Plant Growth Regul (2014) 73:289–297
DOI 10.1007/s10725-014-9889-9

ORIGINAL PAPER

Foliar-applied salicylic acid alleviates heat and high light stress induced photoinhibition in wheat (*Triticum aestivum*) during the grain filling stage by modulating the *psbA* gene transcription and antioxidant defense

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Received: 8 July 2013 / Accepted: 10 January 2014 / Published online: 14 January 2014

Abstract In this study, we investigated the effects of foliar applied salicylic acid (SA) on protecting wheat (*Triticum aestivum*) from heat and high light stress during the grain filling stage. Specifically, the Photosystem II (PSII) photochemistry of wheat flag leaves under the treatment of SA was studied as indicators for stress response. Our results indicated that under stress, SA-supplemented wheat plants maintained higher chlorophyll content, photochemical activity of PSII, and net photosynthetic rate in comparison to non-SA treated plants, and, in addition, the SA-supplemented plants recovered more rapidly from photoinhibition when the stress was removed. SA-treated plants inhibited the decrease of the *psbA* gene transcription that is caused by stress and then recovered to the original control level after the stress was removed. In addition, foliar supplementation of SA could maintain or elevate the activities of antioxidative enzymes, including superoxide dismutase, ascorbate peroxidase, and catalase, which are known to provide protection against oxidative stress for wheat crops. Taken together, our results suggest that foliar application of SA can protect the PSII complex from photo-damage through enhanced transcription of the *psbA* gene (encoding D1 protein), as well as through mitigating photo-oxidation enabled by a high level of antioxidative enzyme activities, which allows for faster functional recovery of PSII from heat and high light stress.

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Keywords Wheat (*Triticum aestivum*) · Heat and high light stress · Photoinhibition · *psbA* gene transcription · Antioxidant defense

Abbreviations

ANOVA	One-way analysis of variance
APX	Ascorbate peroxidase
CAT	Catalase
C_T	Cycle threshold
EDTA	Ethylene diamine tetraacetic acid
ETR	Electron transfer rate of PSII
FM	Fresh weight
Fm	Maximal fluorescence
Fo	Initial fluorescence
Fv	Variable fluorescence in dark-adapted leaves
Fv/*Fm*	The ratio of variable to maximum chlorophyll fluorescence
Fv/*Fo*	The ratio of variable to initial chlorophyll fluorescence
MDA	Malondialdehyde
NBT	Nitro blue tetrazolium
Pn	Net photosynthetic rate
PPFD	Photosynthetic photon flux density
PSII	Photosystem II
PVP	Polyvinylpyrrolidone
ROS	Reactive oxygen
SA	Salicylic acid
SOD	Superoxide dismutase
TBA	Thiobarbituric acid

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important food crops planted worldwide. In the major wheat

producing areas of the Northern Hemisphere, wheat plants often suffer from heat stress combined with high irradiance during the grain-filling stage, which normally takes place in May. The stress leads to damages in the photosynthetic apparatus, early decline of leaf function, stunt of seed, death of leaf cells, changes in antioxidants, lipid peroxidation, and protease activity in wheat leaves (Hameed et al. 2012), and finally reduction of yield (Ferris et al. 1998; Altenbach 2012). Corresponding to the prospective global warming, the quantity of wheat suffering from heat and high light stress is expected to increase gradually (Ortiz et al. 2008). Moreover, heat stress, in combination with other stresses, is a common constraint that causes composite damage during the anthesis and grain-filling stages in many cereal crops located in temperate regions (Wang et al. 2010b; Wang et al. 2010a; Monjardino et al. 2005). Therefore, much attention is currently being focused upon the effect of heat and high light stress on the photosynthesis of wheat plants during the grain-filling period, as well as its exogenous regulation.

It is well known that, in the photosynthesis system of plants, the reaction center in photosystem II (PSII) is the key site where damage is incurred by various stresses, such as heat and light, and the extent of this damage depends upon the balance between injury and repair (Adir et al. 2003; Murata et al. 2007; Baker 2008). PSII is a protein complex composed of more than 25 kinds of subunits, of which the D1 protein, encoded by the chloroplast *psbA* gene, is the target site damaged by many environmental stresses (Yamamoto et al. 2008). Hence, fast turnover of D1 protein is considered a premise for functional recovery of PSII (Yamamoto et al. 2008). Although electron flow driven by moderate light is necessary for the D1 protein turnover and PSII recovery (Marutani et al. 2012), a previous study has shown that heat and high light combined stress caused a significant decrease in content of D1 protein in grain filling wheat plants (Zhao et al. 2011).

