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ORIGINAL PAPER

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SALICYLIC ACID INDUCES PHYSIOLOGICAL AND BIOCHEMICAL CHANGES IN PEONY UNDER WATERLOGGING STRESS

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ABSTRACT

In this study, the effects of salicylic acid to antioxidative activity and photosynthetic characteristics in waterlogging stress of two peony cultivars ('Fengdanbai' and 'Mingxing') were investigated. 4-year-old peony grown in different levels of waterlogging stress and then different concentration prepared SA (0.0, 0.1, 0.5 and 1.0 mmol L^{-1}) sprayed on fresh leaves of peony. The antioxidative enzymes activities include superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), chlorophyll content, relative conductivity and MDA content were measured in leaves about different waterlogging treatment, the photosynthetic characteristics were also measured using photosynthetic measurement system. The results showed that waterlogging stress decreased the chlorophyll content in all peony cultivars leaves, but with SA treatment can inhibit the decrease of chlorophyll content. Relative conductivity increased as the extension of waterlogging time in two cultivars. SA treatment could effectively inhibit the increase of relative conductivity, and 0.5 mmol L^{-1} of SA was the most suitable concentration. SOD, POD, CAT activity increased first and then decreased in different waterlogging condition, SA significantly increased the activity of various enzymes. MDA content was increase as the expansion of waterlogging time in two cultivars. SA inhibits the increase of MDA content. Of all concentration of SA, 0.5 mmol L^{-1} was the best concentration to inhibit the waterlogging stress. For the photosynthetic characteristics, the net assimilation rate (Pn), stomatal conductance (Gs), transpiration rate (Tr) and intercellular CO_2 (Ci) were decreased under different waterlogging condition. SA treatment can increase Pn, Gs, Tr and Ci of peony.

Key words: peony, salicylic acid, antioxidant enzymes, photosynthetic physiology

Abbreviations: SA – salicylic acid, REC – relative electrolyte conductivity, ROS – reactive oxygen species, CAT – catalase , POD – peroxidase, SOD – superoxide dismutase, MDA – malondialdehyde, Pn – net assimilation rate, Gs – stomatal conductance, Tr – transpiration rate, Ci – intercellular CO_2

INTRODUCTION

Tree peony (*Paeonia suffruticosa* Andr.) is a one of ten ornamental flowers in China. It has excellent ornamental and medicinal value. It originated in China and has a history of more than 2000 years [Picerno et al. 2011, Li et al. 2012]. Because of its large flowers, range of colors, attractive shape and fragrance, tree peony has attracted increasing attention around the world both as a pot plant and cut flower production [Beruto et al. 2004, Han et al. 2008]. Four large tree peony groups have been described, with nearly 3000 cultivars found in China [Zhang et al. 2007]. Of these, the Jiangnan group is mainly distributed

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in Shanghai, Jiangsu, Zhejiang, Anhui Province [Li et al. 2003].

Jiangnan of China has a subtropical to tropical monsoon climate, with abundant rain in this region. As a result, the duration of waterlogging is relatively long, often up to 6 months (from April to September). Rainfall is also frequent and heavy during the peony growing period, especially in the rainy season, causing characteristics of waterlogging and seriously affecting growth. Only about 20 Jiangnan peony cultivars remain, with some rare cultivars such as 'Ziyunfang' and 'Fengwei' on the verge of extinction [Wang 2009].

Understanding the characteristics of waterlogging tolerance in peony is important in terms of selecting cultivars suitable for growth in Jiangnan regions of China. In line with this, determining the physiological and biochemical characteristics under waterlogging stress is important from both a theoretical and practical viewpoint. Recently, waterlogging tolerance has attracted considerable attention. Waterlogging is a general environmental stress in rainy areas and wetlands, and areas with improper irrigation management [Colmer et al. 2010, Mutlu et al. 2013]. As mentioned above, the main cause of waterlogging in Jiangnan of China is frequent rain in summer. Waterlogging affects plant morphology, physiology and metabolism, mainly due to secondary stress in the form of low oxygen or anoxia [Irfan et al. 2010, Tang et al. 2016]. Waterlogging stress significantly inhibits plant growth and development, as well as inducing premature senility [Sairam et al. 2008]. Therefore, waterlogging results in an anaerobic environment, which inhibits aerobic respiration of the mitochondria, inducing anaerobic respiration in root system. Because of the aerobic respiration, plant electron transport is hindered and ATP synthesis suppressed, a large amount of reactive oxygen species (ROS) accumulated in the plant cell. ROS has active chemical properties that react with biological macro-molecules in plant leaves, destroying their active conformation and affecting normal cell metabolism. In turn, this causes an increase in malondialdehyde (MDA) production, damaging the membrane structure [Pandey et al. 2013]. Elimination of ROS is an important plant response to waterlogging stress. Accordingly, plants have evolved various enzymatic and non-enzymatic protection systems to eliminate ROS to reduce cell damage [Nakai and Kisanuki 2007]. For example, changes in SOD, POD and CAT activity and antioxidant enzymes were found to be correlated with waterlogging resistance [Vasantha et al. 2008]. The initial ecophysiological response of most plants to waterlogging stress was leaves wilting and stomata closing in one or two days. In water-intolerant plants, a significant reduction in Pn, Gs and chlorophyll content has also been observed in response to waterlogging [Mishra et al. 2008]. The relationship between antioxidant capacity and waterlogging tolerance has been measured in some species, also including peony [Zhu et al. 2017].

