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Effect of salicylic acid on the antioxidant system and photosystem II in wheat seedlings

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Abstract

To study the effects of application of salicylic acid (SA) on the antioxidant system and photosystem II (PS II) in wheat seedlings we used two different experiments. The first method was carried out by immersing roots in Hoagland's nutrient solution containing 0, 0.25, or 2.5 mM SA, and the second method was performed by spraying two-week-old seedlings with the same SA concentrations. After 24 h, chlorophyll fluorescence, thylakoid membrane proteins, antioxidant enzyme activities, and reactive oxygen species were measured. The low concentration of SA caused a significant increase in the antioxidant enzyme activities. However, the treatment with 2.5 mM SA resulted in an increase in the non-photochemical quenching coefficient and a decrease in the antioxidant enzyme activities, the quantum yield of PS II photochemistry, and the photochemical quenching, especially in the first method of application. All these results indicate that the effects of SA on PS II and the antioxidative defense system were dependent on the concentration used and the method of application.

Additional key words: ascorbate peroxidase, catalase, chlorophyll fluorescence, glutathione reductase, reactive oxygen species, superoxide dismutase.

Introduction

Salicylic acid (SA) is monohydroxybenzoic acid and a naturally occurring plant hormone that regulates various physiological and biochemical functions in plants (Janda and Ruelland 2014). It also plays an important role in tolerance to biotic and abiotic stresses as signaling molecule (Li *et al*. 2014). Detailed evidence implicates SA in pathogenesis-related gene expression, systemic acquired resistance, and hypersensitive response (Yuan and Lin 2008). In addition, SA is involved in activation of the stress-induced antioxidant system when plants are exposed to many abiotic stresses such as chilling, heat, heavy metals, osmotic stress, and salinity (Janda and Ruelland 2014). Khan *et al*. (2014) reported that SA application alleviates the adverse effects of salt stress in mungbean through the improvement of plant photosynthesis and also enhancing antioxidant system. Previous studies have indicated that SA treatment influences a wide variety of plant processes including plant growth and yield (Javaheri *et al*. 2012), induction of antioxidant enzymes (Ghasemzadeh and Jaafar 2013), and regulation of some photosynthetic reactions (Arfan *et al*. 2007, Li *et al*. 2014). However, the effects of SA on plant physiological and biochemical processes vary depending upon species, developmental stage, mode of application, SA concentration, and environmental conditions (Shraiy and Hegazi 2009). For instance, a high concentration of SA increases oxidative damage generated by NaCl in *Arabidopsis* (Borsani *et al*. 2001). On the other hand, when applying SA at appropriate concentrations, it enhances the efficiency of the antioxidant system (Alam *et al*. 2014).

Reactive oxygen species (ROS) are formed as natural byproduct of normal metabolism and play important roles in signaling (Bailey-Serres and Mittler 2006). However, excessive amounts of the ROS can cause membrane lipid peroxidation (Gunes *et al*. 2007), damage to chloroplasts,

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Abbreviations: APX - ascorbate peroxidase; AsA - ascorbate; CAT - catalase; Chl - chlorophyll; DAB - 3-diaminobenzidine; DHA - dehydroascorbate; F_m - maximum fluorescence; F_v - variable fluorescence; GPX - glutathione peroxidase; GR - glutathione reductase; MDA - malondialdehyde; NBT - nitroblue tetrazolium; NPQ - non-photochemical quenching; POD - peroxidase; PS II - photosystem II; qP - photochemical quenching; ROS - reactive oxygen species; RWC - relative water content; SA - salicylic acid; SOD - superoxide dismutase; TCA - trichloroacetic acid; Φ_{PSII} - quantum yield of PS II photochemistry.
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inhibition of photochemical reactions, and decrease in photosynthesis (Li *et al*. 2014). For surviving, plants adopted several mechanisms to counteract the adverse effects of stress-induced ROS. To scavenge the ROS, plants develop enzymatic and non-enzymatic antioxidants (Gill and Tuteja 2010). Plant defence responses are activated by SA, and it can participate in regulating the content of ROS by changing antioxidant enzyme activities under different stress conditions (Klessig and Malamy 1994).

Previous studies have indicated that SA as signaling molecule is in a low concentration and the optimal range for the highest stress tolerance is usually from 0.1 to 0.5 mM for most plants (Shi *et al*. 2006, Yuan and Lin 2008). However, plants usually suffer from oxidative stress when exogenous SA is more than 1 mM. Previously, we investigated the effects of high SA concentrations on photosynthetic parameters and antioxidative enzymes (Yuan and Lin 2008). At present, the effect of the mode of SA application on physiological processes has been investigated. The first method used immersing roots in Hoagland's nutrient solution containing different concentrations of SA according to Kang *et al*. (2013). The second method used spraying shoots with an SA solution (War *et al*. 2011, Ghasemzadeh and Jaafar 2013). However, there is no study on comparison of the antioxidant system and photosystem II (PS II) under two different methods of SA application yet. We compared differences of ROS production, chlorophyll fluorescence, PS II proteins, and antioxidant enzyme activities. The results could be helpful in understanding the physiological functions of SA in plants which cope with biotic and abiotic stresses.

Materials and methods

Plants, growth conditions, and treatments: The seeds of wheat (*Triticum aestivum* L. cv. Chuanmai 42) were obtained from the Crop Research Institute, the Sichuan Academy of Agricultural Science, Chengdu, China. They were sterilized with 5 % (m/v) sodium hypochlorite for 10 min, thoroughly rinsed with distilled water, placed in Petri dishes with wetted filter paper, and germinated in the dark at 25 °C in a growth chamber. After 72 h, the uniformly germinated seeds were transferred into sand with with half strength Hoagland's solution and the seedlings were grown at a temperature of 25 ± 1 °C, a relative humidity around 70 %, a 12-h photoperiod, and a photosynthetic photon flux density of 250 μmol m^{-2} s^{-1}.

