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Effects of Salicylic Acid on Physiological Characteristics of Strawberry Leaves under Drought Stress

C. H. Sun, D. Wang, Y. L. Hu, X. H. Li, W. D. Zhang, J. Sun and X. L. Gao
(College of Life Science, Jiangsu Normal University, China)

Summary

The effects of the salicylic acid (SA) on the content of soluble carbohydrates, free proline, malonaldehyde (MDA) and the activity of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) in strawberry seedling leaves under drought stress were studied. The results showed that under drought stress, suitable concentration of SA increased the content of osmotic adjustment substances such as soluble carbohydrates and free proline of strawberry leaves. Meanwhile, it induced the activity of antioxidant enzymes, alleviated membrane lipid peroxidation as well as membrane system damage, thus improving the drought resistance of strawberry plants.

Key words. strawberry – drought stress – salicylic acid – drought resistance

Introduction

Plants are frequently exposed to a variety of abiotic stresses such as drought, which hamper plant growth and crop productivity worldwide (Li 2007). Water deficit is a crucial factor of restraint factor at the initial phase of plant growth and establishment (Shao et al. 2008). Understanding how plants react to these stresses is a prerequisite for developing strategies to improve plants stress tolerance (Ashraf 2010). Drought stress causes considerable yield reduction in most crops including strawberry. Strawberry is one of the crops that require adequate irrigation (Serrano et al. 1992) to achieve satisfying commercial yields. In regions prone to water deficits due to with scanty rainfall and limited water resources for irrigation, the strawberry is highly likely to experience water deficits (Klamkowski and Treder 2008) that limit the production.

Antioxidant enzymes are the most important components of the ROS scavenging system (Meloni et al. 2003). Several studies have demonstrated that salt-tolerant species increase their antioxidant enzyme activities and antioxidant components in response to salt stress while salt-sensitive species fail to do so (Koca et al. 2007; Jin et al. 2009).

Lucrezia et al. (2012) reported that In salt stress conditions, the activity of antioxidant enzymes (APX, CAT, POD, SOD) in wild chicory was enhanced, thus wild chicory has a high capacity to activate protective mechanisms against the oxidative damage generated by salt stress.

Salicylic acid (SA) is a common plant-produced signal molecule that is responsible for inducing tolerance to various biotic and abiotic stresses (Arfan et al. 2007). The exogenous application of SA was reported to have an effect on a wide range of physiological processes including increased cold germination tolerance in pepper (Korkmaz et al. 2007), chilling tolerance in cucumber (Kang and Saltveit 2002) and maize (Janda et al. 2000), salinity tolerance in barley (El-Tayeb 2005), improved heat shock tolerance in mustard (Dat et al. 1998), decreased inhibitory effect of drought stress in tomato and beans (Senaratna et al. 2000) and wheat (Sakhabutdinova et al. 2003). During drought stress, endogenous SA increased the production of *Phillyrea angustifolia* up to five-fold. (Munne-Bosch and Penuelas 2003). In barley, SA content in the root was increased by water deficit. When barley was treated with SA before drought stress, the damaging effect of water deficit on the cell membranes in the leaves was reduced (Bandurska and Stroinski 2005). Luo et al. (2010) considered,exogenous SA and SA-binding protein combination, thereby reducing the CAT activity; Duner and Klessing (1996) proposed the SA that depresses ascorbate peroxidase (APX) activity, resulting in increased H_2O_2 content which causing mild oxidative stress, and in turn improves the activity of antioxidant enzymes, enhancing overall antioxidant capacity (Kang et al. 2003). The aim of the present study was to investigate the effects of SA on the tolerance of strawberry against drought stress and provide some theoretical basis for drought tolerance cultivation.

Materials and Methods

Plant material and treatments

The experiment was conducted using Chunxiang strawberry cultivar (*Fragaria* × *ananassa* Duch. cv. 'Toyonoka') at Jiangsu Normal University. The strawberry seedlings (provided by the experimental field of the College of Life Sciences, Jiangsu Normal University in Xuzhou, Jiangsu Province, China) were cultivated in pots and grown in greenhouse (average day temperature being 25–27 °C, resp. 15–17 °C at night; natural light with a maximum light intensity of 1087 μmol m^{-2} s^{-1} and a relative atmospheric humidity of 40–65 %). Strawberry plants with identical growth (5 leaves) were chosen for experiments and divided into five groups (Table 1).