Finding a plant growth regulator to reduce plant waterlogging is an important process for understanding how plants adapt to waterlogging environmental. SA is an endogenous plant growth regulator, which regulates the metabolic and physiological responses of plants and affects the growth and developmental processes of plants. It has been shown that SA has very effective in reducing the adverse effects of water. It considered as a signaling molecule that induces protective mechanisms against biological or abiotic stresses in plants [Sayyari et al. 2013, Hayat et al. 2013]. Several researches showed that SA can alleviate the waterlogging damage [Singh et al. 2003]. Pretreatment with SA can enhanced tolerance towards abiotic stress including waterlogging stress and enhanced antioxidant capacity or induce multiple signal transduction pathways [Janda et al. 2012]. The role of SA in plant growth and development, photosynthesis, flowering and stomatal regulation have examined in previous studies [Li et al. 2014]. The role of SA in drought [Mehran et al. 2013], salinity [Mutlu et al. 2009b], temperature [Tasgin et al. 2006] and heavy metal [Jamali et al. 2011] stress were studied in the past. However, effects of SA on peony under waterlogging stress has not been reported. The influence mechanism of SA treatment on physiological and biochemical characteristics of peony under the stress of water was not clarified.

Waterlogging and high temperature are two important environmental factors inhibiting peony growth in Jiangnan of China. It is of great significance to study the correlation between waterlogging and the exogenous SA. However, the exogenous SA has not yet been reported on the study of the threat to the peony. The main objective of this study is to determine the alleviation roles of SA on two peony under waterlogging stress. This study can supply the difference in response to waterlogging between peony cultivars in SA induced protection.

MATERIALS AND METHODS

Plant materials and experimental design. Experiments were conducted in a controlled environment room in the College of Jiyang, Zhejiang Agriculture and Forestry University, Zhejiang Province, China (29°71'N, 120°23'E), in May 2016. The temperature was controlled at 26 ±0.5°C and 65 ±5% relative humidity. Four-year-old healthy homogenous 'Fengdanbai' (FDB) and 'Mingxing' (MX) seedlings were obtained from Heze, Shandong Province of China and transplanted in plastic containers (top diameter: 27 cm, bottom diameter: 17 cm, height: 22 cm). Each container was filled with a mixed matrix consisting of garden soil, sand and perlite (v/v/v = 5 : 3 : 2, pH 6.4), and grown in good conditions with normal water and fertilizer management.

The flooded depth of two peony cultivars was 4 cm above the soil surface in the plastic containers throughout the experiment. Then freshly prepared SA solutions (0.1 mmol L^{-1}, 0.5 mmol L^{-1}, 1 mmol L^{-1}, pH 6.4) were sprayed on the leaves of the seedlings every day until water drop from leave tips. Using distilled water as control. After measuring the physiological characters, collecting the plant leaves on day 3, 6 and 9 to determine the activities of the antioxidant enzymes. Repeat the same treatment three times when measure the enzyme activity. Physiological parameters such as photosynthesis, stomatal conductance and transpiration were analyzed using repeated-measures for three times.

Chlorophyll content. 0.1 g chopped leaves samples of peony were treated using 95% alcohol at 4°C for 24 h in dark and then shook three or four times until blanched. The chlorophyll content was determined by a spectrophotometer (Shimadzu UV-2550, Kyoto, Japan), the absorbance was measured at 646 and 663 nm after centrifugation, then chlorophyll concentrations calculated according to the standard method [Arnon 1949] and expressed in mg g^{-1} fresh weight (FW).

Cell membrane permeability. According to Shi [2008], we measured the relative electrolyte conductivity of peony leaves to estimate the membrane permeability. Using deionized water rinsed leaves (0.2 g) briefly first and then immersed with 30 mL deionized water for 12 h. Subsequently electrical conductivity (initial EC) of the leach liquor was measured using a conductivity meter (Model DJS-1C). The leaves were then heated 20 min at 100°C and re-read the conductivity (final EC) in the bathing solution. Membrane permeability was calculated as EC (%) = 100(initial EC/final EC).

SOD, POD and CAT activities. We cut leaves (0.5 g) into pieces and then ground in 10 mL of 50 mmol phosphate buffer (pH 7.8) containing 1% (w/v) polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 10,000 × g for 15 min at 4°C, and the supernatant used to determine SOD and POD activities.

The measure principle of SOD activity based on superoxide radicals inhibit nitroblue tetrazolium reduction to blue formazan. The reaction fluid (3 mL) consisted of 39.15 mol methionine, 0.225 mol nitroblue tetrazolium, 0.006 mol riboflavin, 50 mmol potassium phosphate buffer (pH 7.8) with 0.3 mol ethylene diaminetetraacetic acid and 0.05 ml enzyme extract. The reaction was conducted for 15 min at 25°C under 4000 lx. One unit of SOD activity was defined as the amount of enzyme resulting in 50% inhibition of nitroblue tetrazolium reduction. SOD activity was expressed as U g^{-1} protein.