Salicylic acid was dissolved in absolute ethanol and then added drop-wise to water (ethanol:water, 1:1000, v/v) (Li *et al.* 2014). Two-week-old wheat plants were gathered, washed with tap water, and dried briefly with filter paper to remove surface water. Two different SA applications were conducted. The first method (I) was that the wheat seedlings were transferred into 350 cm^3 plastic beakers containing half strength Hoagland's solution supplemented with 0.25 or 2.5 mM SA (Kang *et al*. 2013). As control we used the plants grown in the half strength Hoagland's solution. The second method (II) was that SA was sprayed on the shoots at concentrations of 0.25 or 2.5 mM with a hand sprayer. The control plants were sprayed with a similar solution without SA. The leaves were sprayed every 8 h and the measurements were done after 24 h.

In addition, some seedlings were transferred into a 16 % (v/v) polyethylene glycol 6 000 solution with an osmotic potential of -0.6 MPa for 72 h after the SA pretreatment for 24 h. Then relative water content (RWC) and malondialdehyde (MDA) content were measured.

Chlorophyll content, RWC, electrolyte leakage, and malondialdehyde content: Chlorophyll (Chl) *a* and *b* content was measured according to Porra *et al*. (1989). Fresh leaves (0.5 g) were cut, homogenized and extracted with 80 % (v/v) acetone at room temperature. The extract was filtered through two layers of filter paper. After filtering, the absorbances of the solution were read at 645 and 663 nm using a spectrophotometer *U2000* (*Hitachi*, Tokyo, Japan). The RWC was measured according to Li *et al*. (2014).

Lipid peroxidation in leaves was estimated by measuring MDA content as described by Luo *et al*. (2009) with a minor modification. Fresh leaf tissue (0.5 g) was homogenized in 5 cm^3 of 5 % (m/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 5 000 *g* and 4 °C for 10 min. To 2 cm^3 of the supernatant, 2 cm^3 of the 5 % TCA containing 0.67 % (m/v) thiobarbituric acid was added. The assay mixture was heated at 95 °C for 30 min and then quickly cooled in an ice bath. The mixture was centrifuged at 5 000 *g* and 4 °C for 10 min. The absorbance of the supernatant was read at 532 nm and corrected for non-specific turbidity by subtracting the absorbance of the same at 600 nm. Electrolyte leakage was determined according to Cao *et al*. (2009). After measuring conductivity, the samples were boiled for 15 min to achieve a 100 % electrolyte leakage when the membrane structure was damaged completely.

Chlorophyll fluorescence visualization: Chlorophyll fluorescence images were obtained using a modulated imaging fluorometer (*Imaging PAM M-Series*, *Heinz-Walz Instruments*, Effeltrich, Germany) according to the instructions provided by the manufacturer. The wheat samples were dark adapted for 30 min prior to fluorescence measurements. Values of minimum fluorescence and maximum fluorescence (F_m) were averaged to improve the signal-to-noise ratio. Image data acquired in each experiment were normalized to a false color scale. Maximum efficiency of PS II photochemistry in the dark-adapted state (F_v/F_m), photochemical quenching (qP), quantum yield of PS II photochemistry

(Φ_{PSII}), and non-photochemical quenching (NPQ) were visualized (Maxwell and Johnson 2000).

Histochemical detection of ROS: Superoxide and H_2O_2 were detected with nitroblue tetrazolium (NBT) and 3,3-diaminobenzidine (DAB), respectively, as described previously (Yang *et al*. 2004) with some modifications. The seedlings were excised at the base with a razor blade and immersed into 6 mM NBT in a 50 mM HEPES buffer (pH 7.5) for 2 h or into 5 mM DAB in a 10 mM MES buffer (pH 3.8) for 8 h in the dark. The leaves were then decolorized in boiling ethanol (90 %, v/v) for 0.5 - 2 h. At least five leaves were used for each treatment.

Determination of H_2O_2 and O_2^- content: Content of H_2O_2 was determined by the method of Okuda *et al*. (1991). Approximately 0.5 g of fresh leaf tissue was cut into small pieces and homogenized in an ice bath with 5 cm^3 of 0.1 % (m/v) TCA. After centrifugation at 12 000 *g* and 4 °C for 20 min, 0.5 cm^3 of the supernatant was added to 0.5 cm^3 of a 10 mM potassium phosphate buffer (pH 7.0) and 1 cm^3 of 1 M KI. Absorbance was recorded at 390 nm. Finally, the content of H_2O_2 was calculated using a standard curve. Production of O_2^- was estimated according to Elstner and Heupel (1976) by monitoring nitrate formation from hydroxyl amine.

Enzyme extraction and assays: For enzyme measurements, 0.5 g of fresh leaf tissue was homogenized with 5 cm^3 of an ice-cold 25 mM HEPES buffer (pH 7.8) containing 0.2 mM Na_2EDTA, 2 mM ascorbate and 2 % (m/v) polyvinylpyrrolidone using a chilled mortar and pestle. The homogenate was centrifuged at 12 000 *g* and 4 ºC for 20 min. After centrifugation the supernatant obtained was used for determination of specific enzymatic activities. Peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GPX), and glutathione reductase (GR) were measured as described previously by Cao *et al*. (2009). One unit of specific POD activity was defined as the amount of the enzyme that caused an absorbance change of 0.01 unit per minute. One unit of specific SOD activity was defined as the amount of enzyme that caused a 50 % inhibition of the photochemical reduction of NBT per minute. One unit of CAT activity was defined as the amount of the enzyme that consumed 1 μmol of H_2O_2 per minute. One unit of APX activity was defined as the amount of the enzyme that catalyzed oxidation of 1 μmol of ascorbate per minute at 290 nm. One unit of GPX activity was defined as the amount of the enzyme that oxidized 1 μmol of NADPH per minute. One unit of GR activity was defined as the amount of the enzyme that consumed 1 mmol of NADPH per minute at 340 nm. All the activities were expressed as specific activities. The protein content was measured according to Lowry *et al*. (1951).

Content of ascorbate (AsA) and dehydroascorbate (DHA) were determined according to Kampfenkel *et al*. (1995). The assay is based on reduction of Fe^{3+} to Fe^{2+} by AsA. The Fe^{2+} then forms complexes with bipyridyl giving a pink color that absorbs at 525 nm. The content of AsA and AsA+DHA were estimated using L-ascorbate as standard. Then, the DHA content was obtained as AsA+DHA minus AsA.