Moreover, recent research has suggested that the indirect effects of heat stress and/or excessive illumination caused damage to PSII and were closely associated with the production of reactive oxygen species (ROS). Namely, ROS damages D1 protein (Yamamoto et al. 2008) and inhibits the repair of photodamaged PSII by suppressing the de novo synthesis of D1 protein (Murata et al. 2007). Furthermore, the oxygen evolution complex in PSII can be inactivated by the ROS that is generated by stress treatment through lipid peroxidation (Yamashita et al. 2008; Yamauchi and Sugimoto 2010).

Salicylic acid (SA) plays an important role as a signal molecule in abiotic stress tolerance, and considerable interest has been focused on SA due to its ability to induce a protective effect on a plant under stress (Horváth et al. 2007). Inhibitors of SA biosynthesis in pea (*Pisum sativum* L.) plants reduced the plants' tolerance to heat stress (Pan et al. 2006). As well as, the induction of thermotolerance of exogenous SA in grapevine (*Vitis vinifera* L.) was related to changes in the antioxidant enzyme activities (Wang and Li 2006). There are already several studies which report that SA increases wheat's resistance to salt (Arfan et al. 2007), freezing (Taşgın et al. 2006) stress, etc. Although the exact mechanism of SA regulated thermotolerance was not fully elucidated, it is evident that SA may either directly influence the activity of certain enzymes or may induce genes responsible for protective mechanisms (Horváth et al. 2007). Furthermore, a current study in wheat showed that, exogenous SA treatment could retard the degradation of D1 protein and PSII functional injury during heat and high light stress (Zhao et al. 2011). However, little is known regarding the amelioration mechanism of SA on grain filling wheat plants suffering from heat and high light stress, especially regarding the correlation between the photoinhibition alleviation and *psbA* transcription, and antioxidant defense.

In this study, the photosynthetic response to heat and high light stress and the subsequent recovery of wheat during the grain filling stage were presented. Moreover, the result of supplementing a stressed wheat plant with foliar SA was also evaluated in order to understand a possible protective effect against photoinhibition induced by SA, especially through modulating the *psbA* gene transcription and antioxidant defense.

Materials and methods

Plant materials and treatments

Wheat (*T. aestivum* L. cv. Aikang) seeds of uniform size were selected and surface-sterilized with 1 % sodium hypochlorite solution for 10 min and followed by washing several times with sterile distilled water. The seeds were then sown in Petri plates (9 cm) lined with six layers of filter paper that had been moistened with 10 mL of distilled water for germination for 2 days. Three germinated seedlings were then placed into each plastic pot (height 25 cm and diameter 20 cm) containing quartzite and grown under controlled conditions (300 μmol photon m^{-2} s^{-1} of photosynthetic photon flux density (PPFD) light; 75–85 % relative humidity; a 12/12 h day/night cycle; a continuous day/night temperature of 25/20 °C, respectively). Fifteen days after the anthesis (grain filling stage), the plants were foliar pretreated with 0.1, 0.3 and 0.5 of salicylic acid (SA) with water as the control. Three days later, the pretreated plants were subjected to heat and high light stress (light, 1,800 μmol photon m^{-2} s^{-1} of PPFD; temperature, 39 ± 2 °C) for 2 h and then recovered under non-stressed

conditions (light, 300 μmol photon m^{-2} s^{-1} of PPFD; temperature, 25 ± 2 °C) for 3 h in the climate chamber. Five or more flag leaves from all of the treated plants were sampled and analyzed immediately. The experiment was repeated three times under the same conditions.

Determination of chlorophyll content

Chlorophyll content was determined by taking fresh leaf samples (0.5 g) randomly from selected wheat plants. The samples were homogenized with 10 mL of acetone (80 % v/v) using a pre-cooled pestle and mortar. Then the homogenate was centrifuged at 5,000×*g* for 10 min. The absorbance was measured with a UV–visible spectrophotometer at 663 and 645 nm (Beckman, CA, USA). Chlorophyll contents were calculated using the equations proposed by Arnon (1949).

Chlorophyll fluorescence parameters and net photosynthetic rate

Chlorophyll fluorescence parameters were measured using a portable Chl fluorometer, (FMS-2, Hansatech, UK). Leaves were kept in dark for 20 min before measurement. Actinic light intensity was 400 μmol m^{-2} s^{-1}, and saturated flash intensity was 8,000 μmol m^{-2} s^{-1}. Some parameters were calculated with the following formulas: $Fv/Fm = (Fm - Fo)/Fm$, $Fv/Fo = (Fm - Fo)/Fo$, where *Fo* is initial fluorescence, *Fm* is maximum fluorescence, and *Fv* is variable fluorescence. *Fv/Fm*, the ratio of variable to maximum chlorophyll fluorescence, represents the maximal photochemical efficiency. *Fv/Fo*, the ratio of variable to initial chlorophyll fluorescence, represents potential photochemical efficiency. *ETR* represents the electron transfer rate of PSII. Net photosynthetic rate (*Pn*) was determined using a LI-6400 photosynthesis equipment (Li-cor, USA).