We use the guaiacol method to determine the POD activity [Sun et al. 2011]. The reaction mixture (3 mL) contained 2.75 mL of 50 mmol phosphate buffer (pH 7.0), 0.1 mL of 1% H_2O_2, 0.05 mL enzyme extract and 0.1 mL of 4% guaiacol solution. The increase in absorbance at 470 nm due to guaiacol oxidation was recorded for 2 min then one unit of enzyme activity defined as the amount of enzyme causing a change in absorbance of 0.01 per min. Specific POD activity was expressed as U g^{-1} protein.

CAT activity was determined by tracking the consumption of H_2O_2 at 240 nm for 3 min [Aeby 1984]. The assay mixture (3 mL) consisted of 100 mmol potassium phosphate buffer (pH 7.0), 15 mmol H_2O_2 and 50 µL leaf extract. Specific CAT activity was expressed as U g^{-1} protein.

MDA content. According to the method of Zhang [Zhang 1992], we determined the MDA content. The degree of lipid peroxidation was determined from the content of 2-thiobarbituric acid (TBA) reactive metabolites. Fresh leaf tissue was homogenized then extracted in 10 mL 0.25% (w/v) TBA dissolved in 10% (w/v) trichloroacetic acid (TCA). The extract was heated for 30 min at 95°C then cooled quickly on ice. Then measure the absorbance of the supernatant at 532 nm after centrifugation at 10,000 × g for 10 min. The correction of non-specific turbidity carried out by subtracting the absorbance at 600 nm. Lipid peroxidation was expressed as nmol per g fresh weight. Enzyme activities were measured simultaneously after 15-days treatment.

Analysis of gas exchange. The healthy fully developed leaves which treated with different concentration of SA for 3, 6 and 9 days randomly selected from one branch were chosen for gas exchange measurements, which were measured on annual shoot attached using a portable photosynthesis measuring instrument (LI-6400, LiCor, Inc. Lincoln, NE, USA) equipped with an 6400 chamber at a concentration of 400 µmol mol^{-1} CO_2 and 50% relative humidity with natural light source. The temperature of chamber was kept at 22–25°C. Physiological parameters were analyzed using repeated-measures ANOVA with Duncan test for mean comparison among treatments.

Statistical analysis. Data were subjected to analysis of variance by SPSS (20.0 for windows) statistical program. Mean and standard error (SE) values of three replicates were calculated using SPSS20.0.

RESULTS

Effects of SA on peony chlorophyll content under waterlogging stress. To understand the chlorophyll content state of two peony seedlings under waterlogging conditions with SA and without SA, the chlorophyll content of two peony cultivars were analyzed in this experiment. In the study, we found that chlorophyll content decreased as the extension of waterlogging time, SA treatment could effectively inhibit the decrease of chlorophyll content, and 0.5 mmol L^{-1} was the most effective concentration (Fig. 1). Generally, the chlorophyll content was declined after waterlogging stress occurred. After 3 days, there were no obvious differences among different treatments, the chlorophyll content was higher with SA treatment compared to without SA treatment. There were significantly difference among the treatments after 6 days waterlogging. The chlorophyll content of peony treated with SA was higher than normal and the concentration of 0.5 mmol L^{-1} was highest of all. On day 9 after waterlogging, the chlorophyll content of all treatments significantly decreased. The results showed that SA can alleviate waterlogging stress on chlorophyll content of peony and the concentration of 0.5 mmol L^{-1} was the most effective concentration. In the same treatment time, the chlorophyll levels are in the order of 0.5 mmol L^{-1} > 1.0 mmol L^{-1} > 0.1 mmol L^{-1} > 0 mmol L^{-1}. The chlorophyll content of ‘Fengdanbai’ was higher than ‘Mingxing’ at the same treatment.

Effects of SA on peony cell membrane permeability under waterlogging stress. Generally, when waterlogging stress occurs, the fatty acid phospholipids composition of cell membrane changed [Mirdehghan et al. 2007] and the damages of membrane initiate a cascade of secondary reactions leading to disruption of cell structures. We can measure electrolyte leakage to evaluate the extent of damage. The electrolyte leakage in this study was significantly higher in control than SA treated. The results show a role of SA in maintaining membrane integrity. With respect to electrolyte leakage, SA led to significantly lower relative conductivity than control (Fig. 1). The results showed that relative conductivity increased as the extension of waterlogging time in two peony cultivars. SA treatment could effectively inhibit the increase of relative conductivity and 0.5 mmol L^{-1} of SA was the most effective concentration. We can see that SA treatment alleviate effects of waterlogging and maintain membrane integrity, without significant differences between 0.1 and 1 mmol L^{-1} concentrations. Similar results have been reported in tomato [Aghdam et al. 2014].