Protein gel blotting analysis: Thylakoid membrane proteins were isolated, solubilized, and subjected to sodium dodecylsulphate polyacrylamide gel electrophoresis (6 %, m/v, acrylamide stacking gel + 15 %, m/v, separation acrylamide gel with 6 M urea) as described by Fristedt *et al*. (2011). Immunoblot analysis was performed as described previously (Chen *et al.* 2009). Antibodies used (all raised in rabbits) were anti-Arabidopsis D1, CP43, LHCB1, LHCB2, LHCB3, LHCB4, LHCB5, and LHCB6. These antibodies were purchased from *Agrisera* (Umea, Sweden). Quantification of the immunoblots was done using the *Quantity One* software.

Statistical analysis: All experiments were repeated three times, and mean values were presented with standard deviations (SDs; $n = 3$). Student's *t*-test or Duncan's multiplication range test were used for comparison between different treatments. A difference was considered to be statistically significant when $P < 0.05$.

Results

We found that all the physiological parameters were not significantly different between the two controls in the experiments I and II (data not shown). Therefore, only one control data are shown. Using the two different methods of application, both concentrations of SA caused a significant increase in total Chl content compared with the control ($P < 0.05$), especially in the first method of application (Fig. 1*A*). However, the higher concentration of SA (2.5 mM) resulted in a lower total Chl content compared with the lower SA concentration. In addition, the Chl *a*/*b* ratio remained almost unchanged at 0.25 mM SA, but 2.5 mM SA caused a significant decrease in Chl *a*/*b* ratio as compared to the control (Fig. 1*A*). Furthermore, we found that the soluble protein content slightly increased under 0.25 mM SA but slightly decreased under 2.5 mM SA (data not shown).

The maximum photochemical efficiency of PS II in the dark-adapted state (F_v/F_m) was not significantly different between the control and SA-treated plants (Fig. 2). The non-photochemical quenching (NPQ), quantum yield of PS II photochemistry (Φ_{PSII}), and photochemical quenching (qP) were not significantly influenced by 0.25 mM SA, however, 2.5 mM SA caused a decrease in Φ_{PSII} and qP whereas an increase in NPQ

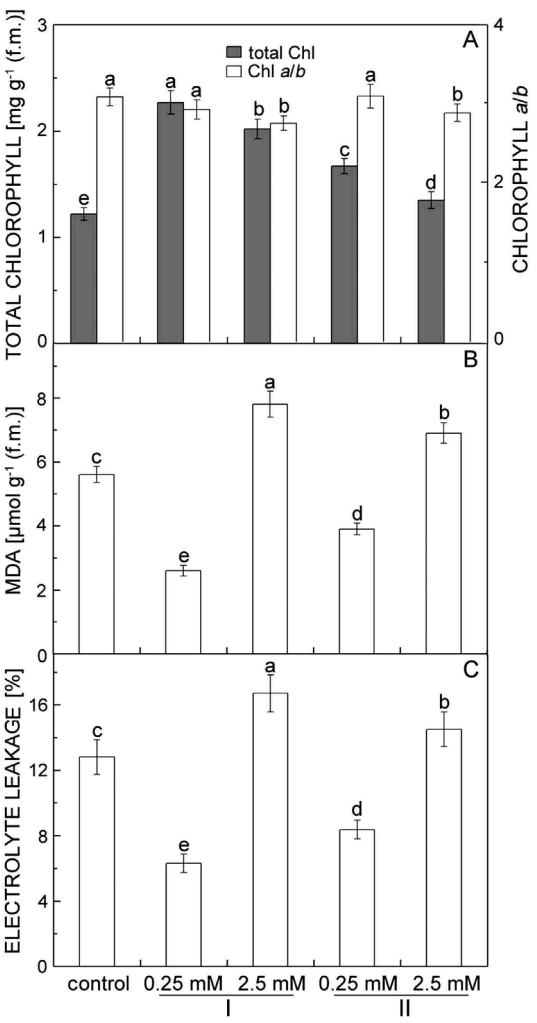

Fig. 1. Effects of SA concentration and method of application on total chlorophyll (Chl) content and Chl *a*/*b* ratio (*A*), MDA content (*B*), and electrolyte leakage (*C*). Means ± SDs, $n = 3$; different letters indicate significant differences according to Duncan's multiple range test at $P < 0.05$. I - the first method (root immersion), II - the second method (leaf spraying).

(Fig. 2) together with significant changes of leaf color compared with the control. At the same time, the results show that the root immersion was more effective in changing chlorophyll fluorescence than leaf spraying.

We also detected O_2^- and H_2O_2 produced under the different SA applications by histochemical staining the leaves using NBT and DAB, respectively. Slight staining was observed in the control leaves and intensive staining in the 2.5 mM SA-treated plants (Fig. 3*A*,*B*). To confirm these results, we measured the content of H_2O_2 and O_2^-. Under both the methods of SA application, the 0.25 mM SA treatment resulted in a slight increase and 2.5 mM SA in a significant increase in content of H_2O_2 and O_2^- as compared with the control plants (Fig. 3*C*,*D*). The content of MDA and electrolyte leakage under the 0.25 mM SA concentration was lower than in the control. However, the 2.5 mM SA treatment resulted in a significant increase in MDA content and electrolyte leakage as compared with the control (Fig. 1*B*,*C*). The first method of SA application induced more obvious changes in MDA content and electrolyte leakage than the second one. Under the PEG-induced water stress, the 0.25 mM SA pretreatment increased the RWC and decreased MDA as compared with the non-pretreated seedlings, and the effect was more pronounced under the root immersion than leaf spraying (Fig. 4). However, compared to the non-pretreated seedlings, the 2.5 mM SA pretreatment decreased the RWC and increased MDA more using first than second method of application (Fig. 4).

The effects of different SA concentrations and applications on specific activities of antioxidant enzymes and on non-enzymatic antioxidants in wheat are presented in Fig. 5. Compared with the control plants, the 0.25 mM SA-treated plants showed a significant increase in specific activities of POD, SOD, CAT, APX, GPX, and GR. However, the application of 2.5 mM SA resulted in an increase in specific activity of GR and a decrease in specific activities of SOD, CAT, and APX using both the methods of application. However, the root immersion caused usually more obvious changes in specific activities of antioxidant enzymes than leaf spraying.

