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Effects of Exogenous Silicon on the Activities of Antioxidant Enzymes and Lipid Peroxidation in Chilling-Stressed Cucumber Leaves

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Abstract

In order to increase vegetable productivity by improving environmental conditions, this article investigates the effects of exogenous silicon on the activities of major antioxidant enzymes and on lipid peroxidation under chilling stress, and it examines whether silicon-induced chilling tolerance is mediated by an increase in antioxidant activity. *Cucumis sativus cv*. Jinchun 4 was hydroponically cultivated to the two-leaf stage, at which point seedlings were watered with different concentrations of silicon (0, 0.1 and 1 mmol L^{-1}) and separately exposed to normal (25/18°C) or chilling (15/8°C) temperatures for six days under low light (100 μmol m^{-2} s^{-1}). Data were collected from the second leaves on the percentage of withering and the levels of endogenous silicon, malondialdehyde (MDA), hydrogen peroxide (H_2O_2), superoxide radical ($O_2^{\cdot-}$), superoxide dismutase (SOD, EC 1.15.1.1), glutathione peroxidase (GSH-Px, EC 1.11.1.9), ascorbate peroxidase (APX, EC 1.11.1.11), monodehydroascorbate reductase (MDHAR, EC 1.6.5.4), glutathione reductase (GR, EC 1.6.4.2), reduced glutathione (GSH) and ascorbate (AsA). Compared to normal temperatures, chilling resulted in partially withered leaves and increased MDA content. When 0.1 or 1 mmol L^{-1} exogenous silicon was combined with chilling, the withering of the cucumber leaves was reduced relative to the original chilling treatment, while the endogenous silicon content was increased, antioxidants such as SOD, GSH-Px, APX, MDHAR, GR, GSH, and AsA were more active, and the levels of H_2O_2, $O_2^{\cdot-}$, and MDA were lower. We propose that exogenous silicon leads to greater deposition of endogenous silicon and thereby increases antioxidant activities and reduces lipid peroxidation induced by chilling.

Key words: antioxidant enzyme, chilling, *Cucumis sativus*, lipid peroxidation, silicon

INTRODUCTION

Some chilling-sensitive vegetable cultivars such as *Cucumis sativus cv*. Jinchun 4 are widely grown in the fields of China. However, it is well known that larger seedlings bear fruits earlier. Before being transferred to the field, the seedlings are thus cultivated in early spring using sunlight-heated greenhouses where they are often subjected to chilling temperatures and low light. Chilling and low light inhibit the growth and development of plants (Xu *et al*. 2008). Thus, understanding the biochemical and physiological mechanisms of chilling and low-light stresses is essential for improving environmental conditions to increase vegetable productivity.

Chilling and low light conditions induce the production of reactive oxygen species (ROS) such as superoxide radical ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) (Xu *et al*. 2008). The accumulation of ROS damages membrane lipids and can lead to the death of plant cells (Molassiotis *et al*. 2006). In order to scavenge ROS, plants possess enzymatic antioxidants, including superoxide dismutase (SOD), catalase (CAT), guaiacol per-

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oxidase (GPX), glutathione peroxidase (GSH-Px), ascorbate peroxidase (APX), glutathione reductase (GR), dehydroascorbate reductase (DHAR) and monodehydroascorbate reductase (MDHAR), as well as non-enzymatic antioxidants such as reduced glutathione (GSH) and ascorbate (AsA) (Asada 1992).

Silicon, the second-most abundant element in soil (Lewin and Reimann 1969), can increase the antioxidant activities of plants and thereby alleviate ROS damage induced by stresses such as salt (Liang 1999; Liang *et al*. 2003; Zhu *et al*. 2004; Qian *et al*. 2006), drought (Gong *et al*. 2005; Li Q F *et al*. 2007), manganese (Iwasaki *et al*. 2002; Shi *et al*. 2005; Feng *et al*. 2009), sodic-B toxic soil (Gunes *et al*. 2007a, b, c, d), arsenic (Guo *et al*. 2005), cadmium (Treder and Cieslinski 2005), powdery mildew (Wei *et al*. 2004), sheath blight (Zhang *et al*. 2006), and rust (Li G J *et al*. 2007). Liang *et al*. (2008) reported that silicon enhances the resistance to freezing stress in winter wheat.

In general, freezing temperatures are lower than chilling temperatures, and plants respond differently to the two kinds of low temperatures (Kojima *et al*. 1998). We hypothesized that exogenous silicon can scavenge chilling-induced ROS by changing antioxidant activities and thus enhance plants' ability to resist chilling temperatures under low light. In China, seedlings of the chilling-sensitive cucumber cultivar Jinchun 4 were treated with different temperatures and silicon concentrations under low light conditions to investigate the effects of exogenous silicon on antioxidant activities and lipid peroxidation. Our work may help to reveal how exogenous-silicon-induced chilling tolerance is mediated by elevated antioxidant activities.

MATERIALS AND METHODS

Plant materials and growth conditions

Cucumber seeds (*Cucumis sativus cv*. Jinchun 4) were germinated on moist pledget for 2 d and then transplanted into 10-cm plastic pots filled with sand. The cucumber seedlings were grown at 25°C with 12 h light (600 μmol m^{-2} s^{-1})/12 h dark and watered twice daily with Hoagland nutrient solution containing 5 mmol L^{-1} $Ca(NO_3)_2$, 5 mmol L^{-1} KNO_3, 1 mmol L^{-1} $NH_4H_2PO_4$, 2 mmol L^{-1} $MgSO_4$, 70 μmol L^{-1} Fe-EDTA, 10 μmol L^{-1} $MnSO_4$, 50 μmol L^{-1} H_3BO_3, 0.7 μmol L^{-1} $ZnSO_4$, 0.2 μmol L^{-1} $CuSO_4$, and 0.01 μmol L^{-1} $(NH_4)_6Mo_7O_{24}$.