The changes of SOD, POD and CAT activities after SA treatment under waterlogging stress. This study examined mutual and separate effects of exogenous SA (0, 0.1, 0.5 and 1 mmol L^{-1}) and waterlogging stress on the activities of SOD, POD and CAT in the leaves of ‘Fengdanbai’ and ‘Mingxing’. In control conditions, treatment with SA can increased SOD activity. Waterlogging treatments without SA also stimulated SOD activity at first period and then decreased as the extension of waterlogging time (Fig. 1). In all species, SOD activity rise in the first stage and then

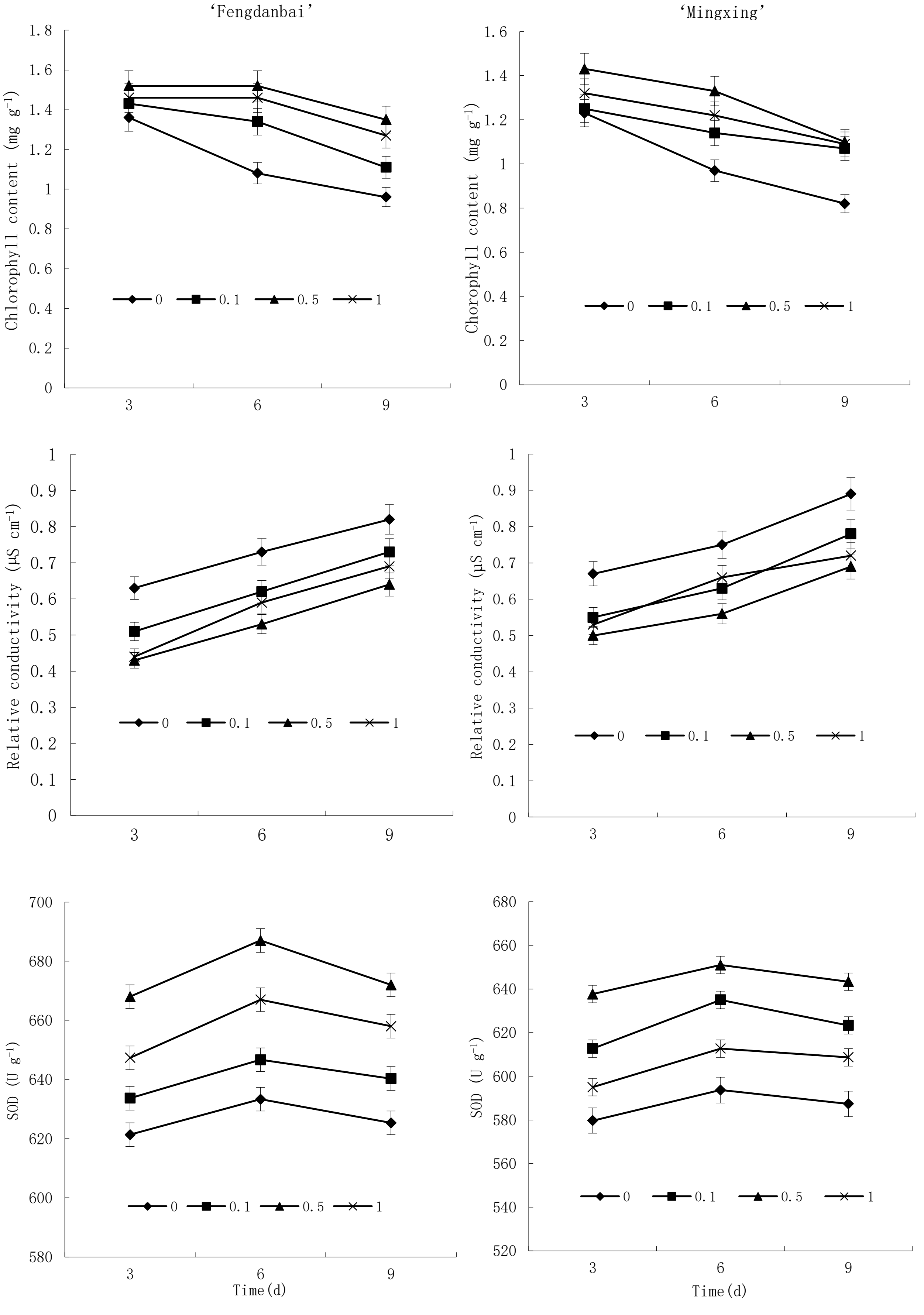

Fig. 1. Effects of salicylic acid at a concentration of 0, 0.1, 0.5, 1 mmol L^{-1} on chlorophyll content, relative conductivity and SOD in peony 'Fengdanbai' and 'Mingxing'