We also measured AsA and DHA content because they are important antioxidants. Compared with the control, 0.25 mM SA increased the leaf AsA content only when applied to roots whereas 2.5 mM SA decreased the AsA content when using both methods of application (Fig. 5*G*). By contrast, the SA treatment decreased the content of DHA in comparison to the control and the two different methods did not show any significant differences (Fig. 5*H*).

To observe the effects of SA treatment on PS II proteins, immunoblotting of several thylakoid membrane proteins and quantitative analysis of these proteins were performed. We found that the 0.25 mM SA treatment mostly did not change the content of thylakoid proteins and only the 2.5 mM SA application increased the content of D1, Lhcb3, Lhcb5, and Lhcb6 proteins using the second method of SA application as compared with the control. However, using the first method, both the 0.25 mM SA application and especially the 2.5 mM SA application induced an increase in the content of D1, Lhcb3, and Lhcb6 proteins (Fig. 6). The most striking increase was in content of Lhcb6 ($P < 0.01$) after the 2.5 mM SA treatment.

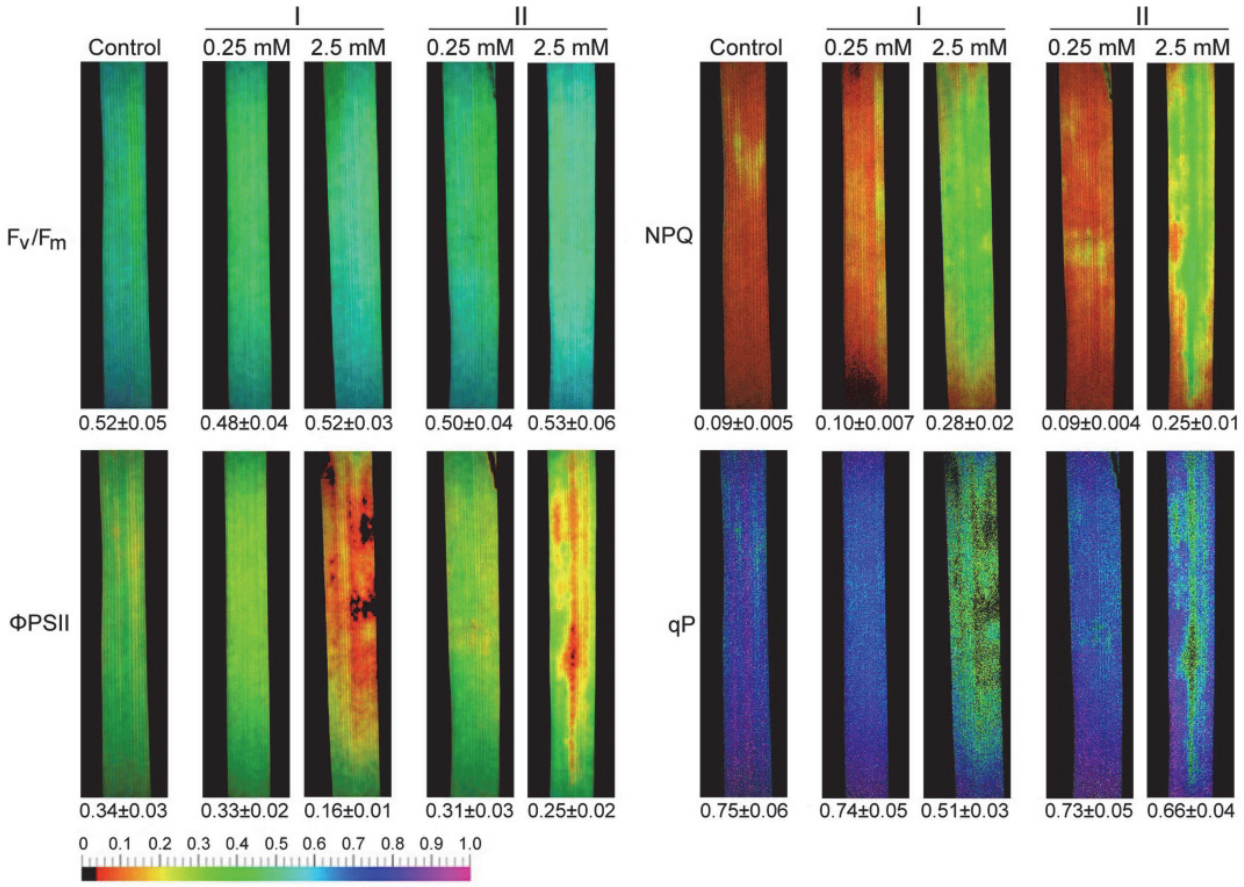

Fig. 2. Effects of SA concentration and method of application on chlorophyll fluorescence parameters (F_v/F_m - maximum photochemical efficiency of PS II in the dark-adapted state, qP - photochemical quenching, NPQ - non-photochemical quenching, Φ_{PSII} - quantum yield of PS II photochemistry). Quantitative values ± SDs are shown *below* each fluorescence image. Other is the same as in Fig. 1.

Discussion

Salicylic acid has been shown to work in plants through different pathways. It enhances activity of the antioxidant system by inducing antioxidative enzymes or altering expression of their genes (Kang *et al*. 2013, Janda and Ruelland 2014). In addition, it is now becoming clear that SA interacts both negatively and positively with other major signaling pathways including those regulated by jasmonic acid and ethylene (Singh and Gautam 2013, Janda and Ruelland 2014). In the present study, we investigated the effects of two different methods of SA application on PS II and the antioxidant system. The results show that the total Chl content significantly increased by the SA application. In agreement with our results, treatment with SA increases photosynthetic pigment content in some plants under normal or stress conditions (Singh and Usha 2003, Javaheri *et al*. 2012). We suggest that the SA application increased protection of the photosynthetic apparatus also by an enhanced antioxidant capacity, especially in the lower SA concentration, and the root immersion was more effective than leaf spraying. It seems that the wheat seedlings absorbed SA more rapidly through the root system than through the leaves with wax and trichomes on the surface. Also under the water stress, the SA pretreatment by the first method had more obvious effects on MDA and RWC than by the second method.