At the two-leaf stage, six groups of uniform seedlings were selected and placed in growth chambers. Kept under normal temperature conditions (25/18°C, day/night), the first three groups of cucumber seedlings were watered independently with one of three solutions: Hoagland nutrient solution only, Hoagland nutrient solution containing 0.1 mmol L^{-1} K_2SiO_3 and Hoagland nutrient solution containing 1 mmol L^{-1} K_2SiO_3. The groups were designated S_0, $S_{0.1}$, and S_1, respectively. The other three groups of seedlings were kept in chilling conditions (15/8°C, day/night) and treated separately with one of the three types of nutrient solutions as described above, and designated L_0, $L_{0.1}$, and L_1, respectively. In nutrient solutions where K_2SiO_3 introduced additional K, the K was subtracted from the KNO_3 and the resultant nitrate loss was supplemented with dilute nitric acid. The pH of the nutrient solutions was adjusted to 6.0 using H_2SO_4 before transplanting (Zhu *et al*. 2004). All six groups of plants received 12 h light (100 μmol m^{-2} s^{-1})/12 h dark. After 6 days of treatment, samples of the second leaf were harvested in triplicate.

Determination of silicon concentration

The silicon concentration in cucumber leaves was determined according to the modified method of Gunes *et al*. (2007d). In porcelain crucibles, 0.2 g of dry leaves was incinerated at 300°C for 3 h and then at 550°C for 4 h. When the temperature fell to 70°C, 2 drops of deionized water were added to the samples. After being warmed at 70°C for 1 h, the leaf ash was heated to 550°C for 3 h. It was then washed into polyethylene bottles with 5 mL of nitric acid where it was heated at 90°C for 10 min. A total of 15 mL of 10% (w/v) sodium carbonate was added and the solution of leaf ash was heated at 90°C for 5 min. The pH value was adjusted to 1.8 with 5 mol L^{-1} sodium hydroxide and 1 mol L^{-1} nitric acid after 10 mL of hydrochloric acid was added to the ash solution. Following the addition of 10 mL of 4% (w/v) boric acid, the solution volume was brought to 50 mL with deionized water. 1.5 mL of the solution was mixed with 1.5 mL of color reagent (containing 0.75 mL of 0.08 mol L^{-1} sulfuric acid and 0.75 mL of 0.016 mol L^{-1} ammonium

heptamolybdate). Then 1.5 mL of 3.3% (w/v) tartaric acid and 1.5 mL of 0.4% (w/v) AsA were added, and the mixture was allowed to set for 25 min. The absorbance was measured at 811 nm and the silicon concentration was calculated from a standard curve using a silicon standard solution (Merck).

Determination of malondialdehyde, $O_2^{\cdot-}$ and H_2O_2 contents

The concentration of malondialdehyde (MDA) was measured according to the method of Xu *et al*. (2008), and the absorbance was measured at 450, 532, and 600 nm (Zhang *et al*. 2005).

Superoxide radical was determined according to the method of Elstner and Heupel (1976) with modifications. Leaves (0.3 g) were homogenized in 3 mL of 65 mmol L^{-1} phosphate buffer (pH 7.8) on an ice bath and were then centrifuged at 4°C and 5 000 × g for 10 min. The supernatants (0.75 mL) were mixed with 0.675 mL of 65 mmol L^{-1} phosphate buffer (pH 7.8) and 0.07 mL of 10 mmol L^{-1} hydroxylamine chlorhydrate and were placed at 25°C. After 20 min, 0.375 mL of 17 mmol L^{-1} sulfanilamide and 0.375 mL of 7 mmol L^{-1} α-naphthylamine were added, and the mixture was placed at 25°C for another 20 min before it was mixed with 2.25 mL of ether. The absorbance was measured at 530 nm and the $O_2^{\cdot-}$ concentration was calculated from a standard curve of $NaNO_2$.

The level of H_2O_2 was determined according to the modified method of Bernt and Bergmeyer (1974). Leaves (0.1 g) were homogenized with cold (-20°C) acetone and were centrifuged at 3 000 × g for 10 min. The supernatant (1 mL) was mixed with 0.1 mL of 95% (v/v) hydrochloric acid [containing 20% (v/v) titanium tetrachloride] and 0.2 mL of ammonia. After being centrifuged at 10 000 × g for 10 min, the sediment was washed with acetone repeatedly and then was dissolved in 3 mL of 1 mol L^{-1} H_2SO_4. The absorbance was measured at 410 nm and the H_2O_2 content of the leaves was calculated from a standard curve of H_2O_2.

Extraction and assay of antioxidant enzymes

After being ground with liquid nitrogen, 0.3 g of leaves was suspended in 3 mL of ice-cold HEPES buffer (25 mmol L^{-1}, pH 7.8) containing 0.2 mmol L^{-1} EDTA and 2% (w/v) PVP. The homogenate was centrifuged at 4°C and 12 000 × g for 20 min, and the resulting supernatants were used for determination of SOD (EC 1.15.1.1), CAT (EC 1.11.1.6), GPX (EC 1.11.1.7), DHAR (EC 1.8.5.1), MDHAR (EC 1.6.5.4) and GR (EC 1.6.4.2) (Ramiro *et al*. 2006). To extract GSH-Px (EC 1.11.1.9), 0.3 g of ground leaves was suspended in 0.3 mL of HEPES buffer (25 mmol L^{-1}, pH 7.8) containing 0.2 mmol L^{-1} EDTA and 2% (w/v) PVP. HEPES buffer (25 mmol L^{-1}, pH 7.8) containing 0.2 mmol L^{-1} EDTA, 2% (w/v) PVP and 2 mmol L^{-1} AsA was used for APX (EC 1.11.1.11) extraction.

The activities of SOD, CAT, GPX, and GR were measured according to the methods of Xu *et al*. (2008). One unit of SOD activity was defined as the amount of enzyme that would inhibit 50% of NBT photoreduction as determined at 560 nm. CAT activity was calculated by the disappearance of H_2O_2 (ε = 40 mol L^{-1} cm^{-1}) in 1 min at 240 nm, while the GPX activity was determined by following the change of absorbance due to guaiacol oxidation (ε = 6.39 mmol L^{-1} cm^{-1}) at 470 nm. In the GR enzymatic reaction, NADPH oxidation (ε = 6.2 mmol L^{-1} cm^{-1}) was recorded as the decrease in absorbance at 340 nm in 1 min.