Preparation of total RNA and real-time PCR quantification of *psbA* transcription

Total RNA was extracted from flag leaves by Trizol RNA extraction solution (Invitrogen, Inc., Carlsbad, CA, USA) following the manufacturer's recommendations. Approximately 4 μg of total RNA was reverse transcribed using an oligo (dT) primer and RevertAid™ First Strand cDNA Synthesis Kits (Fermentas Life Sciences, MD, USA) according to the manufacturer's recommendations.

Real-time PCR was performed using a Real-Time PCR system (7,500 model, Applied BioSystems, USA) with the Power SYBR® Green PCR master mix (Applied BioSystems, USA) according to the manufacturer's recommendations. Primers for the *psbA* gene (accession number NC002762) (psbA-F: 5′-GGAGGGGCAGCGATGAAGGC-3′ and psbA-R: 5′-GCCTGTGGGGTCGCTTCTGC-3′) and *18S rRNA* (accession number AJ272181) (18S-F: 5′-GTGACGGGTGACGGAGAATT-3′ and 18S-R: 5′-GACACTAATGCGCCCGGTAT-3′) were designed using the Primer Express 3.0 software (Applied BioSystems, USA). The primers were synthesized by Shanghai (China) Sangon Biological Engineering Technology & Services Co. Ltd. Data analysis was performed using the $2^{-\Delta\Delta CT}$ method, where $\Delta\Delta C_T = \Delta C_T$ (treated sample) − ΔC_T (untreated sample), $\Delta C_T = C_T$ (*psbA*) − C_T (*18S rRNA*), and C_T is the threshold cycle value for the amplified gene (Livak and Schmittgen 2001).

Measurement of lipid peroxidation, $O_2^{\cdot-}$ production rate and H_2O_2 content

The malondialdehyde (MDA) level was assayed by the thiobarbituric acid (TBA) reaction as described by Wang et al. (2008). The rate of $O_2^{\cdot-}$ generation was determined by monitoring the inhibition of the photochemical reduction of nitro blue tetrazolium (NBT) according to a previously described method by Cakmak and Marschner (1992). The production of H_2O_2, determined according to a method described by Sairam and Srivastava (2002), was estimated by measuring the spectrum absorbance of the titanium–hydroperoxide complex and using a standard curve plotted with a known concentration of H_2O_2.

Assays of antioxidant enzyme activities

Total superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) activities were determined as described by Prochazkova et al. (2001). Samples [1 g fresh weight (FM) of flag leaves] were excised and ground with a pestle in an ice-cold mortar with 8 ml of extract buffer as described by Wang et al. (2011). The buffer for the extractions of SOD and CAT was a 50 mM phosphate buffer (pH 7.8) containing 0.1 mM ethylene diamine tetraacetic acid (EDTA), 0.5 % (m/v) polyvinylpyrrolidone (PVP), and 0.1 % Triton X-100. The buffer for extraction of APX contained 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA, 5 mM ascorbate, 0.5 % (m/v) PVP, 0.1 % (v/v) Triton X-100, and 0.05 % (v/v) b-mercaptoethanol. The homogenates were filtered through four layers of gauze and then centrifuged at 12,000×*g* for 10 min at 4 °C. The supernatants were collected and used to assay antioxidative enzymatic activities.

Statistical analysis

Each treatment included at least three replicate plots with three plants per replicate pot. All the figures were drawn

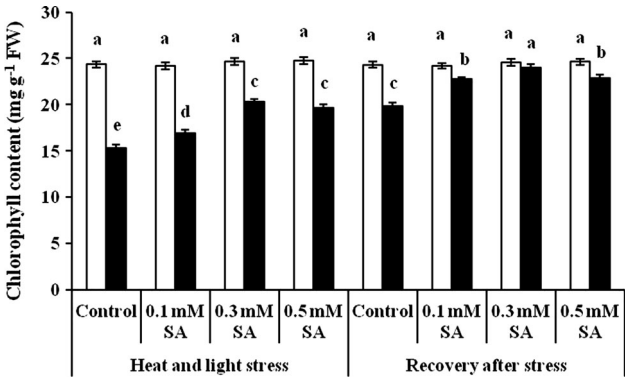

Fig. 1 Effects of SA on the chlorophyll content in wheat leaves under heat and high light stress during grain filling stage. The *open rectangle* represented the wheat plants under 25 ± 2 °C and 300 μmol m^{-2} s^{-1} of PPFD (non-stressed); the *solid rectangle* represented the wheat plants under 39 ± 2 °C and 1,800 μmol m^{-2} s^{-1} of PPFD (heat and high light stressed). Values are mean ± SE (n = 3). Means denoted by the same *letter* did not significantly differ at $P < 0.05$ according to Duncan's multiple range tests