Previous studies suggested that SA treatment can protect the function of PS II and also increase the Φ_{PSII} and F_v/F_m under environmental stresses (Shi *et al*. 2006, Habibi 2012). In the present study, we did not find any significant difference in F_v/F_m between the control and SA-treated plants. The decreased Φ_{PSII} and qP, and increased NPQ suggest that the high concentration of SA induced a damage to photosynthesis to some extent. In addition, we also found that the decrease of Φ_{PSII} and qP values was more obvious using the first method than the second one, which can be interpreted as a rapid response to SA in the rooting medium. This is in accordance with the effect of SA on photosynthetic activity of tomato and wheat under salt stress (Arfan *et al*. 2007, Poor *et al*. 2011).

Salicylic acid content and ROS production are closely connected, and SA inhibits ROS signaling in transcriptional activation of defense related genes (Xu and Brosche 2014). However, several experiments indicated that SA treatment results in an accumulation of O_2^- and H_2O_2 (Rao *et al*. 1997, Agarwal *et al*. 2005, Harfouche *et al*. 2008). Also, the present study shows that 2.5 mM SA induced the accumulation of O_2^- and H_2O_2 in the wheat leaves (Fig. 3) accompanied with the accumulation of MDA and with the electrolyte leakage which are often used as measure of damage to cell membranes (Halliwel and Gutteridge 1984). Therefore, our results indicate that SA at the high concentration resulted in the oxidative damage of cell membranes.

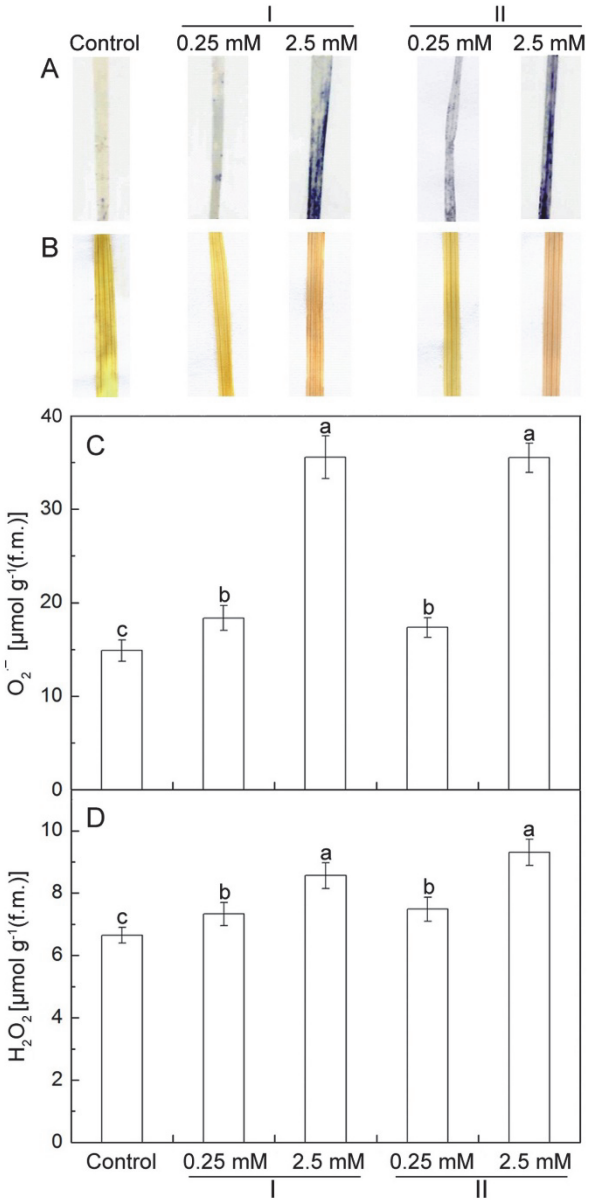

Fig. 3. Effects of SA concentration and method of application on O_2^- (*A*,*C*) and H_2O_2 (*B*,*D*). Histochemical detection (*A*,*B*) and content (*C*,*D*). Other is the same as in Fig. 1.

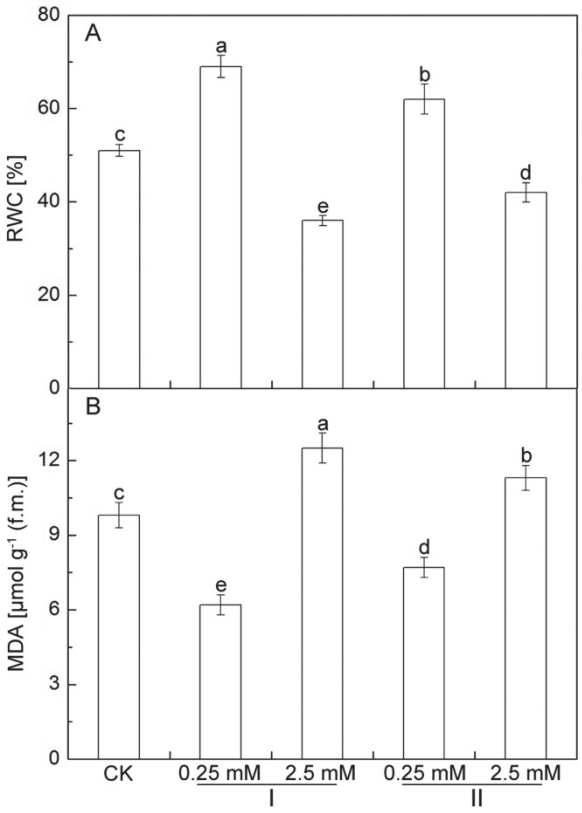

Fig. 4. Effects of SA pretreatment for 24 h and of water stress for 72 h on relative water content (RWC) (*A*) and malondialdehyde (*B*). Two SA concentrations and two methods of application were used. CK - seedlings without SA pretreatment but under a water stress for 72 h. Other is the same as in Fig. 1.

There are data supporting that SA increases activities of antioxidant enzymes and in turn protects plants against ROS generation and lipid peroxidation (Li *et al*. 2014). In contrast, it was also reported that SA treatment induces accumulation of ROS by inhibiting activity of some antioxidant enzymes such as SOD, APX, and CAT (Klessig *et al*. 2000, Horvath *et al*. 2007). In this work, we show that the low concentration of SA increased the specific activities of POD, SOD, CAT, GPX, APX, and GR (Fig. 5*A*-*F*). However, the high SA concentration decreased the specific activities of SOD, CAT, and APX. Therefore, the effect of SA on antioxidant enzyme activities depended on the concentration of SA, the mode of application, and plant species. Ascorbic acid is a soluble compound and ubiquitous in photosynthetic organisms, acting as important antioxidant in plants (Noctor and Foyer 1998). The enhanced AsA content under the low concentration of SA also indicates that the low SA concentration was favorable for promoting oxidative protection.