The activity of GSH-Px was measured by using H_2O_2 as the substrate (Xue *et al*. 2001). A total of 40 μL of enzyme extract was mixed with 40 μL of 1 mmol L^{-1} GSH for the enzymatic reaction. For the non-enzymatic reaction, 40 μL of enzyme extract was boiled for 25 min and was then mixed with 40 μL of 1 mmol L^{-1} GSH. The mixtures were heated at 37°C for 5 min and then 20 μL of 1.5 mmol L^{-1} H_2O_2 was added to initiate the reaction. The reaction was allowed to run for 3 min and was terminated by adding 400 μL of 1.67% (w/v) metaphosphoric acid [containing 0.05% (w/v) EDTA and 28% (w/v) NaCl]. The mixtures were centrifuged at 2 000 × g for 10 min and then 400 μL of the supernatant was mixed with 500 μL of 0.32 mol L^{-1} Na_2HPO_4 and 100 μL of 1 mmol L^{-1} 5,5-dithio-bis(2-nitrobenzoic acid) for 5 min. The absorbance was measured at 412 nm and the enzyme activity was calculated as the decrease in GSH in the enzymatic reaction as compared to the non-enzymatic reaction.

To determine APX activity, 50 μL of enzyme extract was mixed with 850 μL of 25 mmol L^{-1} phosphate buffer (pH 7.0, containing 0.1 mmol L^{-1} EDTA), 50 μL of

5 mmol L^{-1} AsA and 50 μL of 20 mmol L^{-1} H_2O_2 (Zhu *et al*. 2004). The H_2O_2-dependent oxidation of AsA (ε = 2.8 mmol L^{-1} cm^{-1}) was followed by the decrease in absorbance at 290 nm. To measure the activity of DHAR, 50 μL of enzyme extract was mixed with 850 μL of 25 mmol L^{-1} phosphate buffer (pH 7.0, containing 0.1 mmol L^{-1} EDTA), 50 μL of 70 mmol L^{-1} GSH, and 50 μL of 8 mmol L^{-1} dehydroascorbate and its activity was determined by the formation of AsA (ε = 14 mmol L^{-1} cm^{-1}) in 1 min at 265 nm (Doulis *et al*. 1997). MDHAR activity was assayed by monitoring the change in absorbance at 340 nm due to NADPH oxidation (ε = 6.2 mmol L^{-1} cm^{-1}) for 1 min. The reaction mixture contained 50 mmol L^{-1} Tris-HCl buffer (pH 7.5), 0.2 mmol L^{-1} NADH, 2.5 mmol L^{-1} AsA, 0.15 U ascorbate oxidase and 50 μL of enzyme extract (Hoque *et al*. 2007).

The protein concentration of each enzyme extract was determined according to the method of Bradford (1976).

Determination of non-enzymatic antioxidant content

Leaf tissues (0.6 g) were homogenized in 6 mL of 1% (w/v) oxalic acid and were centrifuged at 4°C and 8 000 × g for 10 min. The supernatant was used for determination of AsA and total ascorbate.

The level of AsA was measured according to the methods of Klein and Perry (1982) and Raghu *et al*. (2007) with modifications. A total of 500 μL of ascorbate extract was mixed with 250 μL of 0.1% (w/v) 2,6-dichlorophenol and 625 μL of xylene for 20 s, and the absorbance was measured at 500 nm within 1.5 min.

The level of total ascorbate was determined according to Mukherjee and Choudhuri (1983) with slight modifications. Charcoal (0.2 g) was activated with 15 mL of 1 mmol L^{-1} HCl and was then suspended in 2.5 mL of ascorbate extract for 1 min. After filtration, 200 μL of filtrate was mixed with 200 μL of 2% (w/v) thiourea and 100 μL of 2% (w/v) 2,4-dinitrophenylhydrazine. It was mixed at 37°C for 3 h and then was cooled to room temperature. After 0.5 mL of 85% (v/v) H_2SO_4 was added to the mixture on an ice bath, the absorbance was recorded at 500 nm. The levels of AsA and total ascorbate were calculated from a standard curve of ascorbic acid.

The levels of total glutathione and oxidized glutathione (GSSG) were measured using the GSH and GSSG Assay Kit (Beyotime Institute of Biotechnology, China), and GSH was estimated from the difference between total glutathione and GSSG.

Data analysis

Data were expressed as means ± standard errors. Differences were analyzed with two-way ANOVA using SAS software (SAS Institute) and means were compared by Duncan's multiple range tests (Hsu and Kao 2007). P values < 0.05 were considered to be significant.

RESULTS

Leaf withering

Under chilling conditions (15/8°C, day/night), the second leaf withered in some cucumber seedlings, while no leaves withered at normal temperatures (25/18°C). In the L_0 treatment, (46.2 ± 3.8)% of the second leaves were withered, which was significantly (P < 0.01) more than in the $L_{0.1}$ (25.0 ± 7.2)% and L_1 (8.3 ± 4.2)% treatments. We conclude that exogenous silicon supplementation significantly (P < 0.01) reduces the percentage of cucumber leaves that wither.

Silicon content

The silicon content of cucumber leaves was lower (P < 0.01) in the L_0 treatment than in the S_0 treatment. In the $S_{0.1}$ and S_1 treatments, the silicon contents were higher (P < 0.01) than in the S_0 treatment. Compared to the L_0 treatment, the silicon contents in $L_{0.1}$ and L_1 treatments were higher (P < 0.01) (Fig.1-A). According to the ANOVA analysis, chilling decreases (P < 0.01) the silicon content of cucumber leaves, while exogenous silicon supplementation increases it (P < 0.01).

MDA content

The MDA content in leaves from the L_0 treatment was higher (P < 0.01) than in those from the S_0 treatment.

