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Salicylic acid alleviates cold-induced photosynthesis inhibition and oxidative stress in *Jasminum sambac*

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Abstract: Salicylic acid (SA) is a signal molecule that mediates many biotic and abiotic stress-induced physiological responses in plants. In the current study the protective effects of SA on cold stress-caused oxidative damage and photosynthesis inhibition in jasmine plants (*Jasminum sambac*) were examined. Jasmine seedlings were pretreated with 100 µM SA for 3 days and then subjected to cold stress (4 °C) for 15 days. The amounts of superoxide radicals ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) significantly increased in leaves of plants exposed to cold stress and resulted in oxidative damage, indicated by an increase in malondialdehyde accumulation and membrane leakage. The total chlorophyll content, stomatal conductance (g_s), photochemical efficiency (F_v/F_m), and net photosynthesis rate (P_n) were significantly reduced in response to the cold treatment. However, plants pretreated with exogenous SA (100 µM) exhibited significantly higher photosynthesis-related parameters and antioxidant enzyme activities (peroxidase and catalase) and lower reactive oxygen species levels compared with the cold control. It was therefore concluded that SA could alleviate the cold treatment-caused oxidative stress and photosynthesis decline by enhancing the activities of antioxidant enzymes in leaves of jasmine.

Key words: Cold stress, jasmine, salicylic acid, photosynthesis, oxidative damage

1. Introduction

As sessile organisms, plants encounter various environmental stressors throughout their lives. Among them, low temperature is a major stress in limiting the distribution of native flora and reducing agricultural productivity (Heidarvand and Amiri, 2010; Yadav, 2010). Photosynthesis is one of the most sensitive physiological processes to cold stress in green plants, mediated by a decrease of stomatal conductance (g_s), photochemical efficiency of the photosystem, thylakoid electron transport rate, enzyme activity, carbon metabolism, and photosynthetic pigment complex systems and membrane lipids (Ensminger et al., 2006; Öquest, 2006). Moreover, the negative effects of cold stress on plants are also associated with overproduction of reactive oxygen species (ROS). Excessive production of ROS can cause oxidative damage as evidenced by peroxidation of membrane lipids, leakage of solutes from cells, and the bleaching of chlorophyll (Mittler, 2002; Apel and Hirt, 2004).

Salicylic acid (SA) is a phytohormone with ubiquitous distribution in plants and plays an important role in the regulation of plant growth and development (Vicente and Plasencia, 2011). In addition to facilitating plant growth, it has also been found that SA plays a role during the plant's response to pathogen infection (Mandal et al., 2009) and abiotic stress, such as drought (Kadioglu et al., 2011), heavy metal toxicity (Koç et al., 2013), salinity (Dong et al., 2011), and high and low temperature conditions (Wang et al., 2010; Aydın and Nalbantoğlu, 2011). These reports show that SA application may cause a temporary accumulation of ROS in plants, which acts as a redox signaling message, improving the antioxidative capacity of plants and helping to activate the antioxidant system in plant cells and thereby the acclimation to stress (Horváth et al., 2007). In addition, exogenous application of SA or acetylsalicylate has been shown to improve photosynthetic capacity in grapevine under high temperature stress (Wang et al., 2010) and in *Phillyrea angustifolia* under drought stress (Munne-Bosch and Penuelas, 2003).

Jasmine (*Jasminum sambac*) is one of the most important cultivated plant species in China and other countries for its ornamental, medicinal, and edible values (Cai et al., 2007; Deng et al., 2012a, 2012b). However, jasmine is a tropical and subtropical plant and significantly loses its productivity under extremely cold winter temperatures. In the present study we investigated the effect of exogenous

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SA application on photosynthetic characteristics such as photosynthetic capacity, photosynthetic pigments content, and chlorophyll fluorescence, as well as on the activities of antioxidant enzymes in the leaves of jasmine seedlings during cold stress.

