresh leaf sample of a known weight (1 g) was extracted with 10 ml of 5 % (v/v) m-phosphoric acid and centrifuged at 12,000 × g for 15 min. AsA was determined in a reaction mixture consisting of 0.2 ml of supernatant, 0.5 ml of 150 mM phosphate buffer (pH 7.4, containing 5 mM EDTA) and 0.2 ml of deionized water. Colour was developed in reaction mixtures with the addition of 0.4 ml of 10 % (w/v) TCA, 0.4 ml of 44 % (v/v) phosphoric acid, 0.4 ml of α, α-dipyridyl in 70 % (v/v) ethanol and 0.2 ml of 3 % (w/v) FeCl$_3$. The reaction mixtures were incubated at 40 °C for 40 min and quantified spectrophotometrically at 525 nm. Ascorbate standards were between 1 and 50 mmol ascorbate in 5 % (v/v) m-phosphoric acid.

2.8 Statistical analysis

Experiment was performed according to a factorial design on the basis of Completely Randomized Design (CRD) with 10 pots as 10 independent replications. Statistical analyses were carried out using Sigma Stat (3.5) with Fisher LSD test (P < 0.05).

3 RESULTS

In the absence of chilling stress, Si had no effect on the growth of maize seedlings (Table 1). A significant loss of FM was observed in maize plants under cold stress, i.e., in 96 h after recovery. However, the decrease extent in the Si treatment was less than that in the non-Si treatment. Chilling stress caused significant reduction of RWC, although Si application ameliorated this effect and decreased significantly damaging effects of cold on RWC. Reduction of RWC under chilling stress was alleviated by Si application, accompanied by an increase in FM (Table 1). Chilling stress dramatically increased the necrotic leaf area, while Si supplementation significantly decreased it. As shown in Table 1, cold alone increased necrotic leaf area by 8.6 % after treatment for 96 h recovery, but the increase was only 2.2 % when Si was applied. Concentration of Chl$\alpha$ and b were significantly decreased when the plants were exposed to cold shock in comparison with the non-stressed plants. Si-supplied plants showed the higher anthocyanin and carotenoid concentration as compared with those without application of Si under chilling-stress conditions. The concentration of soluble sugars in the leaves was increased by chilling stress (Table 1). The concentration of starch decreased significantly after 96 h of chilling stress. No significant increase of starch was found by Si application under normal temperature. Under chilling-stress conditions, plants showed an increase in amino acid concentration in the leaves when treated with Si while this change for protein content was negligible. Si supplementation dramatically increased the leaf Si concentration, but the Si concentration was not affected by chilling stress during all treatment periods.
Table 1: Effect of Si supplementation on the shoot fresh mass (SFM), shoot dry mass (SDM), relative water content (RWC), necrotic leaf area, and the concentration of chlorophyll $a$ and $b$, carotenoid, anthocyanin, soluble sugars, starch, total free $\alpha$-amino acids, protein and Si in maize plants grown with or without Si under chilling-stress conditions

<table>
<thead>
<tr>
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<th>Non-stressed</th>
<th>Chilling-stressed</th>
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<tr>
<td></td>
<td>$-\text{Si}$</td>
<td>$+\text{Si}$</td>
</tr>
<tr>
<td>SFM (g plant$^{-1}$)</td>
<td>13.9±1.22$^a$</td>
<td>14.1±1.36$^a$</td>
</tr>
<tr>
<td>SDM (g plant$^{-1}$)</td>
<td>2.87±0.36$^a$</td>
<td>2.94±0.44$^a$</td>
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<tr>
<td>RWC (%)</td>
<td>70±3.2$^a$</td>
<td>73±1.8$^a$</td>
</tr>
<tr>
<td>Necrotic leaf area (%)</td>
<td>0.00±0.00$^a$</td>
<td>0.00±0.00$^a$</td>
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<tr>
<td>Chl $a$ (mg g$^{-1}$ FM)</td>
<td>4.10±0.72$^a$</td>
<td>3.90±0.47$^a$</td>
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<tr>
<td>Chl $b$ (mg g$^{-1}$ FM)</td>
<td>1.78±0.32$^ab$</td>
<td>2.46±0.57$^a$</td>
</tr>
<tr>
<td>Carotenoid (mg g$^{-1}$ FM)</td>
<td>0.98±0.33$^b$</td>
<td>1.16±0.87$^b$</td>
</tr>
<tr>
<td>Anthocyanin (µmol g$^{-1}$ FM)</td>
<td>4.78±0.78$^b$</td>
<td>5.46±0.57$^b$</td>
</tr>
<tr>
<td>Soluble sugars (mg g$^{-1}$ FM)</td>
<td>12.3±2.6$^c$</td>
<td>14.2±3.35$^bc$</td>
</tr>
<tr>
<td>Starch (mg g$^{-1}$ FM)</td>
<td>164±17.6$^b$</td>
<td>172±19.6$^a$</td>
</tr>
<tr>
<td>Amino acids (µmol g$^{-1}$ FM)</td>
<td>3.45±0.65$bc$</td>
<td>3.02±1.04$^c$</td>
</tr>
<tr>
<td>Protein (mg g$^{-1}$ FM)</td>
<td>10.7±2.11$^a$</td>
<td>11.4±2.01$^a$</td>
</tr>
<tr>
<td>Leaf Si (mg g$^{-1}$ DM)</td>
<td>0.79±0.22$^b$</td>
<td>2.16±0.85$^a$</td>
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Samples were taken 96 h after recovery after cold treatment. Data of each row indicated by the same letters are not significantly different ($P < 0.05$).