In comparison to the S_0 treatment, the MDA content of cucumber leaves was lower ($P<0.01$) in the S_1 treatment, but was not significantly lower in the $S_{0.1}$ treatment ($P>0.05$). Compared to the L_0 treatment, the MDA content in leaves from the $L_{0.1}$ and L_1 treatments was significantly ($P<0.01$) lower (Fig.1-B). Therefore chilling increases ($P<0.01$) the MDA content of leaves, while exogenous silicon supplementation has the opposite effect ($P<0.01$).

$O_2^{\cdot-}$ formation rate and H_2O_2 content

The formation rate of $O_2^{\cdot-}$ (Fig.1-C) and the content of H_2O_2 (Fig.1-D) were both higher ($P<0.01$) in leaves from the L_0 treatment than in those from the S_0 treatment. In $S_{0.1}$ and S_1 treatments, the $O_2^{\cdot-}$ level did not change significantly ($P>0.05$), while the H_2O_2 content of leaves was lower ($P<0.01$) than that of the S_0 treatment. In comparison with the L_0 treatment, the level of the two ROSs was significantly ($P<0.01$) lower in the $L_{0.1}$ and L_1 treatments. Therefore, chilling increases the levels of $O_2^{\cdot-}$ ($P<0.01$) and H_2O_2 ($P<0.05$) in cucumber leaves, and exogenous silicon supplementation significantly ($P<0.01$) decreases them.

Antioxidant enzyme activity

Compared to the S_0 treatment, the activities of antioxi-

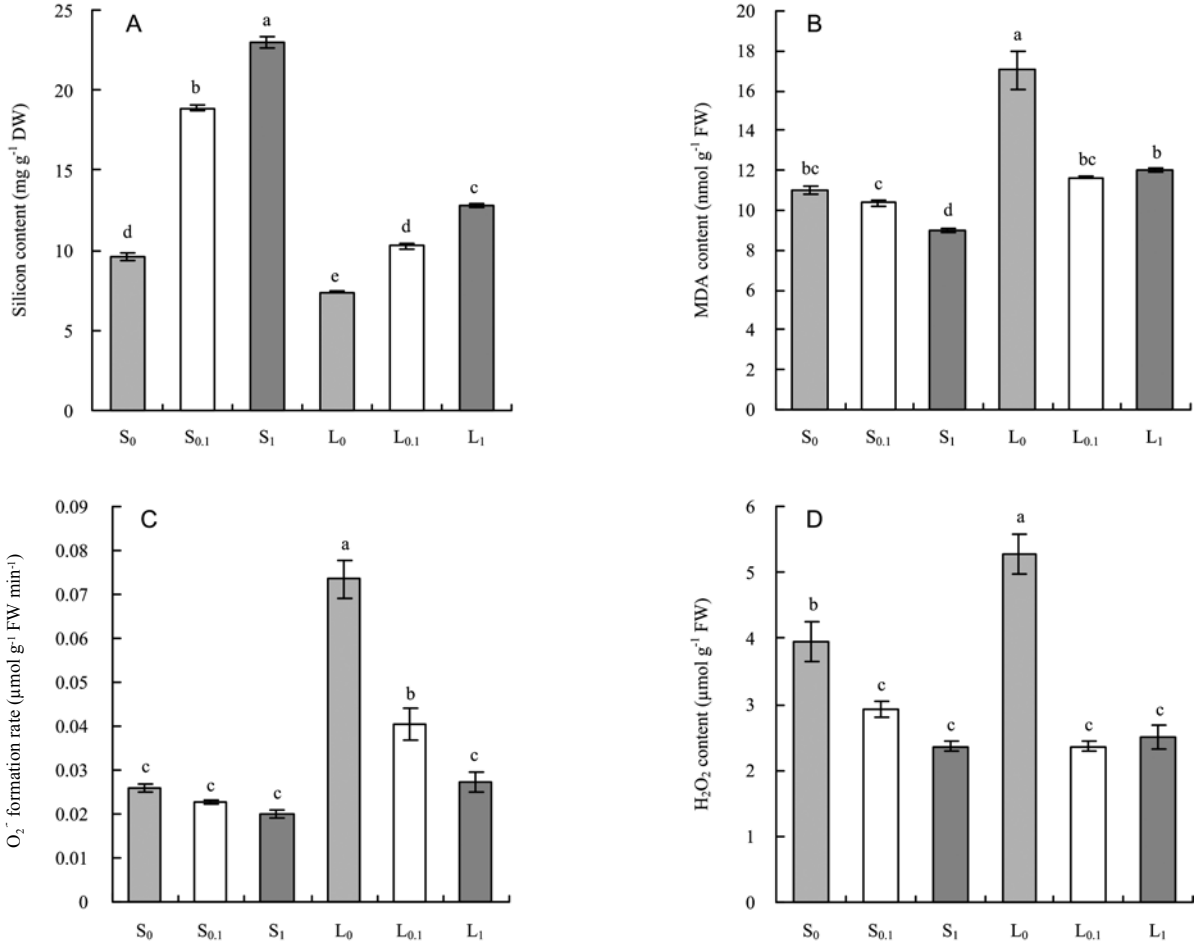

Fig. 1 Effects of exogenous silicon on the levels of silicon (A), MDA (B), and the formation rate of $O_2^{\cdot-}$ (C) and H_2O_2 (D) in chilling-stressed cucumber leaves under low light (100 μmol m^{-2} s^{-1}). S_0, watering with Hoagland nutrient solution at normal temperature (25/18°C); $S_{0.1}$, watering with Hoagland nutrient solution containing 0.1 mmol L^{-1} K_2SiO_3 at normal temperature (25/18°C); S_1, watering with Hoagland nutrient solution containing 1 mmol L^{-1} K_2SiO_3 at normal temperature (25/18°C); L_0, watering with Hoagland nutrient solution at chilling temperature (15/8°C); $L_{0.1}$, watering with Hoagland nutrient solution containing 0.1 mmol L^{-1} K_2SiO_3 at chilling temperature (15/8°C); L_1, watering with Hoagland nutrient solution containing 1 mmol L^{-1} K_2SiO_3 at chilling temperature (15/8°C). Means with the same letters are not significantly different. Error bars represent the standard error. The same as below.