The weighing method was employed to control the field moisture capacity at 75–80 % (plants were watered every morning). Drought stress settings were created by retaining above standards for 10 d, then stopping the watering completely. The contents of soluble carbohydrates, free proline, malonaldehyde (MDA) and the activity of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) were measured at 0, 2, 4, 6, 8 and 10 d respectively after stopping the watering. The third to fifth functional leaves away from the center were considered experimental material. The parameter of each leaf was measured three times randomly (three replicate samples are processed simultaneously), and the average was calculated; then the average of three pots was used.

Determination of osmoregulation substances

Soluble carbohydrates were determined using the anthrone method according to the protocols reported previously (Pence et al. 2005), with glucose as a standard for the assay. Free proline accumulation was determined as described by Bates et al. (1973). Proline concentrations were calculated using proline standards (0–50 mg ml^{-1}) in an identical manner.

Table 1. Treatments of strawberry.

Grouping	Treatment
1. control	normal watering
2. drought	drought stress
3. drought + 0.1 mM SA	sprayed with 0.1 mmol L^{-1} SA one day prior to drought stress
4. drought + 1.0 mM SA	sprayed with 1.0 mmol L^{-1} SA one day prior to drought stress
5. drought + 10.0 mM SA	sprayed with 10.0 mmol L^{-1} SA one day prior to drought stress

Determination of MDA content

The level of cellular malonaldehyde (MDA) in leaves was determined by the thiobarbituric acid (TBA) assay as previously reported (El-Tayeb 2005).

Determination of antioxidant enzyme activities

The activity of superoxide dismutase (SOD) was determined by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium (NBT), according to the methods of Beyer and Fridovich (1987). One unit of SOD was defined as the amount of enzyme which produced a 50 % inhibition of NBT reduction under the assay conditions. Peroxidase (POD) activity was determined using the guaiacol test at 470 nm (Chance and Maehly 1995). One unit of enzyme activity was defined as the amount of enzyme that caused a change of 0.001 in absorbance per minute. The activity of catalase was measured by following the decrease in the H_2O_2 concentration spectroscopically at 240 nm according to Adam et al. (1995).

Statistical analysis

The Statistical analysis was conducted with the SPSS-13.0 for windows statistical software package (Standard released version 13.0 for Windows; SPSS Inc., IL, USA). Analyses of variance (ANOVA) were used to evaluate the effects of treatment. Significant differences among plants from each treatment were calculated at the $P \leq 0.05$ level, using Duncan's tests.

Results

Effects of salicylic acid (SA) on the osmoregulation in strawberry leaves under drought stress

Changes in content of soluble carbohydrates. The soluble carbohydrates content of all the drought stress treatment increased in strawberry leaves compared to control treatment (Fig. 1A). Under drought stress, the soluble carbohydrates content in the leaves without spraying SA showed a steady increasing trend with the prolongation of drought stress time and approximately was 2.47-fold that of the control on the tenth day. A similar pattern was observed in the leaves sprayed with 0.1 mM SA, about 3.03-fold of the control on the tenth day. The soluble carbohydrate content in the leaves treated with 1.0 mM and 10.0 mM SA, the dramatic increase was observed. The soluble carbohydrate content of these leaves increased sharply on the second day of drought stress. The content was 3.50- and 3.19-fold respectively, compared to that of the control set.

Changes in content of free proline. As the stress time extended, free proline accumulation of strawberry leaves under