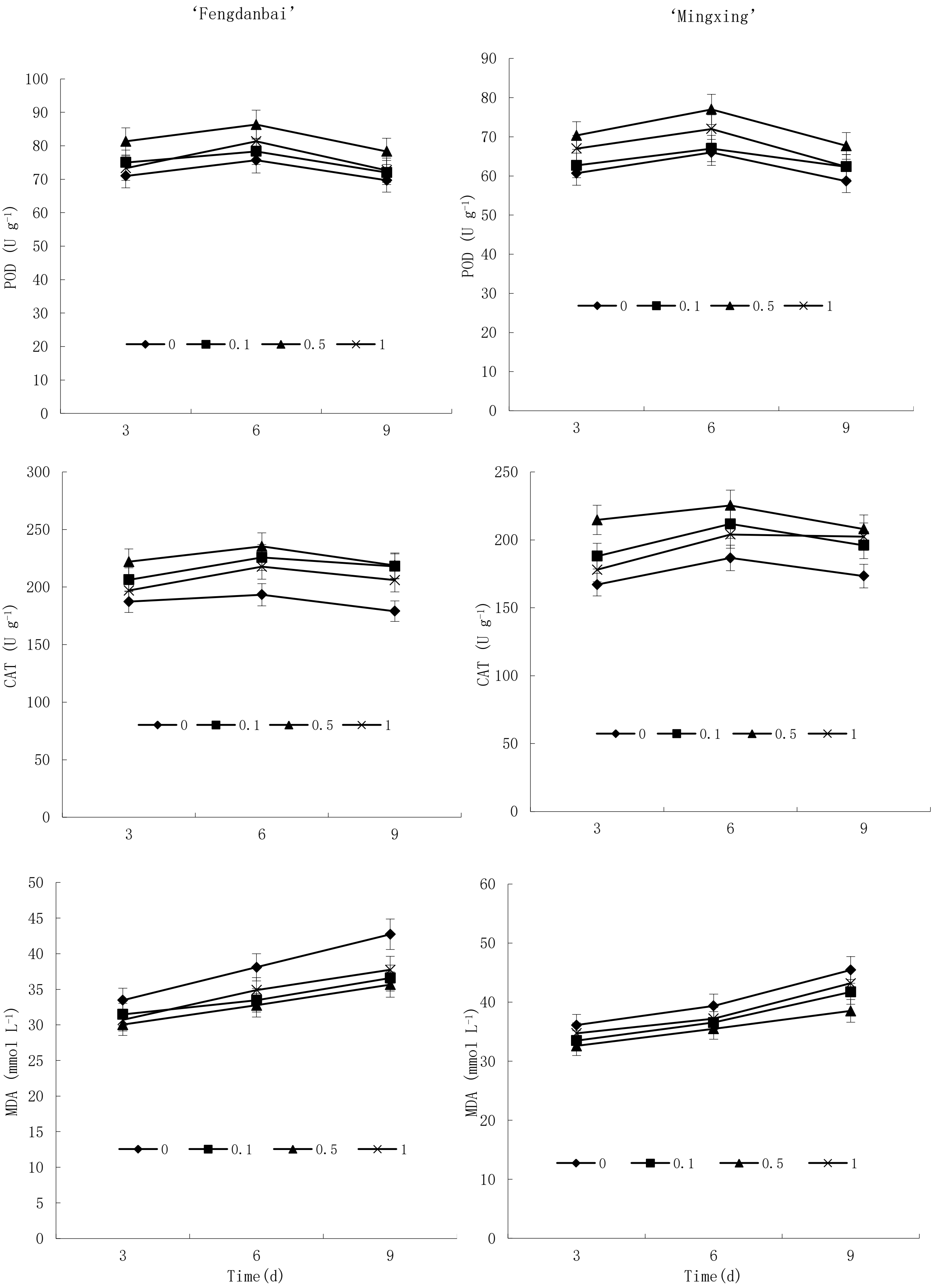

Fig. 2. Effects of salicylic acid at a concentration of 0, 0.1, 0.5, 1 mmol L^{-1} on POD, CAT, and MDA in peony ‘Fengdanbai’ and ‘Mingxing’

decrease, two peony cultivars SOD activity increased dramatically after 6 d of waterlogging treatment (Fig. 1). As waterlogging treatment continued, SOD activity decreased rapidly at 9 d. The SOD activity was higher in ‘Fengdanbai’ than ‘Mingxing’ at the same treatment. As shown in Figure 1, significantly higher SOD content were observed only in 0.5 mmol L^{-1} SA treated leaves and treated with 0.1 mmol L^{-1} and 1 mmol L^{-1} SA were not observed significantly differences compared to control. It indicated that SOD content level was a reflection of leaves waterlogging tolerance.

Under waterlogging stress, peony POD activities in two cultivars increased first and then decreased (Fig. 2). POD activities dramatically increased during 0–6 d respectively, but were relatively low at later treatment stages. After SA treatment, POD activity obviously increased than without SA treatment and the concentration of 0.5 mmol L^{-1} was the most effective concentration. The POD activity was higher in ‘Fengdanbai’ than ‘Mingxing’ at the same treatment.

Under waterlogging stress, activities of CAT increased at the first period in two cultivars, and then decreased at the last period (Fig. 2), SA treatment speed up the increase of CAT and maintained higher enzyme activities when in the waterlogging. The CAT activity was higher in ‘Fengdanbai’ than ‘Mingxing’ at the same treatment. Significantly higher CAT content were observed only in 0.5 mmol L^{-1} SA treated leaves. 0.1 mmol L^{-1} and 1 mmol L^{-1} SA treatment can improve enzyme activity but lower than that of 0.5 mmol L^{-1}.