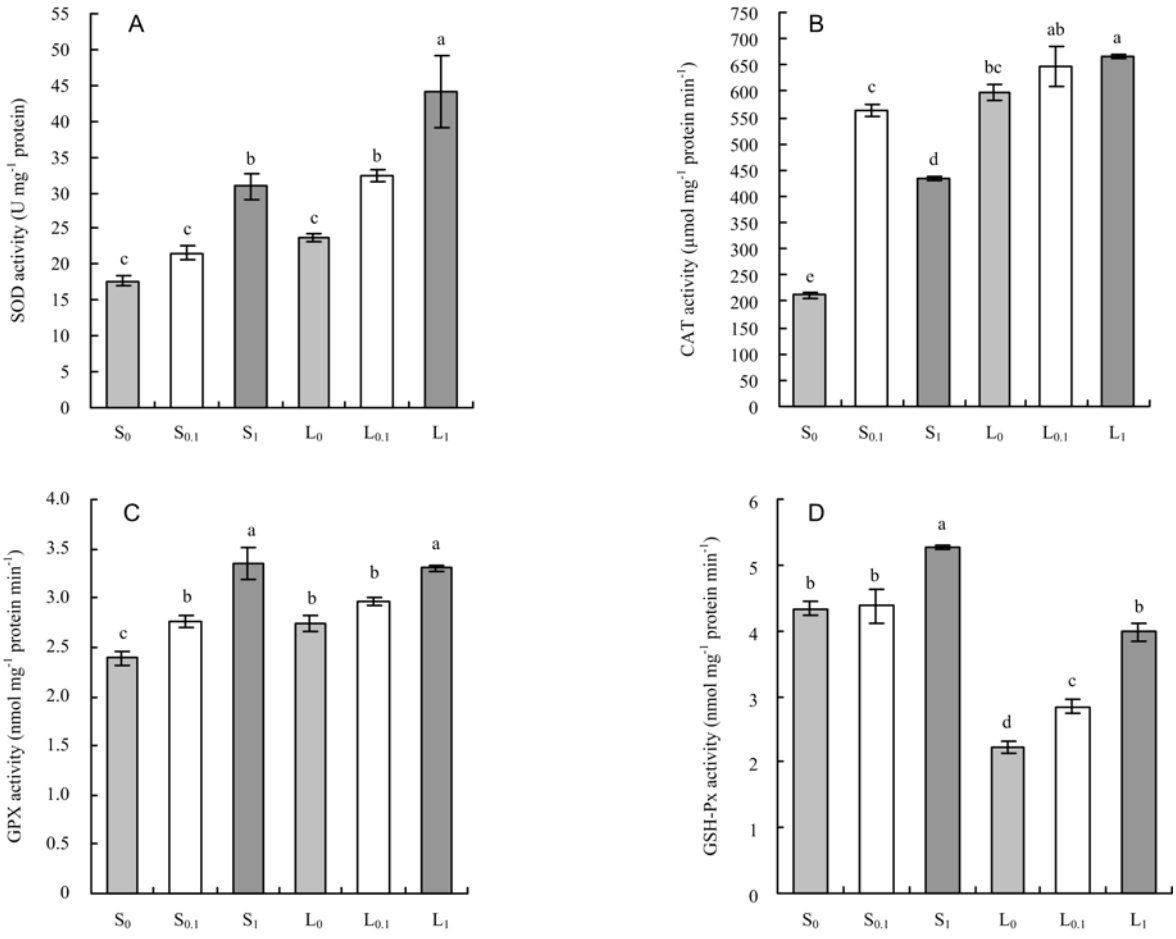

Fig. 2 Effects of exogenous silicon on the activities of SOD (A), CAT (B), GPX (C), and GSH-Px (D) in chilling-stressed cucumber leaves under low light (100 μmol m^{-2} s^{-1}).

dant enzymes such as CAT (Fig.2-B), GPX (Fig.2-C), MDHAR (Fig.3-B), and DHAR (Fig.3-C) were higher ($P<0.05$) in leaves from the L_0 treatment, although the activities of SOD (Fig.2-A), APX (Fig.3-A) and GR (Fig.3-D) were not significantly ($P>0.05$) higher, and in fact GSH-Px (Fig.2-D) activity decreased considerably ($P<0.01$). When comparing the $S_{0.1}$ or S_1 treatments with S_0 treatment, the activities of CAT, GPX, APX, MDHAR, and GR were significantly ($P<0.05$) higher. However, the activities of SOD and GSH-Px were not enhanced significantly ($P>0.05$) in the $S_{0.1}$ treatment, but they were significantly higher ($P<0.01$) in the S_1 treatment. DHAR activity was significantly higher ($P<0.05$) in $S_{0.1}$ treatment, and it did not change considerably ($P>0.05$) in the S_1 treatment. Compared with the L_0 treatment, $L_{0.1}$ and L_1 treatments significantly ($P<0.05$) increased the activities of SOD, GSH-Px, APX, MDHAR, and GR, but did not significantly change the DHAR activity ($P>0.05$). The activities of CAT and GPX increased more ($P<0.05$) in leaves from the L_1 treatment than in those from the L_0 treatment, but they were not significantly increased ($P>0.05$) by the $L_{0.1}$ treatment. According to the ANOVA results, chilling increased ($P<0.05$) the activities of the antioxidant enzymes SOD, CAT, GPX, GSH-Px, APX, MDHAR, DHAR, and GR, while exogenous silicon supplementation significantly ($P<0.01$) increased the activities of SOD, CAT, GPX, GSH-Px, APX, MDHAR, and GR.