Previous studies have shown that SA pretreatment can significantly alleviate damages by environmental stresses on the D1 protein (Luo *et al*. 2009, Zhao *et al*. 2011). In the present study, 2.5 mM SA increased the content of

not only D1 but also Lhcb3, Lhcb5, and Lhcb6. Moreover, 0.25 mM SA increased the content of D1, Lhcb3, and Lhcb6. It has been shown that CP29, CP26, and CP24 are involved in the NPQ in *Arabidopsis* (Andersson *et al*. 2001, Kovacs *et al*. 2006). Here, our results suggest that the rise of NPQ under the high concentration of SA was mainly due to the increase in the content of Lhcb4, Lhcb5, and Lhcb6. Therefore, these results indicate that the SA play important role in regulating the thylakoid membrane proteins under different environmental stresses. However, the detailed mechanism needs to be further investigated.

In conclusion, the low concentration of SA applied by the two different methods improved the antioxidative capacity in the wheat seedlings. The SA application by the root immersion was more effective than leaf spraying. In addition, SA probably affected PS II function by regulation of some thylakoid membrane proteins. All the effects of SA depended on the SA concentration and the method of SA application.

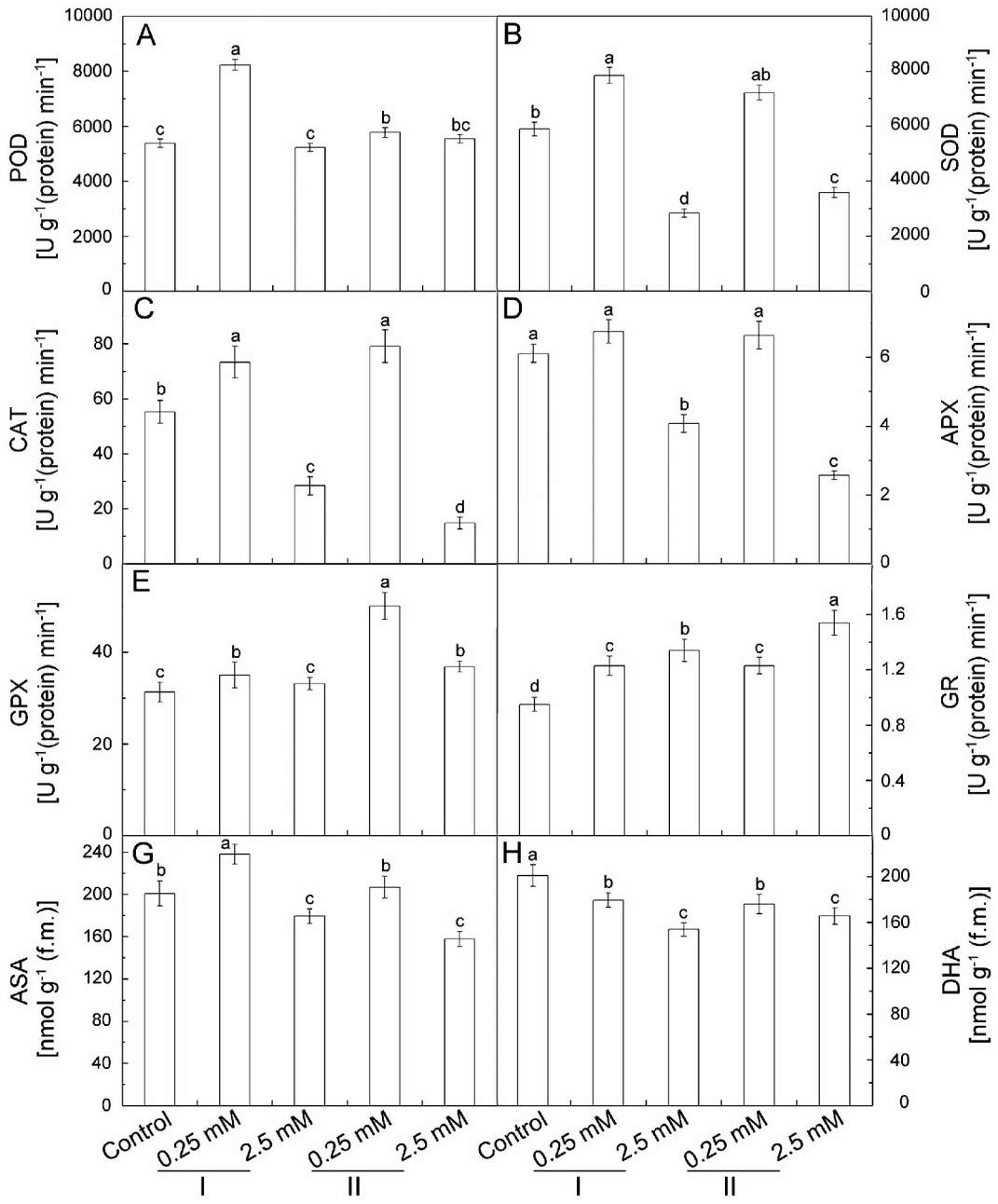

Fig. 5. Effects of SA concentration and method of application on the specific activities of peroxidase (POD) (*A*), superoxide dismutase (SOD) (*B*), catalase (CAT) (*C*), ascorbate peroxidase (APX) (*D*), glutathione peroxidase (GPX) (*E*), glutathione reductase (GR) (*F*), and on the content of ascorbate (AsA) (*G*) and dehydroascorbate (DHA) (*H*). Other is the same as in Fig. 1.