using Excel 2007 software (Microsoft Corporation, USA). Statistical analysis was conducted using the SPSS Statistics 18.0 software (IBM Corporation, NY, USA). One-way analysis of variance (ANOVA) was done with all the data to confirm the variability of data and validity of the results. Differences between the means among treatments were compared using Duncan's multiple range tests at 0.05 probability levels.

Results

Chlorophyll content

The chlorophyll content in the grain filling wheat plants' leaves significantly decreased under heat and high light stress by 37 % compared to the non-stressed control (Fig. 1). Although the chlorophyll content obviously increased after the stress was removed, there was still a 19 % reduction compared to the control. Exogenous SA supplementation showed a concentration dependent protection effect on the chlorophyll content. Stressed wheat plants supplemented by 0.3 mM SA showed 32 % higher chlorophyll content than that under heat and high light stress alone. Similarly, foliar application of SA also showed an improvement of chlorophyll content during the recovery period after stress. 0.3 mM SA treated wheat plants showed no obvious difference compared to the control ($P > 0.05$).

Chlorophyll fluorescence and PSII recovery

As shown in Fig. 2, heat and high light stress led to significant decreases in *Fv/Fm*, *Fv/Fo*, *ETR* and *Pn*, which were 19, 59, 29 and 53 % lower than respective controls. Foliar application of SA was able to retard the decreases in these parameters. For example, foliar application of 0.3 mM SA was able to maintain the *Fv/Fm* at the same level as non-stressed control and keep 79 % of *Fv/Fo*, 86 % of *ETR*, and 71 % of *Pn*, which were 29, 92, 23 and 52 % higher than respective stressed alone wheat plants (without SA, UK).

In the recovery experiments, *Fv/Fm* recovered rapidly to the control level in all treated wheat plants, whether supplemented by exogenous SA or not. However, the parameters *Fv/Fo*, *ETR*, and *Pn* were not able to recover to the control level until 0.3 mM SA was foliar supplemented. Application of a higher concentration of SA (0.5 mM) showed no contribution to the recovery from photoinhibition, except for *ETR*, which was equal to the 0.3 mM SA treated wheat plants.

Relative transcriptional abundance of *psbA* gene

Heat and high light stress resulted in a significant decrease in the *psbA* gene relative transcriptional abundance (Fig. 3). *psbA* transcription was increased by foliar application of SA, and was 242 % higher than the stressed alone plants, but it was still 51 % lower than the non-stressed control. Higher or lower SA concentrations were both adverse to the decreasing inhibition of *psbA* gene transcription.

In the recovery experiments, wheat plants without SA supplement showed a slight but not obvious increase in *psbA* gene transcription (Fig. 3) ($P > 0.05$). Significant recovery enhancement of *psbA* gene transcription was determined in SA supplemented wheat plants. Moreover, there was no significant difference in comparison to the non-stressed control when 0.3 mM SA was foliar applied.

Lipid peroxidation, $O_2^{\cdot-}$ production rate and H_2O_2 content

A sharp increase in MDA content was observed in the grain filling wheat plants that were exposed to heat and high light stress, which was 107 % higher than the control (Fig. 4a). The SA supplemented stressed wheat plants significantly lowered the levels of MDA compared to the plants exposed to stress alone, although they were still obviously higher than their respective controls. After 3 h of recovery, MDA contents of wheat plants under all treatments decreased significantly. The decline of MDA content was enhanced by exogenous SA treatment, and when 0.3 mM SA was supplemented to the heat and high light stressed plants, there was no significant difference in comparison to the control ($P > 0.05$).