2. Materials and methods

2.1. Plants materials and treatment

Stem cuttings of double petal jasmine, one of the main cultivated types of jasmine in China, were rooted in pots containing a mixture of peat moss and perlite (4:6, v/v) and grown for about 2 months in a greenhouse at 75%–85% relative humidity, 25–27 °C/18–20 °C day/night cycle, and photo flux density of 400–500 μmol m^{-2} s^{-1}.

Young jasmine plants with identical growth (10 leaves) were randomly divided into 3 groups. The first group of plants was sprayed with 100 μM SA solution 3 times at an interval of 24 h, and the second group of plants was sprayed with water (hereafter referred to as the cold control). After pretreatment with SA or water for 3 days, the plants were transferred to a growth chamber (MGC-450HP, Shanghai Yiheng Technical Co., Ltd., Shanghai, China) and maintained at 4 °C, 75% relative humidity, photon flux density of 400 μmol m^{-2} s^{-1}, and a 12-h photoperiod for 15 days. The third group of plants, without any treatment or cold stress, was used as a control. Every 3 days during the cold stress, leaves were collected for photosynthetic parameters measurement and biochemical analysis. Each treatment involved 20 pots, each containing 5 plants. All plants were kept well irrigated and protected from bacterial pathogens and weed competition.

2.2. O_2^- and H_2O_2 content assay

The amount of O_2^- was determined following the procedure described by Doke (1983) with some modifications. Leaf samples were homogenized with 65 mM potassium phosphate buffer (pH 7.8) and centrifuged at 6000 × *g* for 25 min. One milliliter of the supernatant was mixed with 0.9 mL of 65 mM phosphate buffer and 0.1 mL of 10 mM hydroxylamine hydrochloride. After incubation at 25 °C for 20 min, 17 mM sulfanilamide and 7 mM α-naphthylamine were added to the mixture and this was incubated for another 20 min. Finally, ethyl ether in the same volume was added and the mixture was centrifuged at 6000 × *g* for 5 min. The absorbance of the supernatant was measured at 530 nm with a spectrometer.

For determination of H_2O_2 concentration, leaf tissues were extracted by homogenizing samples with phosphate buffer (50 mM, pH 6.5) containing 1 mM hydroxylamine. The homogenate was centrifuged at 6000 × *g* for 25 min. The extracted solution was mixed with 0.1% titanium sulfate in 20% (v/v) H_2SO_4 and then centrifuged at 6000 × *g* for 25 min. The absorbance of the supernatant was measured at 410 nm (Wang et al., 2011).

2.3. Lipid peroxidation assay

Lipid peroxidation was determined in terms of malondialdehyde (MDA) content by thiobarbituric acid (TBA) reaction (Wang et al., 2011). The amount of TBA reactive substance was calculated from the difference in absorbance at 532 and 600 nm using an extinction coefficient of 155 mM^{-1} cm^{-1}.

2.4. Membrane leakage measurement

Membrane permeability, expressed as relative electrolyte leakage, was determined by the method of Yang et al. (2011). Disks of leaf tissue were excised with a 10-mm diameter stainless steel cork borer. After being rinsed 3 times with deionized water, 15 disks were placed in 50 mL of deionized water and incubated by shaking at 100 rpm for 30 min. The conductivity of the solution was then measured using a conductivity meter (DDB-6200, Shanghai Leici Apparatus, Shanghai, China). The total electrical conductivity was obtained by boiling the sample for 10 min. The relative electrolyte leakage was expressed as a percentage of the total conductivity.

2.5. Chlorophyll content determination

Total chlorophyll content was determined in 80% acetone extract of leaf tissues and calculated from the absorbance of extract at 645 and 663 nm using the formula of Lichtenthaler (1987).