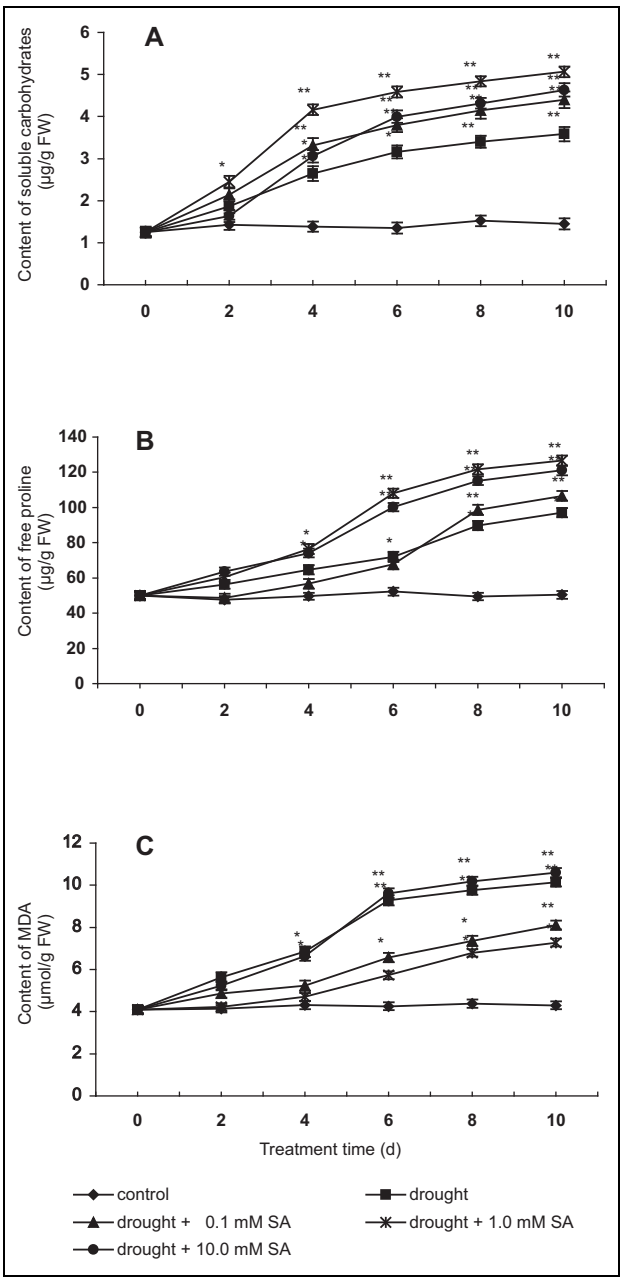


Fig. 1. Effect of the salicylic acid (SA) on (A) soluble carbohydrates content, (B) free proline content and (C) MDA content of strawberry leaves under drought stress. Statistical significance of differences compared to control. * P < 0.05; ** P < 0.01.

drought stress treatment showed rising trend, which was higher than those of the control sets (Fig. 1B). Under drought stress, the changes in free proline content of strawberry leaves without spraying SA were negligible at the early stage of drought. On the tenth day of drought stress, free proline content was about 1.93-fold of the control set. The free proline levels in leaves treated with 0.1 mM SA increased significantly and the free proline content of leaves was approximately 2.47-fold of the control on the tenth day. The free proline levels in leaves treated with1 mM and 10.0 mM SA showed no significant difference compared with the control set on the first two days. On the fourth day, free proline content in leaves increased sharply, and then the amplitude leveled off. The free proline content in these leaves was about 2.51- and 2.40-fold of the control, respectively.

Effect of the salicylic acid (SA) on lipid peroxidation and protective enzyme activities of strawberry leaves under drought stress

Content of Malondialdehyde (MDA). MDA content of all five treatments in strawberry leaves is presented in Fig. 1C. MDA content in the leaves unhandled with SA increased sharply on the fourth day of drought-treatment and has remained at a high level. At the end of the drought, MDA content of these leaves was about 2.36-fold of the control set. MDA content of leaves treated with 0.1 mM and 1.0 mM SA had a less increasing amplitude, and was approximately 1.89- and 1.69-fold of the control respectively on the tenth day. MDA content in the leaves treated with 10.0 mM SA, increased significantly on the sixth day. On the tenth day of drought stress, MDA content was 2.46-fold of the control.

Activity of SOD. Regardless of SA effects, the activity of SOD in strawberry leaves under drought stress, tended to increase first and then to decrease (Fig. 2A). The activity of SOD in the leaves without SA treatment increased on the second day, and the highest enzyme activity was observed on the sixth day. The activity of SOD was about 2.07-fold of the control. A similar pattern was observed in leaves sprayed with 0.1 mM SA. SOD activity reached a maximum, and was 2.50-fold of the control on the sixth day of drought stress. SOD activity in leaves exposed to 1.0 mM and 10.0 mM SA increased at an early stage of drought stress. As the drought time extended, the highest SOD activities, being approximately 2.43- and 2.65-fold of the control respectively, were observed on the fourth day. In strawberry leaves treated with 10.0 mM SA SOD activity decreased rapidly and resembled the control sets' at the end of the drought period.