MDA content. MDA content also increased gradually with the extension of waterlogging time (Fig. 2), both two cultivars MDA was highest of all in control, significantly lower MDA content were observed in 0.5 mmol L^{-1} SA treated peony, treated with 0.1 and 1 mmol L^{-1} SA were also observed lower compared to control. It suggested that MDA content level was a reflection of peony waterlogging tolerance.

Effects of SA on peony gas exchange parameters under waterlogging stress. The results showed that SA treatment can increase the Pn, Gs, Tr and Ci of peony leaves (Fig. 3, 4). As the concentration of SA increased, the Pn increased first and then decreased. And the concentration of 0.5 mmol L^{-1} was the best concentration. Compared to control, the Pn, Gs, Tr and Ci with 0.5 mmol L^{-1} SA treatment dramatically increased under waterlogging stress, and by 13.8% ($P < 0.01$), 46.0% ($P < 0.01$), 15.7% ($P < 0.01$) and 6.5% ($P < 0.01$) with 0.5 mmol L^{-1} SA treatment, respectively.

CONCLUSION AND DISCUSSION

Waterlogging was one of the most critical constraints on the growth and production of trees. In addition, flooding around the world is expected to be more frequent due to increased rainfall [Irfan et al. 2010, Limami et al. 2014]. Therefore, it is very important to improve the waterlogging tolerance of trees. Previous reports have shown that leaf damage is negatively correlated with plant waterlogging tolerance. [Tuo et al. 2015]. Recent studies have shown that SA plays an important role in different biological and abiotic stresses. The regulatory mechanism of SA may directly affect the specific enzyme function, or it may activate genes responsible for the protective mechanism. It has been reported that the application of SA under salinity stress can improve the activity of antioxidant enzyme [Yusuf et al. 2008].

In this study, two peony cultivars were used to show the effect of SA on improvement of peony physiological and biochemical characteristics under waterlogging stress and to determine the most effective concentration of SA application dosage of this chemical. The results showed that ‘Mingxing’ leaves began to wilt after 10 d of waterlogging, ‘Fengdanbai’ leaves began to wilt after 24 d, whereas ‘Fengdanbai’ were more resistant to waterlogging. Furthermore, waterlogging stress decreased the chlorophyll content in all peony leaves, but with SA treatment can inhibit the decrease of chlorophyll content. Relative conductivity increased as the extension of waterlogging time in two cultivars. SA treatment could effectively inhibit the increase of relative conductivity and 0.5 mmol L^{-1} of SA was the most effective concentration. The activity of SOD, POD, CAT increased first and then decreased in the waterlogging condition, SA significantly increased the activity of various enzymes. MDA content was increase as the extention of waterlogging time. SA inhibits the increase of MDA content. Of all concentration of SA, 0.5 mmol L^{-1} was the best concentration to inhibit the waterlogging stress.