2.6. Measurements of leaf gas exchange and chlorophyll fluorescence

Gas exchange was measured using a portable photosynthesis system (LCA-4, ADC, Hoddesdon, UK) equipped with a Parkinson leaf chamber (narrow PLC-2). Measurements were conducted between 1000 and 1200 hours under the approximate photosynthetic photon flux density of 1500 μmol m^{-2} s^{-1}, ambient CO_2 concentration of 380 μmol mol^{-1}, and leaf temperature of 25 °C. Chlorophyll fluorescence was measured using a portable fluorometer (FMS2, Hansatech, King's Lynn, UK) simultaneously with the photosynthetic parameters on the same leaves as above. Maximum fluorescence (F_m), and variable fluorescence (F_v) were recorded after a 30-min dark adaptation (Cai et al., 2007).

2.7. Antioxidant enzymes activities determination

Peroxidase (POD, EC 1.11.1.7) and catalase (CAT, EC 1.11.1.6) activities were assayed using a spectrophotometer following the method of Bai et al. (2009). The POD reaction solution (3 mL) contained 50 mM phosphate buffer (pH 7.8), 25 mM guaiacol, 200 mM H_2O_2, and 0.5 mL of enzyme extract. Changes in absorbance of the reaction solution at 470 nm were determined every 30 s. One unit of POD activity was defined as an absorbance change of 0.01 U min^{-1}.

The CAT reaction solution (3 mL) contained 50 mM phosphate buffer (pH 7.0), 200 mM H_2O_2, and 0.5 mL of

enzyme extract. The reaction was initiated by adding the enzyme extract. Changes in absorbance of the reaction solution at 240 nm were read every 30 s. One unit of CAT activity was defined as an absorbance change of 0.01 U min^{-1}.

2.8. Statistical analyses

One-way analysis of variance (ANOVA) was carried out to estimate differences using SPSS 13, and minimum significant differences were calculated by Duncan's tests ($\alpha = 0.05$). Data were expressed as mean ± standard deviation (SD).

3. Results

3.1. Free radicals production and membrane damage

As shown in Figures 1a and 1b, leaf O_2^- and H_2O_2 contents increased significantly ($P < 0.05$) after exposure to cold stress and were respectively 3.4- and 3.1-fold higher than those of the control after 15 days of cold treatment. Under cold stress, the spraying of jasmine leaves with 100 μM SA significantly ($P < 0.05$) suppressed leaf O_2^- and H_2O_2 production. Maximum suppressions of 67.3% for O_2^- and 58.9% for H_2O_2 were observed on the ninth day of treatment as compared with the cold control.

Damage to the membrane was investigated by monitoring MDA content and relative electrolyte leakage. MDA level and relative electrolyte leakage increased significantly ($P < 0.05$) in leaves of plants immediately after exposure to cold stress and were respectively 7.3 and 6.9 times higher than those of the control after 15 days of cold treatment (Figures 1c and 1d). MDA accumulation and relative electrolyte leakage were considerably reduced by treatment with exogenous SA with the highest reduction of 67.8% for MDA and 74.2% for relative electrolyte leakage observed on the sixth day of treatment as compared with the cold control.

3.2. Activities of antioxidant enzymes

The activities of antioxidant enzymes in jasmine leaves are presented in Figure 2. It can be seen that CAT and POD activities increased significantly ($P < 0.05$) during the first 12 days of cold treatment, followed by a slight decrease with