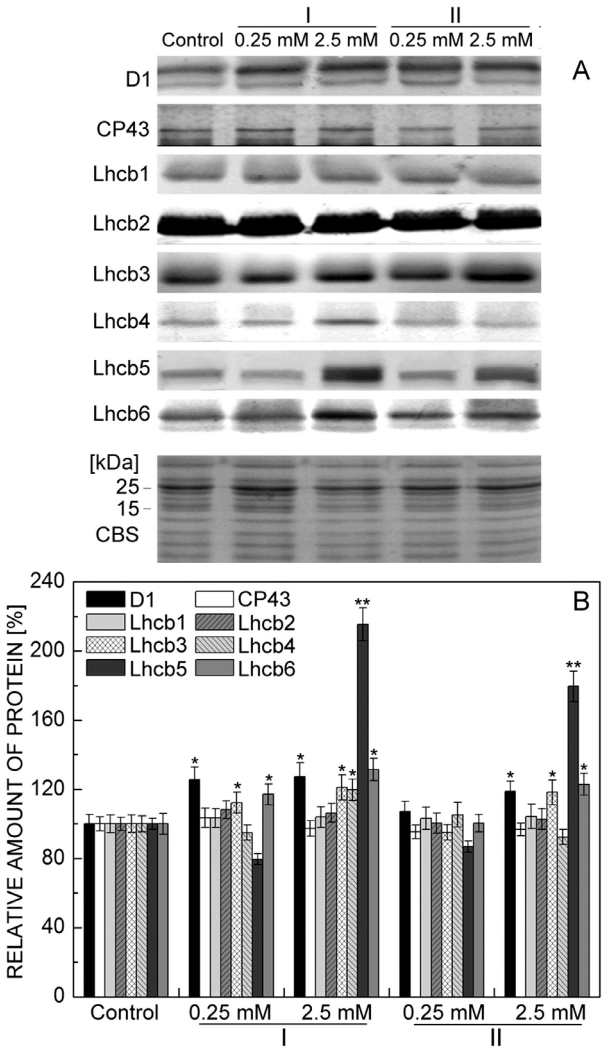

Fig. 6. Effects of SA concentration and method of application on thylakoid membrane proteins. *A* - Immunoblotting was performed with antibodies against D1, CP43, Lhcb1, Lhcb2, Lhcb3, Lhcb4, Lhcb5, and Lhcb6. Loading was according to an equal amount of chlorophyll. Results by SDS PAGE after Coomassie blue staining (CBS) are shown in the *bottom panel*. *B* - The quantification of immunoblot data. Results are relative to the amount in the control (100 %). *, ** indicate statistically significant differences at $P < 0.05$ and $P < 0.01$, respectively, determined using Student's *t*-test. Other is the same as in Fig. 1.

References

Agarwal, S., Sairam, R.K., Srivastava, G.C., Tyagi, A., Meena, R.C.: Role of ABA, salicylic acid, calcium and hydrogen peroxide on antioxidant enzymes induction in wheat seedlings. - Plant Sci. **169**: 559-570, 2005.

Alam, M.M., Nahar, K., Hasanuzzaman, H., Fujita, M.: Alleviation of osmotic stress in *Brassica napus*, *B. campestris*, and *B. juncea* by ascorbic acid application. - Biol. Plant. **58**: 697-708, 2014.

Andersson, J., Walters, R.G., Horton, P., Jansson, S.: Antisense inhibition of the photosynthetic antenna proteins CP29 andCP26: implications for the mechanism of protective energy dissipation. - Plant Cell **13**: 1193-1204, 2001.

Arfan, M., Athar, H.R., Ashraf, M.: Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress? - J. Plant Physiol. **164**: 685-694, 2007.

Bailey-Serres, J., Mittler, R.: The roles of reactive oxygen species in plant cells. - Plant Physiol. **141**: 311, 2006.

Borsani, O., Valpuesta, V., Botella, M.A.: Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in *Arabidopsis* seedlings. - Plant Physiol. **126**: 1024-1030, 2001.

Cao, Y., Zhang, Z.W., Xue, L.W., Du, J.B., Shang, J., Xu, F., Yuan, S., Lin, H.H.: Lack of salicylic acid in *NahG Arabidopsis* protects plants against moderate salt stress, - Z. Naturforsch. C **64c**: 231-238, 2009.

Chen, Y.E., Yuan, S., Du, J.B., Xu, M.Y., Zhang, Z.W., Lin, H.H.: Phosphorylation of photosynthetic antenna protein CP29 and photosystem II structure changes in monocotyledonous plants under environmental stresses. - Biochemistry **48**: 9757-9763, 2009.

Elstner, E.F., Heupel, A.: Inhibition of nitrite formation from hydroxylammonium chloride: a simple assay for superoxide

dismutase. - Anal. Biochem. **70**: 616-620, 1976.

Fristedt, R., Granath, P., Vener, A.V.: A protein phosphorylation threshold for functional stacking of plant photosynthetic membranes. - PLoS ONE **5**: e10963, 2011.

Ghasemzadeh, A., Jaafar, H.Z.E.: Interactive effect of salicylic acid on some physiological features and antioxidant enzymes activity in ginger (*Zingiber officinale* Roscoe). - Molecules **18**: 5965-5979, 2013.

Gill, S.S., Tuteja N.: Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. - Plant Physiol. Biochem. **48**: 909-930, 2010.

Gunes, A., Inal, A., Alpaslan, M., Eraslan, F., Bagci, E.G., Cicek, N.: Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (*Zea mays* L.) grown under salinity. - J. Plant Physiol. **164**: 728-376, 2007.

Habibi, G.: Exogenous salicylic acid alleviates oxidative damage of barley plants under drought stress. - Acta biol. szeged. **56**: 57-63, 2012.

Halliwel, B., Gutteridge, J.M.: Oxygen toxicity, oxygen radicals, transition metal and disease. - Biochem. J. **219**: 1-14, 1984.

Harfouche, A.L., Rugini, E., Mencarelli, F., Botondi, R., Muleo, R.: Salicylic acid induces H_2O_2 production and endochitinase gene expression but not ethylene biosynthesis in *Castanea sativa in vitro* model system. - J. Plant Physiol. **165**: 734-744, 2008.

Horvath, E., Szalai, G., Janda, T.: Induction of abiotic stress tolerance by salicylic acid signaling. - J. Plant Growth Regul. **26**: 290-300, 2007.

Janda, M., Ruelland, E.: Magical mystery tour: salicylic acid signaling. - Environ. exp. Bot. **114**: 117-128, 2015.

Javaheri, M., Mashayekhi, K., Dadkhah, A., Tavallaee, F.Z.: Effects of salicylic acid on yield and quality characters of tomato fruit (*Lycopersicum esculentum* Mill.). - Int. J. Agr. Crop Sci. **4**: 1184-1187, 2012.