Non-enzymatic antioxidant content

When comparing the L_0 treatment with the S_0 treatment, the levels of AsA (Fig.4-A) and GSH (Fig.4-C) and the

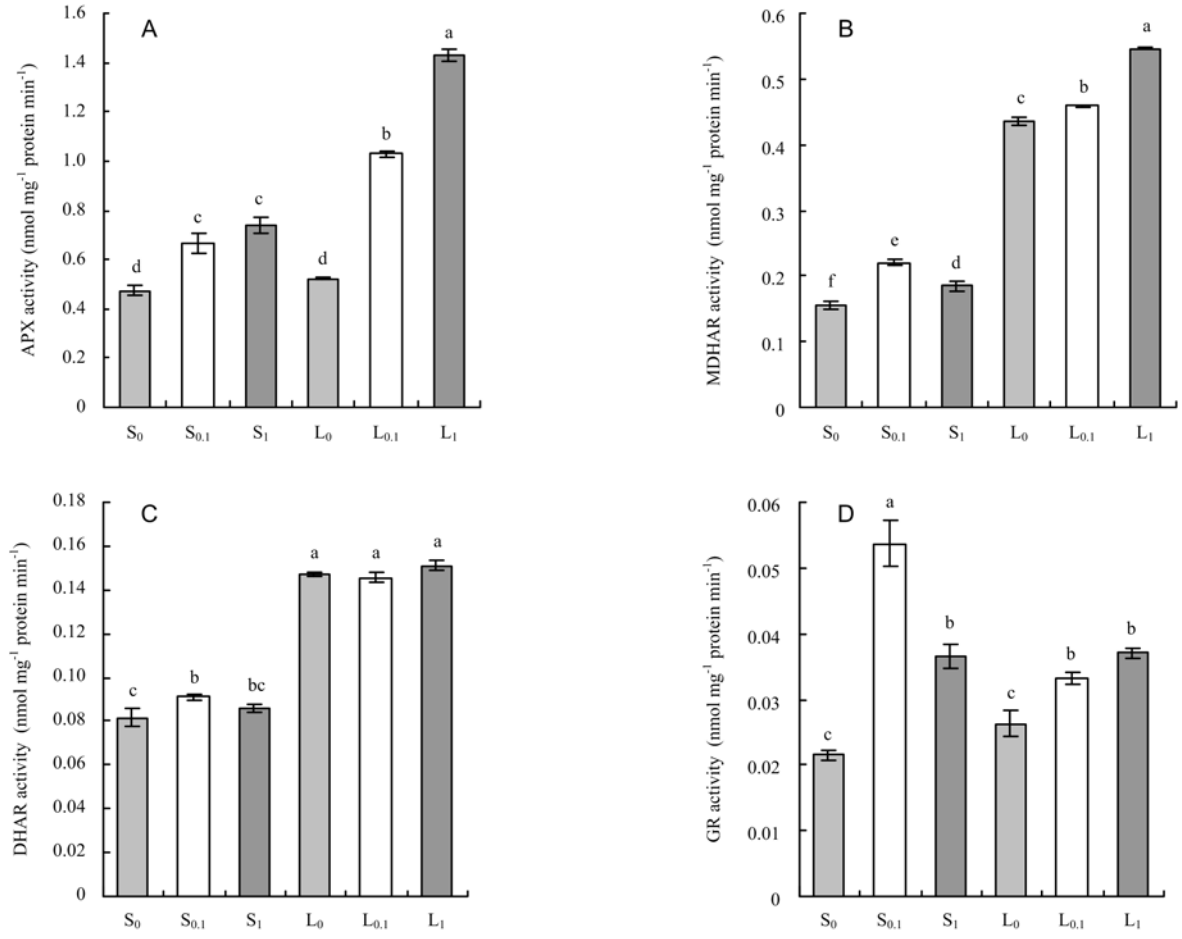

Fig. 3 Effects of exogenous silicon on the activities of APX (A), MDHAR (B), DHAR (C), and GR (D) in chilling-stressed cucumber leaves under low light (100 μmol m^{-2} s^{-1}).

ratio of GSH/total glutathione (Fig.4-D) were higher ($P>0.05$), whereas the ratio of AsA/total ascorbate (Fig.4-B) was lower ($P<0.01$). When comparing the $S_{0.1}$ and S_1 treatments with the S_0 treatment, the AsA content and the ratio of AsA/total ascorbate were higher ($P<0.01$), while the GSH content and the ratio of GSH/total glutathione did not change significantly ($P>0.05$). In comparison with the L_0 treatment, the levels of AsA and GSH were considerably higher ($P<0.05$) in leaves from the $L_{0.1}$ and L_1 treatments. The ratios of AsA/total ascorbate and GSH/total glutathione were enhanced significantly ($P<0.01$) by the L_1 treatment, but they were not significantly changed ($P>0.05$) by the $L_{0.1}$ treatment. The ANOVA results indicate that chilling increased the GSH content and the AsA/total ascorbate and GSH/total glutathione ratios ($P<0.01$), and that exogenous silicon supplementation significantly ($P<0.01$) increased the levels of AsA and GSH, as well as the ratios of AsA/total ascorbate and GSH/total glutathione.

DISCUSSION

Cucumber is a chilling-sensitive vegetable crop (Xu *et al*. 2008). We investigated the second leaves of cucumber plantlets and found that they were withered in some seedlings grown in chilling temperatures (15/8°C). Heat-shock and H_2O_2 pretreatment enhance the survival percentage of maize seedlings under chilling stress (Gong *et al*. 2001). In our case, exogenous silicon supplementation reduced the percentage of withered cucumber leaves. Liang *et al*. (2007) reviewed that silicon can stimulate the antioxidant system in plants and affect the structure of the plasma membrane so as to alleviate abiotic stresses. In order to analyze silicon-induced chilling tolerance, we studied the effect of sili-