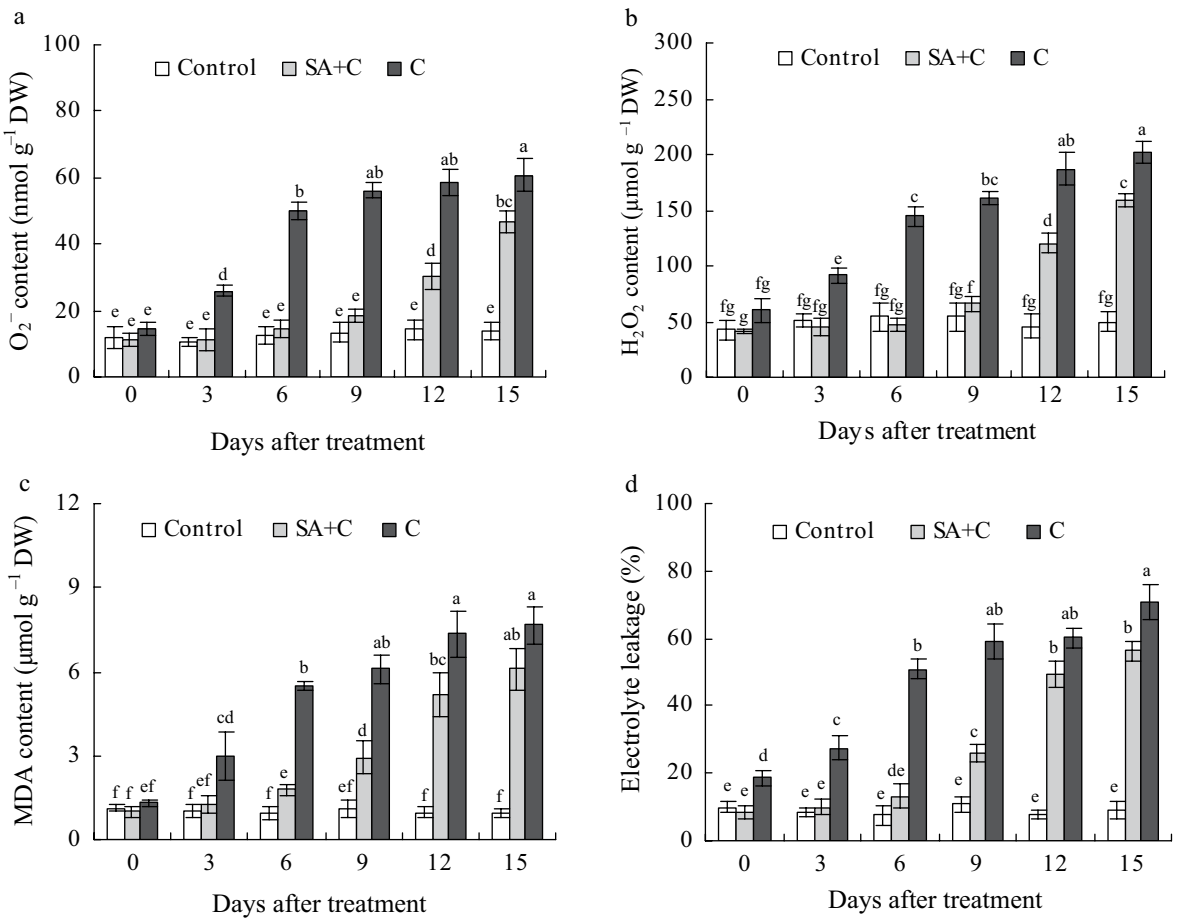

Figure 1. Effects of SA treatment on O_2^- content (a), H_2O_2 content (b), lipid peroxidation (c), and membrane leakage (d) in jasmine leaves under cold stress. Values represent the mean ± SD of 3 independent replicates. Different letters within each parameter indicate statistically significant differences ($P < 0.05$).