Similarly, significant $O_2^{\cdot-}$ production rate and H_2O_2 content were shown in wheat plants that were exposed to heat

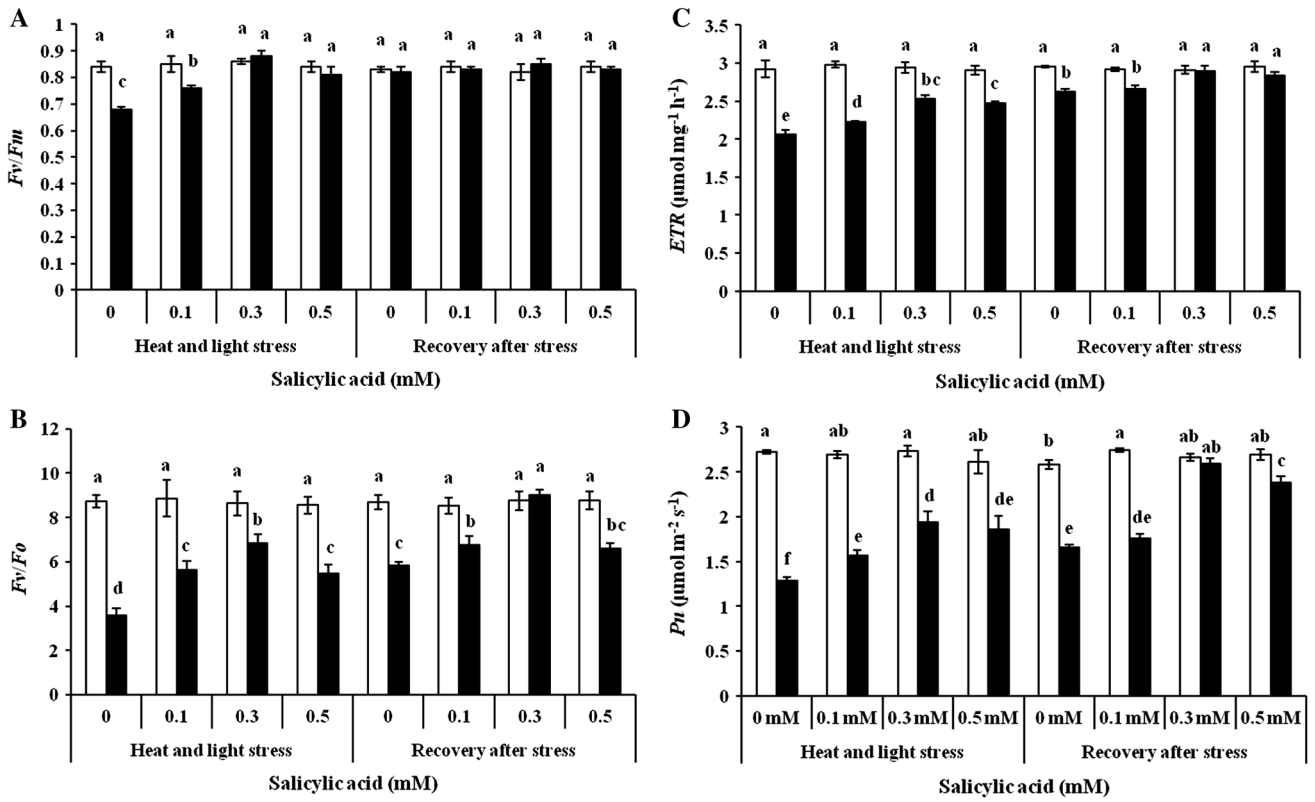

Fig. 2 Effects of foliar application of SA on *Fv*/*Fm* (**a**), *Fv*/*Fo* (**b**), *ETR* (**c**) and *Pn* (**d**) in wheat flag leaves under heat and high light stress during grain filling stage. The *open rectangle* represented the wheat plants under 25 ± 2 °C and 300 μmol m^{-2} s^{-1} of PPFD (non-stressed); the *solid rectangle* represented the wheat plants under 39 ± 2 °C and 1,800 μmol m^{-2} s^{-1} of PPFD (heat and high light stressed). Values are mean ± SE (n = 3). Means denoted by the same *letter* did not significantly differ at $P < 0.05$ according to Duncan's multiple range tests

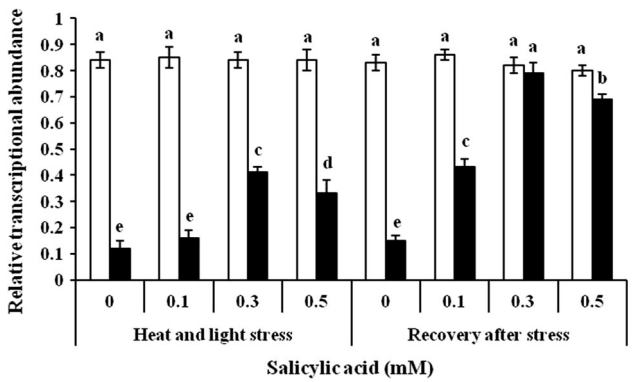

Fig. 3 Effects of foliar application of SA on *psbA* gene relative transcriptional abundance in wheat flag leaves under heat and high light stress during grain filling stage. The *open rectangle* represented the wheat plants under 25 ± 2 °C and 300 μmol m^{-2} s^{-1} of PPFD (non-stressed); the *solid rectangle* represented the wheat plants under 39 ± 2 °C and 1,800 μmol m^{-2} s^{-1} of PPFD (heat and high light stressed). Values are mean ± SE (n = 3). Means denoted by the same *letter* did not significantly differ at $P < 0.05$ according to Duncan's multiple range tests