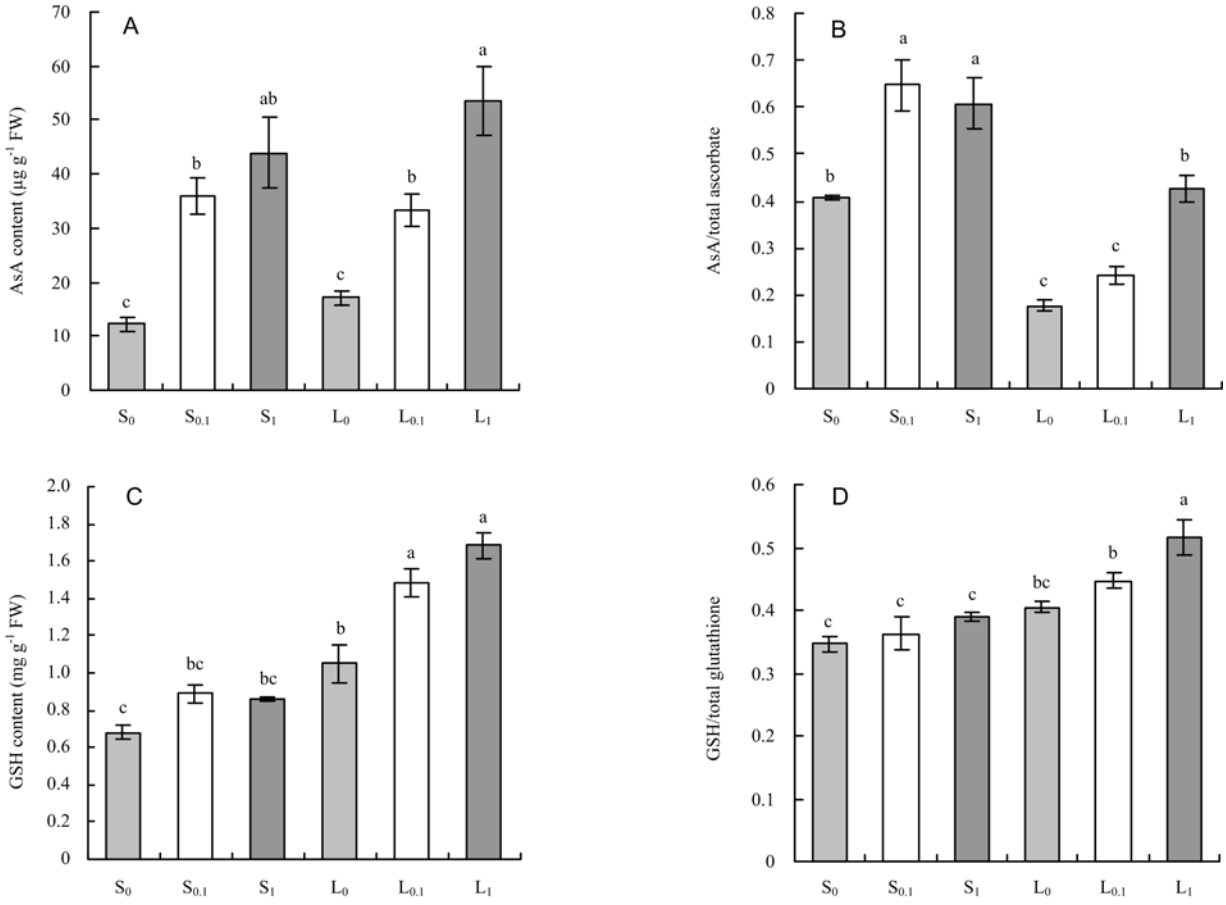

Fig. 4 Effects of exogenous silicon on the AsA content (A), AsA/total ascorbate ratio (B), GSH content (C), and GSH/total glutathione ratio (D) in chilling-stressed cucumber leaves under low light (100 μmol m^{-2} s^{-1}).

con on lipid peroxidation and antioxidant activities in chilling-stressed cucumber leaves.

Application of exogenous silicon increases the silicon concentration of plants (Gunes *et al*. 2007d). In chilling-stressed cucumber leaves, exogenous silicon supplementation also enhanced the endogenous silicon content (Fig.1-A). The higher deposition of endogenous silicon was associated with a decrease in lipid peroxidation (Iwasaki *et al*. 2002). Malondialdehyde is an indicator of lipid peroxidation, and its level is related to the extent of damage in lipid membranes (Hsu and Kao 2007). Temperature stress results in an increase in MDA content (Wang *et al*. 2004). Therefore, low temperature increased the MDA content of cucumber leaves in this study, which indicates that chilling temperature damages the membranes of leaf cells under low light conditions (Xu *et al*. 2008). Exogenous silicon supplementation decreases the MDA content under sodic-B toxic soil stress (Gunes *et al*. 2007d) and also under chilling conditions (Fig.1-B). There is an inverse relationship between silicon content and MDA content in cucumber leaves. These results suggest that exogenous silicon supplementation leads to higher deposition of silicon in leaves of chilling-stressed seedlings and thereby restrains the accumulation of MDA and the damage to lipid membranes (Zhu *et al*. 2004).

Stress conditions induce the overproduction of ROS that can damage lipid membranes and increase the level of MDA (Smirnoff 1993). Pretreatment with 2-aminoethanol results in a correlation between less damage to lipid membranes and the accumulation of fewer ROS, and it also improves the tolerance of barley to drought and paraquat (Mascher *et al*. 2005). Fig.1 shows that chilling increased the levels of $O_2^{\cdot-}$ and H_2O_2. When treated with exogenous silicon, the levels of the two ROSs were lower in chilling-stressed cucumber

leaves under low light, which is consistent with the results regarding MDA content. This suggests that exogenous silicon supplementation decreases the accumulation of $O_2^{\cdot-}$ and H_2O_2 and thereby reduces lipid peroxidation in chilling-stressed leaves.

The tolerance of plants to ROS requires the adaptation of many complex and multifaceted processes. For example, ROS-scavenging enzymes and antioxidant molecules in plants prevent or alleviate the damage from $O_2^{\cdot-}$ and H_2O_2 (Taşgin *et al*. 2006). Superoxide radical can be dismutated into H_2O_2 by SOD (Bowler *et al*. 1992) in chloroplasts, mitochondria, the cytoplasm and peroxisomes. Under salt stress (Liang *et al*. 2003) and drought conditions (Gong *et al*. 2005), the addition of silicon increases SOD activity. In our case, exogenous silicon and chilling both increased SOD activity. However, when silicon was combined with chilling, the enzyme activities were the highest. These results correlate negatively with $O_2^{\cdot-}$ generation. Therefore, an increase in SOD activity can be induced by the addition of silicon, and this will enhance the dismutation of $O_2^{\cdot-}$ in chilling-stressed cucumber leaves under low light.