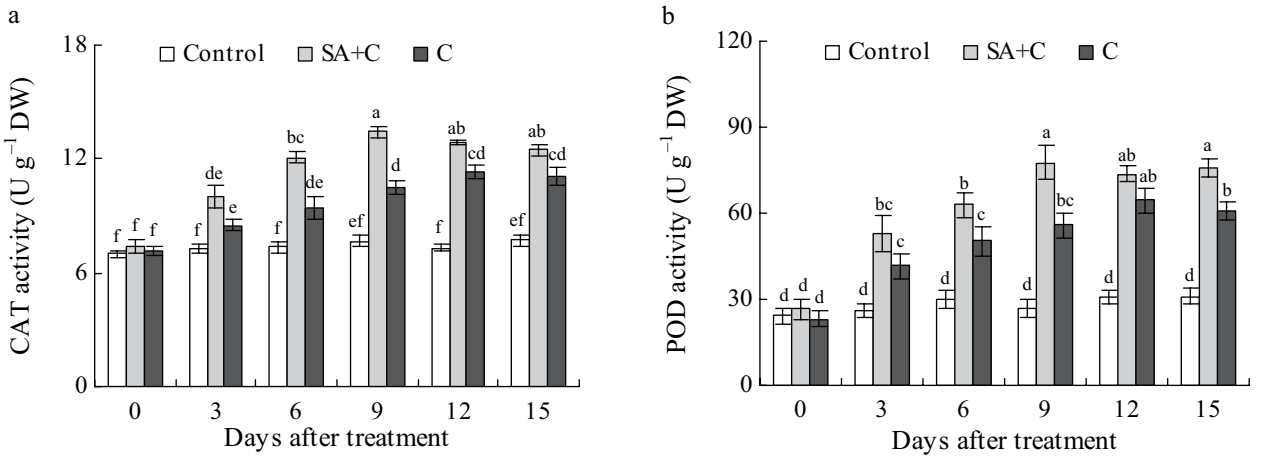

Figure 2. Effects of SA treatment on CAT (a) and POD (b) activities in jasmine leaves under cold stress. Values represent the mean ± SD of 3 independent replicates. Different letters within each parameter indicate statistically significant differences ($P < 0.05$).

prolonged treatment duration. The combination of cold stress and exogenous SA treatment produced much higher antioxidant enzymes activities, with the highest activity of 13.5 U/g DW for CAT and 77.7 U/g DW for POD on the ninth day after cold exposure, which was about 28.3% and 38.7% respectively higher than in the cold control.

3.3. Photosynthetic parameters

As shown in Figures 3a–3d, all photosynthetic parameters, such as pigments, stomatal functioning, photosynthetic rate, and photochemical efficiency, were significantly reduced by the cold treatment ($P < 0.05$). It can be seen that total chlorophyll content significantly decreased after exposure to cold stress and a maximum reduction of 40.1% was recorded at the end of the cold treatment compared to the control (Figure 3a). However, SA application prior to cold treatment significantly enhanced total chlorophyll content by 34.5% after 15 days of cold treatment in comparison with the cold control.

After 15 days of cold treatment, g_s and net photosynthesis rate (P_n) decreased from 0.197 mol m^{-2} s^{-1} and 5.50 μmol m^{-2} s^{-1} to 0.118 mol m^{-2} s^{-1} and 3.22 μmol m^{-2} s^{-1}, respectively (Figures 3b and 3d), and F_v/F_m decreased from 0.647 to 0.494 (Figure 3c). The initial value of F_v/F_m was somewhat low, which may possibly be due to the low light intensity (about 400 μmol m^{-2} s^{-1}) used in this experiment. However, application of 100 μM SA to plants grown under cold stress significantly elevated all of the above photosynthesis-related parameters, where g_s was 41.8%, F_v/F_m was 16.3%, and P_n was 33.9% higher than those respective values in the cold control (Figures 3b, 3c, and 3d).

4. Discussion

The exposure of plants to cold stress as well as other environmental stressors quite often leads to the generation of ROS, which may react with proteins, lipids, and DNA, causing oxidative damage and impairing the normal cellular functions (Gill and Tuteja, 2010). On the other hand, ROS may function as signal molecules and activate the defense responses that protect plants from environmental stressors (Mittler, 2002). The results of the current study showed that cold stress induced the generation of O_2^- and H_2O_2 in the leaves of jasmine plants (Figures 1a and 1b). The application of exogenous SA suppressed O_2^- and H_2O_2 production induced by the cold treatment, indicating that SA alleviated the cold-induced oxidative stress in jasmine plants. Lowering of ROS levels through SA treatment was reported by Mutlu et al. (2013a) for barley and Wang et al. (2009) for rice under cold stress. The decreased ROS accumulation may be partially ascribed to the antioxidant attribute of SA and to the role of SA in the activation of antioxidant responses (Horváth et al., 2007).