and high light stress, which were 81 % and 47 % higher than the respective controls (Fig. 4b, c). The SA supplemented stressed wheat plants maintained significantly lower $O_2^{\cdot-}$ production rate and H_2O_2 content, which was lowest at 0.3 mM SA treatment (21 % of $O_2^{\cdot-}$ production rate and 14 % of H_2O_2 content) as compared to the plants subjected to heat and high light stress without SA. Lower $O_2^{\cdot-}$ production rate and H_2O_2 content were also seen in wheat plants supplemented by SA after 2 h of recovery from heat and high light stress, and the reduction was 35 and 24 % compared to wheat plants without SA, which were almost identical to their respective controls' levels.

Activities of antioxidant enzymes (SOD, CAT, APX and POD)

Compared to the control, there was no significant change of SOD activity in both wheat plants exposed to heat and high light stress and plants recovered from stress alone ($P > 0.05$) (Fig. 5a). Concentration-dependant effects of exogenous SA supplement were shown in stressed and stress-recovered wheat plants, which were 32 and 24 % higher under 0.3 mM SA treatment than heat and high light stress alone (without SA).

A significant decline of CAT activity was evident when only 51 % of the grain filling wheat plants remained after

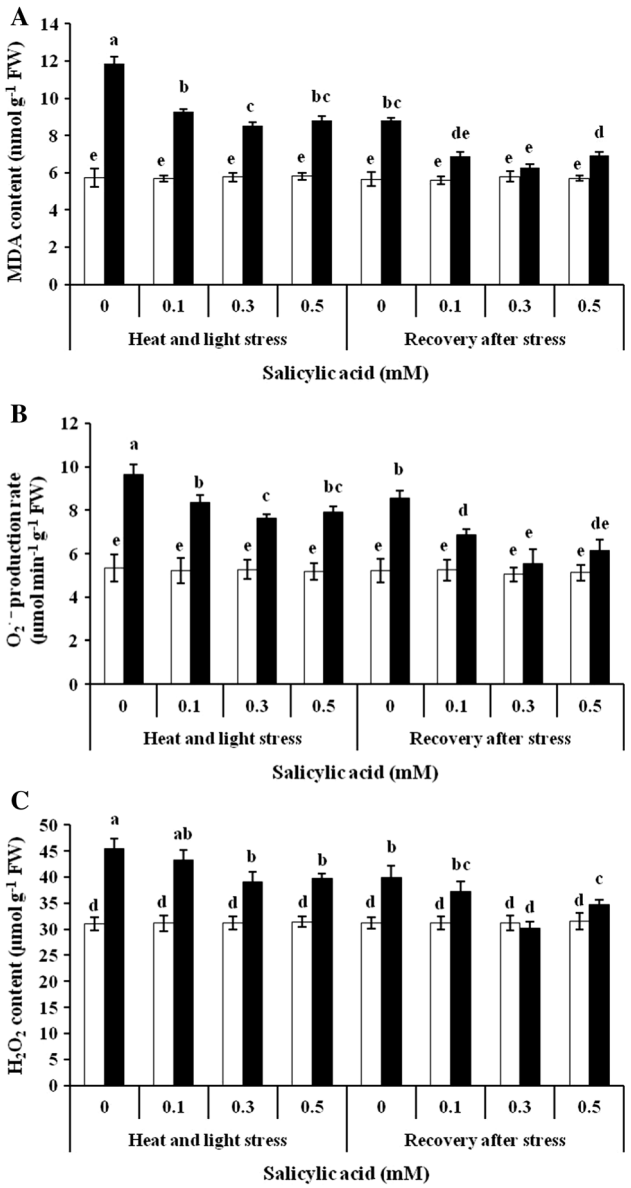

Fig. 4 Effects of SA on the MDA (**a**), $O_2^{\cdot-}$ production rate (**b**) and H_2O_2 contents (**c**) in wheat leaves under heat and high light stress during grain filling stage. The *open rectangle* represented the wheat plants under 25 ± 2 °C and 300 μmol m^{-2} s^{-1} of PPFD (non-stressed); the *solid rectangle* represented the wheat plants under 39 ± 2 °C and 1,800 μmol m^{-2} s^{-1} of PPFD (heat and high light stressed). Values are mean ± SE (n = 3). Means denoted by the same *letter* did not significantly differ at $P < 0.05$ according to Duncan's multiple range tests