Data are the mean ± SD (n = 10).

Si supplementation had no effect on leaf $F_v/F_m$ ratio under the normal condition, but it was decreased by cold treatment after 2 h recovery in the chilling-stressed leaves (Fig. 1a). However, Si quickly and significantly increased the $F_v/F_m$ ratio after 2, 46 and 96 h recovery after cold treatment. The photochemical quenching ($q_P$) of non-Si-supplemented plants showed little change in response to chilling treatment, and Si-supplemented plants showed a marked increase after 2 h recovery. In contrast, the non-photochemical quenching (NPQ) of the maize plants increased significantly after chilling, and the most marked increase in NPQ was occurred for Si-supplemented leaves during 2 h after chilling stress. During recovery, NPQ gradually reduced in the Si-supplemented leaves, but not in the non-Si-treated leaves.
To understand the precise effects of Si on the kinetics of recorded OJIP transients, data collected at water stress periods were analysed in Fig. 2. The effects of drought stress on the maximum quantum yield of primary photochemistry ($F_v/F_m$) and the specific and phenomenological energy fluxes for light absorption, excitation energy trapping and electron transport are also showed in the form of a radar plot (Fig. 2). Chilling stress resulted in the deactivation of reaction centers (RC/CS) and decreased excitation energy trapping (TR$_o$/CS) and electron transport (ET$_o$/CS). Non-Si plants had performance indexes (PI$_{abs}$) of 2-3, however, in Si-supplemented leaves, the PI$_{abs}$ values were respectively 200 % higher than those recorded in non-Si-supplemented leaves (Fig. 2). Si-supplemented plants showed higher PI$_{abs}$ with compared to those without application of Si under cold conditions.

Chilling-stressed plants displayed an increase in lipid peroxidation (Fig. 2) determined by the accumulation of MDA. However, the MDA level was decreased by foliar application of Si under chilling-stress conditions. Under chilling-stress conditions, plants were able to maintain higher GSH and AsA levels, as compared with non-stressed plants, but consistent with the decrease in lipid peroxidation seen in Si-supplied plants during stress, the levels of GSH showed an increment with a concomitant increase in AsA content (Fig. 2).
Effect of foliar-applied silicon on photochemistry, ... and growth in maize plants subjected to chilling stress

Figure 2: Radar plots depicting changes in the phenomenological (per CS) and specific (per RC) energy fluxes of absorption (ABS) excitation energy trapping (TR) and electron transport (ET). The changes in quantum efficiency (Fv/Fm) and the performance indexes (PIabs) are also shown.

Figure 3: Changes in the concentrations of reduced ascorbate (AsA) (a) and glutathione (GSH) (b), and malondialdehyde (MDA) (c) in maize plants grown with or without Si under chilling-stress conditions. Samples were taken 2, 46 and 96 h after recovery (Rec) after cold treatment. Symbols with error bars are the mean ± SD (n = 10), and significant differences (P < 0.05) are indicated by different letters.
4 DISCUSSION

Our results showed that chilling-induced growth inhibition in maize seedlings was partly reversed by Si supplementation (Table 1). These results are in agreement with previous reports regarding the beneficial effects of Si on the growth and yield of *Sorghum bicolor* (L.) Moench under salt stress (Yin et al., 2013) and wheat cultivars under cold-stress (Liang et al., 2008) conditions. Chilling-sensitive plants exposed to low temperature often exhibit signs of water stress due to decreased root hydraulic conductance, leading to associated decreases in leaf water and turgor potential, followed by a reduction of growth (Wasikiewicz et al., 2014). We have already shown that the RWC in the non-Si-treated plants was significantly reduced under chilling stress. In this study, addition of Si helped the plants to maintain a high RWC, and dry matter production. The main mechanism for such roles of Si in maintaining higher water content in leaf tissues is hypothesized to be the reduced transpirational water loss via reduction of both cuticular and stomatal transpiration (Cooke and Leishman, 2011; Sonobe et al., 2011). Leaf necrosis is a typical external sign of chilling injury in chilling-sensitive plants. In this study, pre-Si treatment reduced the leaf necrosis under chilling stress conditions.