The increased levels of O_2^- and H_2O_2 led to cell membrane lipid peroxidation, causing membrane damage and leakage of electrolytes (Bai et al., 2009). In the present study, cold stress induced a dramatic increase in MDA content and relative electrolyte leakage in jasmine leaves, confirming the occurrence of oxidative stress (Figures 1c and 1d). Similar reports were presented by Campos et al. (2003) for *Coffea* and Saltveit (2002) for tomato. However, pretreatment with 100 μM SA obviously reduced MDA accumulation and relative electrolyte leakage in jasmine leaves, exhibiting a potential to alleviate oxidative stress and maintain cell membrane integrity during cold stress. These results are consistent with previous studies in which SA pretreatment decreased MDA content and membrane leakage in cucumber (Zhang et al., 2011) and muskmelon (Korkmaz et al., 2007) after exposure to cold stress. In addition, it is important to note that SA inhibition of ROS

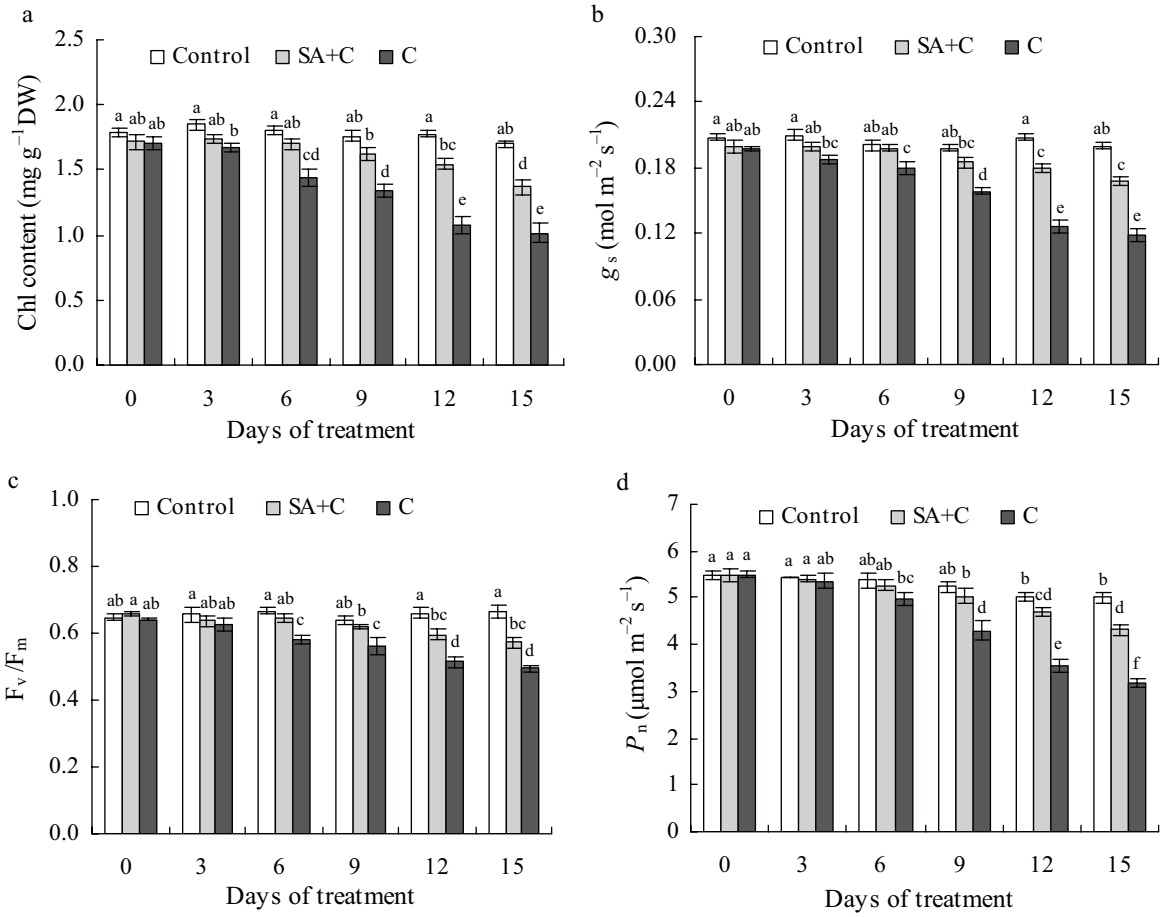

Figure 3. Effects of SA treatment on total chlorophyll content (a), stomatal conductance (b), maximum photochemical efficiency (c), and net photosynthetic rate (d) of jasmine leaves under cold stress. Values represent the mean ± SD of 3 independent replicates. Different letters within each parameter indicate statistically significant differences ($P < 0.05$).