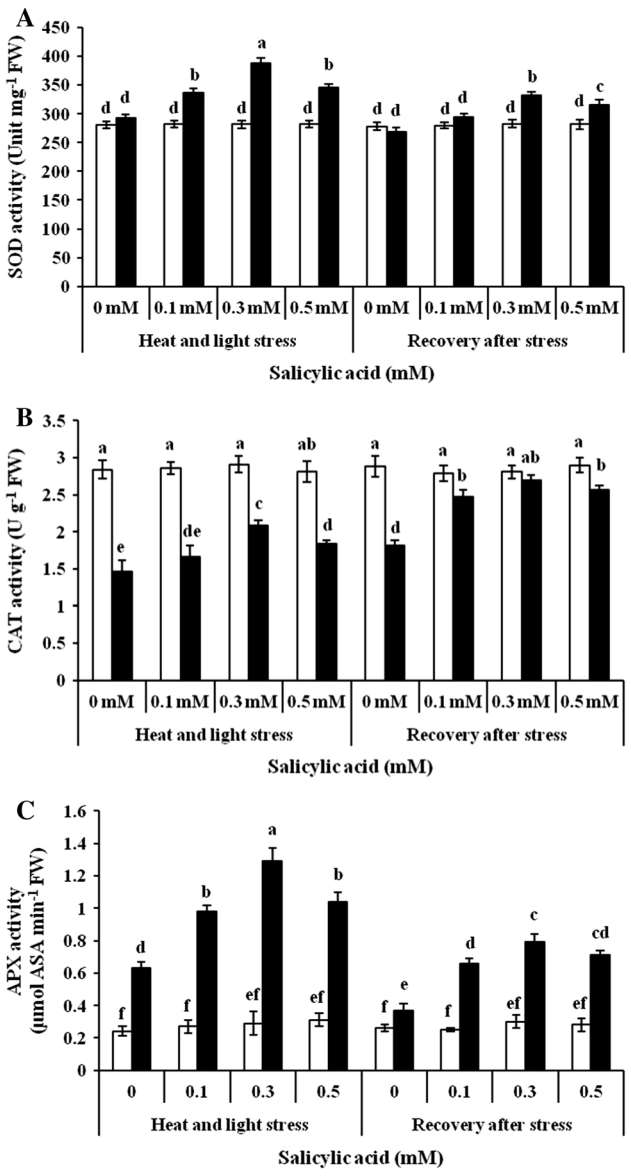

Fig. 5 Effects of SA on the SOD (**a**), CAT (**b**) and APX (**c**) activities in wheat leaves under heat and high light stress during grain filling stage. The *open rectangle* represented the wheat plants under 25 ± 2 °C and 300 μmol m^{-2} s^{-1} of PPFD (non-stressed); the *solid rectangle* represented the wheat plants under 39 ± 2 °C and 1,800 μmol m^{-2} s^{-1} of PPFD (heat and high light stressed). Values are mean ± SE (n = 3). Means denoted by the same *letter* did not significantly differ at $P < 0.05$ according to Duncan's multiple range tests

being exposed to heat and high light stress (Fig. 5b). Exogenous SA supplementation inhibited the stress-induced reduction of CAT activity, which was 42 % higher under 0.3 mM SA treatment than stress alone. During recovery stage after stress, a significant enhancement of the increase in CAT activity comparable to the control level was shown if wheat plants had been supplemented by 0.3 mM SA. Comparatively, 37 % lower CAT activity compared to the control was shown in wheat plants without SA supplement.

As shown in Fig. 5c, a significant increase in APX activity was shown in wheat plants that suffered from heat and high light stress, which was 163 % higher than control. Exogenous 0.1, 0.3 and 0.5 mM SA supplements obviously enhanced the increase of APX activity by 56, 105, and 65 % compared to stressed alone wheat plants (without SA). The increasing enhancement of SA supplement was

also shown in wheat plants during the recovery stage after stress. The APX activity in wheat plants under 0.3 mM SA treatment was 114 % higher than that without SA supplement.