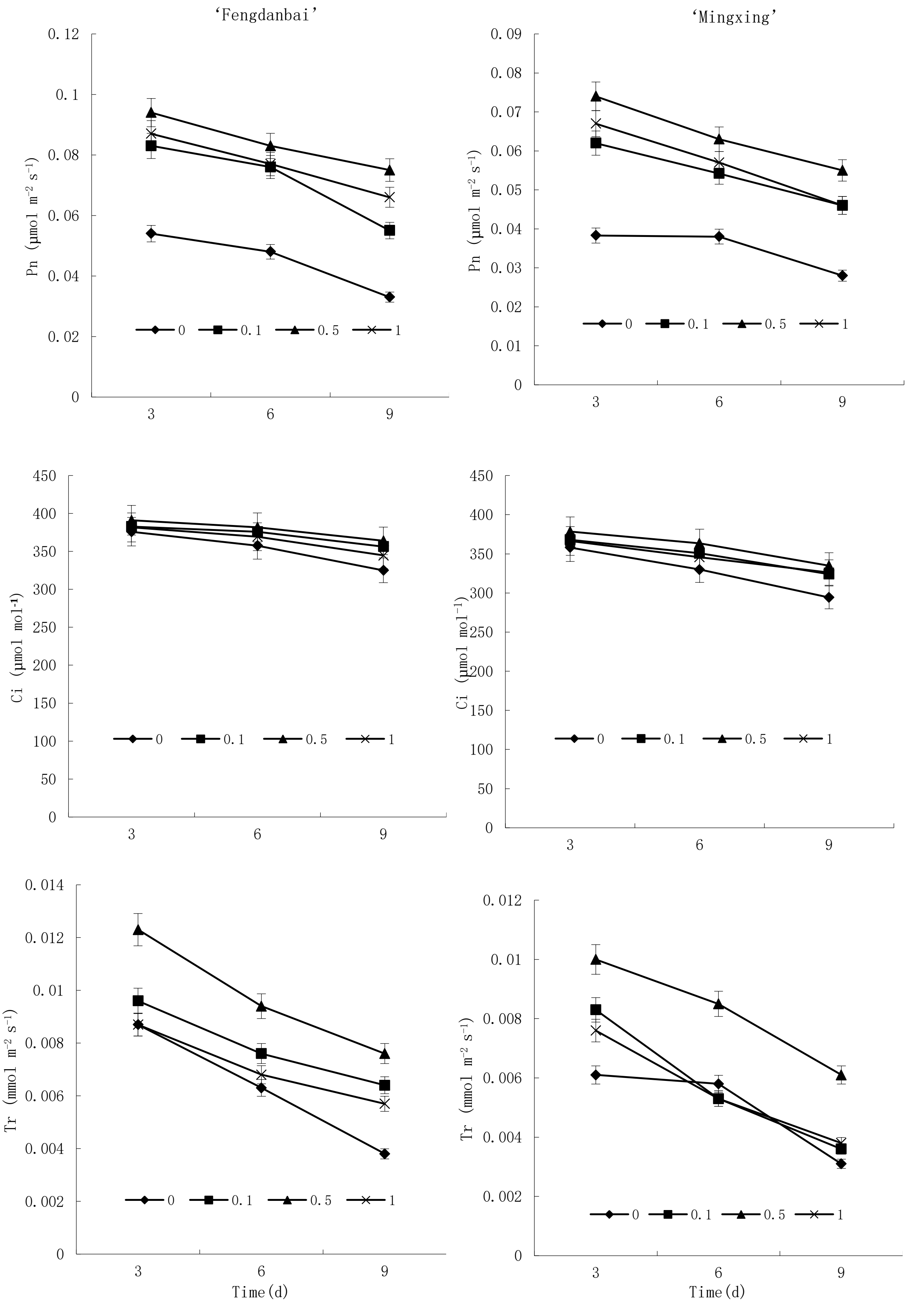

Fig. 3. Effects of salicylic acid at a concentration of 0, 0.1, 0.5, 1 mmol L^{-1} on Pn, Ci and Tr in peony 'Fengdanbai' and 'Mingxing'

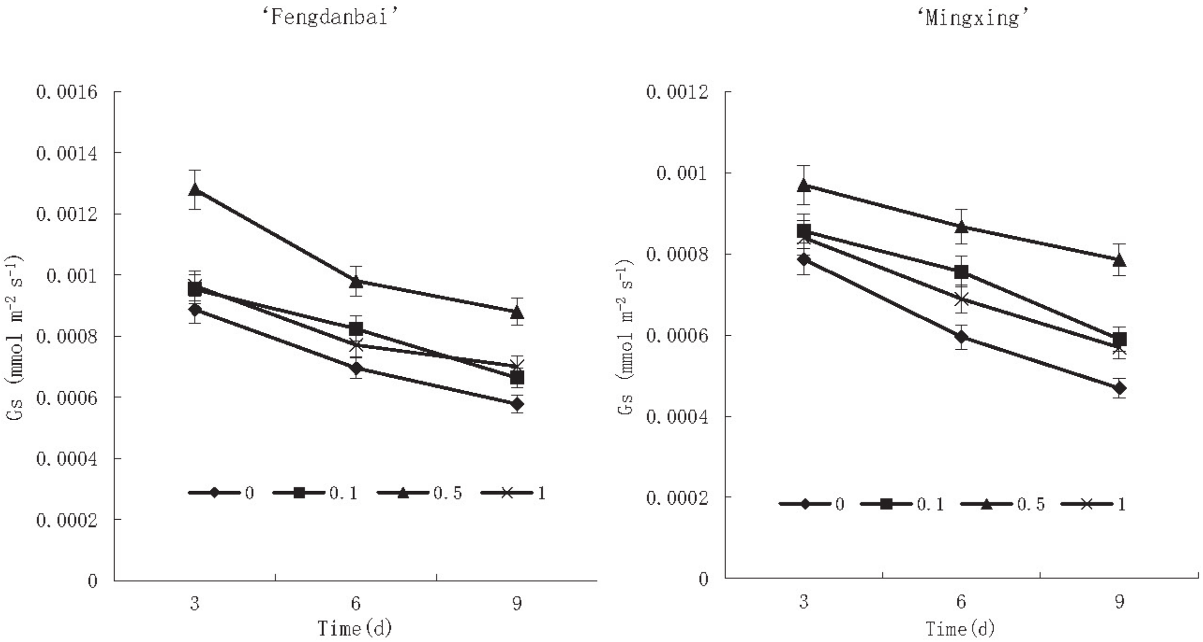

Fig. 4. Effects of salicylic acid at a concentration of 0, 0.1, 0.5, 1 mmol L^{-1} on Gs in peony ‘Fengdanbai’ and ‘Mingxing’

Chlorophyll content is an intuitive expression of photosynthesis, nutrition and aging of plant leaves. Its content is an effective index to identify water damage. Under waterlogging, Chlorophyll content of plants will change, mainly manifested as leaf precocious, senescence and shedding. The balance between the synthesis and decomposition of chlorophyll in plants is affected, and the chlorophyll content is decreased. The decrease of chlorophyll content is the biochemical response of plant leaves to waterlogging stress [Stewart et al. 2010]. It has been reported that SA participating in plant response to abiotic stress [Jayakannan et al. 2013]. SA improves the salt tolerance and drought resistance by enhancing the activity of antioxidant enzyme to reduce the risk of salt stress and water shortage stress in barley [Fayez et al. 2014]. Whether SA can alleviate the waterlogging stress has not been reported in the past. In this study, SA treatment can inhibit the decrease of chlorophyll content, it could effectively reduce waterlogging injury in peony and 0.5 mmol L^{-1} was the most effective concentration.