generation and membrane damage reached peak levels on the ninth day of cold treatment, followed by a gradual decline, indicating a time-dependent effect of SA on alleviating cold damage. This was also observed by Mutlu et al. (2013b) in barley under cold stress.

The improvement of stress tolerance is often related to the enhancement of antioxidant enzyme activities in plants. It has been suggested that the accumulation of ROS triggers the enhancement of the antioxidant defense system, which in turn protects plants from oxidative injury. In this study, the activities of CAT and POD in jasmine leaves under cold stress were shown to increase, followed by a decrease with the duration of cold treatment (Figure 2), indicating that the antioxidant system was activated by cold stress. At the same time, the observed increases in ROS level, MDA content, and membrane leakage imply that leaves were damaged by cold stress (Figure 1). SA plays an important role in the response to abiotic stress and considerable interest has been focused on SA because increased antioxidant enzyme activities induce a protective effect on plants under stress (Horváth et al., 2007). Other researchers noted that SA pretreatment increased antioxidant enzyme activities in cold-stressed watermelon (Yang et al., 2008), eggplant (Chen et al., 2011), and barley (Mutlu et al., 2013a, 2013b). In the present study, application of exogenous SA significantly increased the activities of POD and CAT compared with those in the cold-stressed plants, especially during the first 9 days of cold treatment, which correlated well with the observed changes in ROS level and membrane damage, suggesting that SA plays an important antioxidant role in protecting *J. sambac* from cold-induced oxidative injury.

Cold stress interferes with all cellular physiological processes and specifically with the photosynthetic machinery. The present study also revealed that leaves of plants exposed to cold stress experienced a significant loss in total chlorophyll content; in some cases, the total chlorophyll content was reduced by one-third as compared to that of the control (Figure 3a). Moreover, cold stress lowered the stomatal conductance and photochemical

efficiency (Figures 3b and 3c). These impairing events culminated in a severe decline of the rate of photosynthesis (Figure 3d). However, the application of SA maintained the total chlorophyll content and also enhanced the photosynthetic rate in cold-stressed jasmine plants (Figure 3), suggesting a protective role of SA in minimizing the cold-induced negative effects on jasmine plants. Earlier studies strongly favor these observations. For example, SA proved effective in protecting the photosynthesis machinery in red bayberry (Ying et al., 2013), cucumber (Yildirim et al., 2008), and *Brassica juncea* (Yusuf et al., 2008) against water and salinity stress. The most reasonable explanation of the SA-mediated elevation in photosynthesis may be that SA triggers the antioxidant system and subsequently reduces the cold stress-induced membrane damage, as evidenced by the decreased accumulation of MDA and membrane leakage (Figure 1). These findings take additional strength from the work of Orabi et al. (2010), who showed that exogenous application of SA enhanced the photosynthetic rate and also maintained the stability of the membrane, thereby improving the growth of cold-stressed cucumber plants.

In conclusion, it can be said that SA treatment enhanced the antioxidant enzyme activities in jasmine plants under cold stress. The elevated antioxidant system countered the oxidative stress as well as other direct effects of cold stress, thereby improving the photosynthetic capacity in jasmine plants. This study showed an interesting effect of SA in the stress response that should be important not only for a basic understanding of the role of the hormone, but also for its potential use in agriculture. Characterization of SA-regulated genes or proteins under cold stress using large-scale omics analysis and further determination of the changes in endogenous SA levels in response to low temperature exposure in jasmine plants could be the subjects of future research.

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