Activity of POD. Regardless of SA effects, the POD activity of strawberry leaves under drought stress showed a trend similar to that of SOD activity (Fig. 2B). The POD activity in drought-treated leaves without spraying SA saw a significant rise starting on the fourth day of drought stress, and reached maximum on the sixth day. The POD activity in leaves treated with 0.1 and 1.0 mM SA, increased on the second day. The POD activity of these leaves reached the peak on the fourth day and was 2.59- and 2.91-fold, respectively, of the control sets. The POD activity in leaves treated with 10.0 mM SA under drought was remarkably enhanced on the second day, decreasing sharply thereafter.

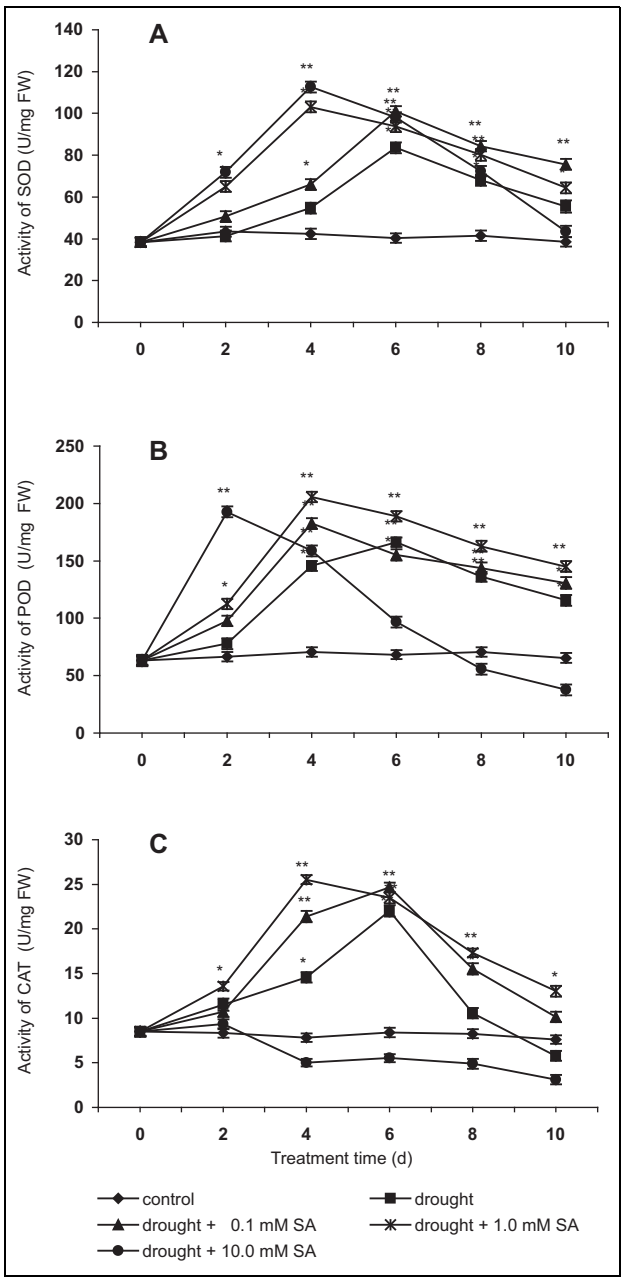


Fig. 2. Effect of the salicylic acid (SA) on (A) SOD activity, (B) POD activity and (C) CAT activity of strawberry leaves under drought stress. Statistical significance of differences compared to control. * P < 0.05; ** P < 0.01.

Activity of CAT. From Fig. 2C, it was found that the increasing amplitude of CAT activity in the leaves without spraying SA was insignificant on the first two days. Leaf CAT activity under drought treatment stress was increased on the fourth day of drought and the highest CAT activity was observed on the sixth day. Then, CAT activity decreased rapidly and was lower than control at the end of the experimental period. The change of CAT activity in these leaves treated with 0.1 and 1.0 mM SA was similar to the drought treatment with the stress time extended. On the second day of drought, the CAT activity was increased significantly, and reached maximum on the sixth day and fourth day respectively. Meanwhile, CAT activity was about 2.94- and 3.27-fold of the control. The CAT activity of strawberry leaves exposed to 10.0 mM SA increased slightly on the first two days. Thereafter, CAT activity was decreased continuously. At the end of the drought, CAT activity reduced the level to 40.8 % of control treatment.