Kampfenkel, K., Van Montagu, M., Inze, D.: Extraction and determination of ascorbate and dehydroascorbate from plant tissue. - Anal. Biochem. **225**: 165-167, 1995.

Kang, G.Z., Li, G.Z., Liu, G.Q., Xu, W., Peng, X.Q., Wang, C.Y., Zhu, Y.J., Guo, T.C.: Exogenous salicylic acid enhances wheat drought tolerance by influence on the expression of genes related to ascorbate-glutathione cycle. - Biol. Plant. **57**: 718-724, 2013.

Khan, M.I.R., Asgher, M., Khan, N.A.: Alleviation of salt-induced photosynthesis and growth inhibition by salicylic acid involves glycinebetaine and ethylene in mungbean (*Vigna radiata* L.). - Plant Physiol. Biochem. **80**: 67-74, 2014.

Klessig, D.F., Durnder, J., Noad, R., Navarre, D.A., Wendehenne, D., Kumar, D., Zhou, J.M., Shah, J., Zhang, S.Q., Kachroo, P., Trifa, Y., Pontier, D., Lam, E., Silva, H.: Nitric oxide and salicylic and signaling in plant defense. - Proc. nat. Acad. Sci. USA **97**: 8849-8855, 2000.

Klessig, D.F., Malamy, J.: The salicylic acid signal in plants. - Plant mol. Biol. **26**: 1439-1458, 1994.

Kovacs, L., Damkjaer, J., Kereiche, S., Ilioaia, C., Ruban, A.V., Boekema, E.J., Jansson, S., Horton, P.: Lack of the light-harvesting complex CP24 affects the structure and function of the grana membranes of higher plant chloroplasts. - Plant Cell **18**: 3106-3120, 2006.

Li, T.T., Hu, Y.Y., Du, X.H., Tang, H., Shen, C.H., Wu, J.S.: Salicylic acid alleviates the adverse effects of salt stress in *Torreya grandis* cv. Merrillii seedlings by activating photosynthesis and enhancing antioxidant systems. - PloS ONE **9**: e109492, 2014.

Lowry, O.H., Rosebrough, N.J., Fan, A.L., Randal, R.J.: Protein measurement with the Folin phenol reagent. - J. biol. Chem. **193**: 265-275, 1951.

Luo, M.H., Yuan, S., Chen, Y.E., Liu, W.J., Du, J.B., Lei, T., Wang, M.B, Lin, H.H.: Effects of salicylic acid on the photosystem II of barley seedlings under osmotic stress. - Biol. Plant. **53**: 663-669, 2009.

Maxwell, K., Johnson, G.N.: Chlorophyll fluorescence – a practical guide. - J. exp. Bot. **345**: 659-668, 2000.

Noctor, G., Foyer, C.H.: Ascorbate and glutathione: keeping active oxygen under control. - Annu. Rev. Plant Physiol. Plant mol. Biol. **49**: 249-279, 1998.

Okuda, T., Masuda, Y., Yamanka, A., Sagisaka, S.: Abrupt increase in the level of hydrogen peroxide in leaves of winter wheat is caused by cold treatment. - Plant Physiol. **97**: 1265-1267, 1991.

Poor, P., Gemes, K., Szepesi, A., Horvath, F., Simon, L.M., Tari, I.: Salicylic acid treatment *via* the rooting medium interferes with the stomatal response, CO_2 fixation rate and carbohydrate metabolism in tomato and decreases the harmful effects of subsequent salt stress. - Plant Biol. **13**: 105-114, 2011.

Porra, R.J., Thompson, W.A., Kriedmann, P.E.: Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. - Biochim. biophys. Acta **975**: 384-394, 1989.

Rao, M.V., Paliyath, G., Ormrod, D.P., Murr, D.P., Watkins, C.B.: Influence of salicylic acid on H_2O_2 production, oxidative stress, and H_2O_2-metabolizing enzymes. Salicylic acid-mediated oxidative damage requires H_2O_2. - Plant Physiol. **115**: 137-149, 1997.

Shi, Q.S., Bao, Z.Y., Zhu, Z.J., Ying, Q.S., Qian, Q.Q.: Effects of different treatments of salicylic acid on heat tolerance, chlorophyll fluorescence, and antioxidant enzyme activity in seedlings of *Cucumis sativa* L. - Plant Growth Regul. **48**: 127-135, 2006.

Shraiy, A.M.E., Hegazi, A.M.: Effect of acetylsalicylic acid, indole-3-butyric acid and gibberellic acid on plant growth and yield of pea (*Pisum sativum* L.). - Aust. J. basic appl. Sci. **3**: 3514-3523, 2009.

Singh, B., Usha, K.: Salicylic acid induced physiological and biochemical changes in wheat seedlings under water stress. - Plant Growth Regul. **39**: 137-141, 2003.

Singh, P.K., Gautam, S.: Role of salicylic acid on physiological and biochemical mechanism of salinity stress tolerance in plants. - Acta Physiol. Plant. **35**: 2345-2353, 2013.

War, A.R., Paulraj, M.G., War, M.Y., Ignacimuthu, S.: Role of salicylic acid in induction of plant defense system in chickpea (*Cicer arietinum* L.). - Plant Signal. Behav. **6**: 1187-1792, 2011.

Xu, E.J., Brosche, M.: Salicylic acid signaling inhibits apoplastic reactive oxygen species signaling. - BMC Plant Biol. **14**: 155, 2014.

Yang, Y.N., Qi, M., Mei, C.S.: Endogenous salicylic acid protects rice plants from oxidative damage caused by aging as well as biotic and abiotic stress. - Plant J. **40**: 909-919, 2004.

Yuan, S., Lin, H.H.: Role of salicylic acid in plant abiotic stress. - Z. Naturforsch. C **63c**: 313-320, 2008.

Zhao, H.J., Zhao, X.J., Ma, P.F., Wang, Y.X., Hu, W.W., Li, L.H., Zhao, Y.D.: Effects of salicylic acid on protein kinase activity and chloroplast D1 protein degradation in wheat leaves subjected to heat and high light stress. - Acta ecol. sin. **31**: 259-263, 2011.