The inhibition effect of waterlogging stress on plant root growth has been widely recognized. Leaf withering and yellowing can also damage the upper part. With the increase of waterlogging stress, water stress leads to the increase of ROS in leaf tissue, thus causing oxidative damage to cell organelles and biomolecules [Li et al. 2011]. To avoid oxidative damage, plant tissues have antioxidant defense systems with important components such as SOD, CAT and POD [Srivastava et al. 2011, Kamal et al. 2016]. The increases caused by SA were higher in the ‘Fengdanbai’ than in ‘Mingxing’ cultivar. Waterlogging treatment alone stimulated SOD activity in two species. Under waterlogging, the treatments with SA increased SOD activity in both ‘Fengdanbai’ and ‘Mingxing’ cultivars at every concentration. Our results showed that SOD was involved in a series of defense reactions caused by SA in peony plants under waterlogging stress. In addition, this difference can be attributed to the diversity of oxidative toxicity damage mechanisms and the subsequent tolerance to waterlogging. SOD plays an important role in the defense of cell injury against environmental stress. In this experiment, the results show that SA can play an important role by regulating the activity of SOD. Pandey [2013] also found that SA induced SOD activity in rice plants under aluminum toxicity [Pandey et al. 2013].

The POD activity of two peony cultivars treated with SA alone was higher than that of the control group. This result is consistent with results obtained from other plant species. On the other hand, POD activity in both the cultivars also increased at first and then decreased after waterlogging treatments alone (Fig. 2). The increase extend in POD activity became more pronounced in ‘Fengdanbai’ than ‘Mingxing’ cultivar. POD activity can be significantly affected by waterlogging stress. Some studies reported that waterlogging resulted in higher activity of POD in plant cells [Yin et al. 2009]. In this experiment, the results show that the SA increases more POD activity by waterlogging in the peony cultivars. SA results in higher activity of POD in plant cells under waterlogging stress in previous studies [Yin et al. 2009]. It is known that POD protects cells against the damaging effects of H_2O_2 during an oxidative-burst response under toxicity. Exogenous SA plays an important role in regulating the response of peony varieties to waterlogging stress by stimulating POD activity, although this effect varies from plant species to plant species.

Catalase is responsible for decomposition and detoxification of H_2O_2 in the peroxisomes. The activity of this enzyme is sensitive to both drought and heat stresses. It was confirmed that SA exogenous application could improve antioxidants activity in plants [Knorzer et al. 1999, Movaghatian et al. 2014]. There was a transitory reduction on CAT activity as a result of SA exogenous treatment. Some studies showed that SA treatment can decreased CAT activity in wheat [Tasgin et al. 2006]. In our experiment, an opposite observation has been determined from peony cultivars (Fig. 2). SA increased CAT activity in Souza and McAdam [2001] study. This result was similar with our results. The findings suggest that CAT activity responds in different degrees to SA, depending on both its concentration and plant cultivar. Our results support the hypothesis that SA effects depending on the degrees of water tolerance of plant cultivars.

The main products of membrane lipid peroxidation was MDA, and its content is positively correlated with plant injury. MDA content level was a reflection of water tolerance degree. Water stress significantly increased leaf MDA content in both cultivars. It has been reported that MDA prevented after SA treatment [Asghari et al. 2010]. With the increase of waterlogging stress, MDA content increases gradually in this study and SA treatment can delay the increase of MDA content. The results showed that a certain concentration of SA can be used to relieve the water damage of the peony.

Stomatal closure is the earliest responses to waterlogging stress, protecting plants from extensive water loss. In the present study it was observed that waterlogging stress reduced Pn, Gs, Tr and Ci value. The decrease in Pn mediated by moderate water stress was the consequence of the closure of stomata, thereby decreasing CO_2 supply as well as Ci and resulting in a decrease in Tr. Plants treated with SA increased in Gs compared to those treated without SA. This proves that the application of SA can reverse the stomatal closure of peony seedlings under flooded water stress and improve the photosynthetic rate. However, under waterlogging stress, Pn of leaves may also have non--stomatal restrictions. Our results showed that Ci, Gs and Tr significantly increased in SA treated seedlings, indicating that the decrease in Pn was mainly the result of non-stomatal restriction. SA reduced the photosynthetic activity of grape leaves under high temperature stress by maintaining high Rubisco activation [Wang et al. 2010].

In conclusion, the results showed that Water stress reduced peony growth and chlorophyll content, increased membrane damage and lipid peroxidation. By increasing chlorophyll content and enhancing the mechanism of plant antioxidant enzyme, SA can effectively improve the negative effects of waterlogging stress on the growth and chlorophyll of peony, thus alleviate the membrane oxidative damage from waterlogging. To our knowledge, this is the first report of water tolerance mechanisms in peony seedlings. The results of this study can also provide useful guidance for peony protection and large-scale planting.

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