Discussion

Osmotic regulation of plants is an important way to reduce osmotic potentials and maintain turgor pressure in order to resist stress, and ultimately adapt to arid environments. Furthermore, osmotic adjustment is physiological characteristic linked to drought tolerance in plants, including the strawberry (Grant et al. 2010). Soluble carbohydrates and proline content are two crucial factors actively involved in osmotic regulation (Pinheiro et al. 2004). Under drought stress, the increase of soluble carbohydrates in plants can reduce the cell osmotic potential, allowing cells to continue the process of absorbing absorb from the soil. Accumulation of proline permits osmotic adjustment, which results in water retention and avoidance of cell dehydration (Blum 2005). The results of the current study showed that under drought stress, with or without spraying SA, soluble carbohydrates and free proline content in strawberry leaves was increased with the prolongation of time, whereas external application of suitable concentration of SA could significantly increase the content of both soluble carbohydrates and free proline (Fig. 1A and B). Therefore, spraying suitable concentration of SA can increase water retention capacity of cells and the cell saps concentration, thus ensuring that the plant able to grow in the normal physiological environment, and improve the drought tolerance.

MDA is one of the main products of lipid peroxidation and MDA content is used to be a measure of lipid peroxidation. The accumulation of MDA in strawberry leaves treated with 0.1 and 1.0 mmol L^{-1} SA was significantly reduced (Fig. 2C), thus reduced the membrane lipid peroxidation, enhance the stability of membrane systems and improve the drought resistance of strawberry.

Reactive oxygen species (ROS) of plants are mainly in the form of superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (·OH). Plants subjected to drought stress tend to overproduce ROS in different plant tissues (Pinheiro et al. 2004). Plants possess a complex antioxidant system to detoxify ROS, including low-molecular mass antioxidants as well as antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) (Foyer and Halliwell 1976). SOD can detoxify O_2^-, while POD and CAT can convert H_2O_2 into H_2O, Some studies indicated that exogenous SA could

induce leaf SOD and POD activity of wheat maize under osmotic stress, and thereby reduce O_2^- production (Shang et al. 2007). The result of the study indicated that certain concentration (0.1–1.0 mmol l^{-1}) of SA applied before drought induced SOD, POD and CAT activity in strawberry leaves at the early stage of stress (Fig. 2) and also improved the drought tolerance of strawberry. However, there exists a limit for plant cells' toleration of ROS. Plants can effectively detoxify ROS by increasing the activities of antioxidant enzymes within the threshold. But stepping beyond the threshold leads to reduced protective enzyme activity due to oxidative damage (Wang and Wang 2000). Accumulation of ROS exceeded enzyme's detoxifying ability, thereby the plants is subjected to oxidative damage (Wang and Wang 2000). The current results showed that under drought stress the three kinds of antioxidant enzyme activity in strawberry leaves were initially stimulated and then decreased with the prolongation of time.

In summary, various concentrations (0.1–1.0 mmol L^{-1}) of SA pretreatment prior to drought could increase soluble carbohydrates and free proline content of strawberry leaves, reduce the accumulation of MDA and induce antioxidant enzyme activities. Meanwhile, spraying with high SA concentration (10.0 mmol L^{-1}) resulted in MDA accumulation of MDA and CAT depression. Therefore, suitable concentration of SA pretreatment could increase the osmotic adjustment ability and antioxidant capacity of strawberry under drought stress. And this reduces the ROS-induced oxidative damage, and thus enhanced drought tolerance in strawberry.

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Addresses of authors: Cunhua Sun (corresponding author), Dan Wang (co-corresponding author), Yulong Hu, Xuehua Li, Wendi Zhang, Jian Sun and Xiali Gao, College of Life Science, Jiangsu Normal University, Shanghai Road 101, Tongshan Qu, Xuzhou, Jiangsu 221116, China, e-mail (corresponding author): chsun193@sohu.com.