In the present study, though an expected enhancement in free amino acid level under chilling stress, Si application caused a significant stimulation in free amino acid level. Accumulation of these metabolites can function as osmolytes to preserve cell turgor and have the ability to protect membranes from stress damage (Krasensky and Jonak, 2012).

$F_v/F_m$ is an indication of overall photosynthetic capacity (Balouchi, 2010). Significant reduction of $F_v/F_m$ in chilling-stressed maize indicated that a proportion of PSII reaction centers is damaged or inactivated following photoinhibition, commonly observed in plants under stress (Baker and Rosenqvist, 2004). In the present study, there was a remarkable decrease in $F_v/F_m$ ratio of Si-treated leaves after chilling treatment and rapid increase during recovery, indicating that the capacity of electron transport was inhibited by chilling stress, and the damage in chlorophyll reaction center was reversible, but not in the non-Si-treated leaves.

Both Si and non-Si treatments showed a significant increase in NPQ just after cold shock, but then declined in Si-treated leaves during recovery, indicating that NPQ capacity of photosynthetic apparatus is changeable over different environments. It may be also suggested that the ability to cope with excess energy and photoinhibition was much improved in Si-treated plants. Based on the current results, it can be concluded that Si application not directly enhance chilling tolerance of maize plants, but it increased recovery ability from chilling injury. Plants have evolved a variety of protective mechanisms against the stress induced damage to cellular components, such as the dissipation of excess excitation energy and the synthesis of protective pigments, such as carotenoids and anthocyanins (Marczak et al., 2008; Huang et al., 2010). In this study, Si application caused a significant stimulation in these pigments under chilling stress. This finding is consistent with other published reports suggesting that the accumulation of carotenoids and anthocyanins is generally well correlated with chilling tolerance (Marczak et al., 2008).

The results presented that the decrease in the $P_{I_{abs}}$ and down-regulation of photochemical activity during chilling stress conditions may be interpreted as evidence for PSII RC deactivation (Ivanov et al., 2006), and the $P_{I_{abs}}$ was much more sensitive than the $F_v/F_m$ ratio. These results indicated that PSII RC’s are functionally altered by Si application through increase in density of active reaction centers, RC/CS.

The magnitude of oxidative damage is usually measured by MDA (an end product of membrane lipid peroxidation), as a marker for the ROS-mediated cell membrane damage (Liu et al., 2009). In the present work, chilling stress caused membrane damage, as assessed by lipid peroxidation. However, Si could enhance antioxidant defense activity in maize plants under chilling stress, resulting in decreased membrane oxidative damage, and improved stability of cell membranes and enhanced stress tolerance. Fu et al. (2014) reported a consistent increase in reduced glutathione and ascorbate in *Elymus nutans* Griseb.in response to an increase in the intensity and duration of chilling stress. In agreement with
the above, in our study, the contents of total GSH and AsA increased under chilling stress. GSH and AsA concentrations were higher in Si-treated plants under chilling stress compared with –Si ones. Our results suggest that improvement of maize tolerance to chilling stress by Si supplementation may be achieved by maintaining a relative high content of GSH and AsA as well as activation of antioxidant defense capacity in cold-stressed plants.

In conclusion, results from this study showed that the foliar application of Si alleviated effects of chilling on plant growth. In the present study, there was a remarkable decrease in Fv/Fm ratio of Si-treated leaves after cold treatment and rapid increase during recovery, indicating that the damage in chlorophyll reaction center was reversible, but not in the non-Si-treated leaves. This can be explained by enhancement of efficiency for dissipation of excess photon energy in the PSII antenna, determined as non-photochemical quenching as well as accumulation of protective pigments, such as carotenoid and anthocyanin leading to the protection of PSII from photo-damage. Our results suggest that improvement of maize tolerance to chilling stress by Si supplementation is achieved by maintaining a relative high content of antioxidants and photochemical reactions.

5 REFERENCES


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Effect of foliar-applied silicon on photochemistry, ... and growth in maize plants subjected to chilling stress