H_2O_2 can rapidly diffuse across membranes and is toxic (Foyer *et al*. 1997). To scavenge this molecule, plants have evolved an antioxidant system including CAT, GPX, GSH-Px, and the ascorbate-glutathione cycle. CAT is the main enzyme that eliminates H_2O_2 in the microbody (Shigeoka *et al*. 2002). In chilling-stressed cucumber leaves, 1 mmol L^{-1} exogenous silicon increased this enzyme's activity significantly, while 0.1 mmol L^{-1} silicon did not enhance it considerably. However, the ANOVA results show that exogenous silicon supplementation significantly influences CAT activity. Thus CAT activity correlates negatively with H_2O_2 content. This indicates that enhanced CAT activity, which is induced by the addition of silicon (Liang *et al*. 2003), may play a role in the removal of H_2O_2.

GPX may act against the accumulation and toxicity of H_2O_2 in the apoplast (Shigeoka *et al*. 2002). The prominent increase in GPX activity at low temperature indicates the ability of plantlets to scavenge H_2O_2 and tolerate freezing temperatures (Janda *et al*. 2003). In our study, chilling increased GPX activity and exogenous silicon led to an even further increase in this enzyme's activity. This finding is complementary to our results regarding the levels of H_2O_2 and MDA. Therefore, GPX plays a role in chilling-stressed cucumber leaves under low light when exogenous silicon reduces lipid peroxidation induced by ROS.

It has been shown that chloroplasts contain GSH-Px, which is important for eliminating H_2O_2 and lipid peroxidation products (Djanaguiraman *et al*. 2005). After the inoculation of *Botrytis cinerea*, a decrease in GSH-Px activity was found in tomato leaves (Kuźniak and Skłodowska 2001). However, elevated GSH-Px activity has been detected in plants under different stress situations (Ben-Hayyim *et al*. 1993). In this paper, chilling decreased GSH-Px activity, but the addition of silicon increased its activity in chilling-stressed cucumber leaves under low light. As shown in Fig.1, exogenous silicon supply lowered the levels of H_2O_2 and MDA. Therefore, GSH-Px may play a role in the removal of H_2O_2 when cucumber seedlings are subjected to silicon treatment and chilling temperatures.

The ascorbate-glutathione cycle is found in chloroplasts, and the cytosol (Foyer *et al*. 1994), mitochondria and peroxisomes (Jiménez *et al*. 1997). In this cycle, APX plays an important role in removing H_2O_2. After treatment with silicon, there is an increase in APX activity in cucumbers stressed by salt (Zhu *et al*. 2004). In this paper, chilling temperatures increased APX activity, and the addition of silicon further enhanced this activity in chilling-stressed cucumber leaves under low light. Thus APX activity correlates negatively with the levels of H_2O_2 and MDA. This indicates that exogenous silicon can scavenge H_2O_2 and reduce the lipid peroxidation via APX.

In order to remove H_2O_2, APX needs the substrate AsA, which can be regenerated from dehydroascorbate by DHAR using GSH as an electron donor or can be transformed from monodehydroascorbate by MDHAR. Moreover, GR, another enzyme of the ascorbate-glutathione cycle, plays a key role in the reduction of GSSG to GSH (Luster and Donaldson 1987). In tobacco plants treated with paraquat, the activities of DHAR and GR are enhanced (Miyagawa *et al*. 2000). Pretreatment with salicylic acid increases the activities of GR, DHAR and MDHAR in heat-stressed mustard seedlings (Dat *et al*. 1998). In chilling-stressed cucumber leaves, 0.1 or 1 mmol L^{-1} exogenous silicon increased the activities of MDHAR and GR and enhanced the levels of AsA and GSH. Meanwhile, 1 mmol L^{-1} exogenous silicon also

increased DHAR activity and the ratios of AsA/total ascorbate and GSH/total glutathione. This indicates that the addition of 1 mmol L^{-1} silicon increases the activities of DHAR, MDHAR, and GR and thereby results in the regeneration of AsA and GSH under chilling and low light conditions. When 0.1 mmol L^{-1} silicon is supplied, AsA and GSH can be regenerated by MDHAR and GR.

AsA and GSH not only act as substrates in the ascorbate-glutathione cycle, but they also act non-enzymatically. Their increased levels alleviate injuries due to ROS (Hsu and Kao 2007). In this paper, 1 mmol L^{-1} exogenous silicon supplementation significantly increased the levels of AsA and GSH and the redox state of AsA and GSH. This indicates that 1 mmol L^{-1} exogenous silicon accelerates the abilities of the two antioxidants to eliminate ROS in chilling-stressed cucumber leaves under low light.

As shown in Fig.1, chilling increased the levels of H_2O_2 and $O_2^{\cdot-}$ in cucumber leaves. It has been shown that ROS increase the antioxidant activities of plants and ameliorates the damage from chilling stress (Yu *et al*. 2002). In this paper, ROS levels and antioxidant activities were higher at chilling temperatures than normal temperatures. When compared to chilling treatment alone, however, antioxidant activities were even higher and the ROS levels lower when chilling was combined with 1 mmol L^{-1} silicon. Therefore, it is exogenous silicon that activates antioxidants such as SOD, CAT, GPX, GSH-Px, APX, DHAR, MDHAR, GR, AsA, and GSH in chilling-stressed cucumber leaves under low light.

CONCLUSION

Chilling increased the percentage of the second leaves that withered and also enhanced the levels of H_2O_2, $O_2^{\cdot-}$, and MDA in cucumber leaves under low light. Compared to chilling treatment, exogenous silicon supplementation reduced the percentage of withered second leaves and increased the silicon concentration of leaves. It enhanced the activities of antioxidants such as SOD, GPX, APX, MDHAR, GR, AsA, and GSH, while it reduced the levels of $O_2^{\cdot-}$, H_2O_2, and MDA. This indicates that exogenous silicon leads to higher deposition of silicon in chilling-stressed cucumber leaves and thereby increases the antioxidant activities and reduces the lipid peroxidation of leaves. Thus silicon supplementation protects chilling-stressed cucumber leaves from being damaged by reactive oxygen species under low light.

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