Discussion

When higher plants were suffering from abiotic stress, there normally occurred a reduction in chlorophyll content, disintegration of chloroplast membranes, disruption of the biochemical reactions of photosystems (Dall'Osto et al. 2006), and a reduction in photosynthetic activity, which can be due to or lead to photoinhibition (Allakhverdiev et al. 2008). Our results indicated that heat and high light stress significantly decreased chlorophyll content in wheat leaves (Fig. 1). During the grain filling stage, the decrease of chlorophyll content under environmental stress should be attributed to the enhancement of chlorophyll degradation (Park et al. 2007). Thus, it could be indicated that the SA-retarded decrease of chlorophyll content might correspond to its protective effect on the degradation of chlorophyll under heat and high light stress.

Photosystem II is believed to play a key role in the response of leaf photosynthesis to environmental disturbances (Murata et al. 2007). *Fv/Fm*, *Fv/Fo*, *ETR*, and *Pn* are usually used as sensitive indicators of plant photosynthetic performance and represent the measure of the functional status of the oxygen evolving complex (Baker 2008). In the present study, when the grain filling wheat plants were exposed to heat stress combined with high irradiance, there were significant declines in *Fv/Fm*, *Fv/Fo*, *ETR*, and *Pn*. However, when stressed wheat plants were foliar sprayed by SA, the declines were inhibited and could be restored to the original control level after recovering from the stress (Fig. 2), suggesting that the PSII complex was better suited to withstand photoinduced inactivation. The results indicated that exogenous SA is able to alleviate photoinhibition caused by heat and high light stress in grain filling wheat leaves.

The strong protective effect of SA on structure and function of the oxygen-evolving complex of PSII against different environmental stresses has been well established in vitro (Horváth et al. 2007). This effect was concentration-dependent, as SA exhibited a protective effect only at low concentrations (Horváth et al. 2007). In the present study, compared among the three concentrations sprayed on wheat leaves, 0.3 mM SA showed the highest photosynthesis protection effect. Comparatively, the worse protection might be caused either by the insufficiency of lower SA concentration or by the ROS redundant of higher SA concentration (Rao et al. 1997). Similar research has also been shown in cucumber plants (*Cucumis sativa* L.); foliar spraying with SA induced heat tolerance, as shown by a higher *Fv/Fm* chlorophyll a fluorescence value, whereas the hydroponic application of higher concentration had an opposite effect (Shi et al. 2006).

The direct protective role of SA on PSII, either through enhancing D1 protein turnover or through alleviating oxidative stress may have additional functions. Under heat and high light stress, the excess light energy absorbed by the antenna pigments must be dissipated safely to avoid severe photoinhibition and photooxidation. Energy dissipation relying on D1 protein turnover is a very important mechanism (Russell et al. 1995). It is known that D1 protein is one of the most important componential and functional proteins in the PSII complex (Edelman and Mattoo 2008; Yamamoto 2001). The *psbA* gene was responsible for the reproduction of new D1 proteins for the replacement of the portion injured by stress, and plays an important role in D1 protein turnover during stress resistance (Mulo et al. 2009; Wang et al. 2011). In present research, transcription of the *psbA* gene was suppressed by heat and high light stress, but could be alleviated by the foliar application of SA (Fig. 3). The inhibition of *psbA* mRNA transcription may decrease the activity of PSII and electron transfer rates in the grain filling wheat plant, which was observed by a decrease in chlorophyll content and *ETR*, as shown in Figs. 1 and 2c, respectively. It is known that the D1 protein turnover includes two important procedures, the degradation of injured partition and the de novo synthesis of new copies (Edelman and Mattoo 2008). Foliar application of 0.3 mM SA has been proven to retard the decrease of D1 protein content in grain filling wheat plants under heat and high light stress (Zhao et al. 2011). In the present study, induced *psbA* transcription by foliar applied SA may enhance the de novo synthesis of the new copies of D1 protein, and then improve the turnover of it and dissipate more excess light energy (Mulo et al. 2012), which are all beneficial for the improvement of PSII tolerance both in heat and high light stressed and recovered wheat plants (Figs. 1, 2).

Like other abiotic stress, high temperature and irradiance stress accelerates the generation and reactions of ROS including $^1O_2$, $O_2^{\cdot-}$, H_2O_2 and $OH^{\cdot}$, thereby inducing oxidative stress (Mittler 2002; Yin et al. 2008). Under heat and high light stress condition, excess energy that has not been used for photosynthesis may produce large amounts of ROS, which may cause oxidative damage to chloroplasts and other cell structures (Singh and Singhal 2001). Pretreatment by a foliar spray of SA may have a signaling function that plays a role in the stimulation of heat tolerance in the grain filling wheat plants as indicated by the decreases in MDA content, $O_2^{\cdot-}$ production rate, and H_2O_2 concentration (Fig. 4). Low concentrations of ROS, especially H_2O_2, are known to act as signal molecules initiating several protective resistance mechanisms against pathogens, chilling, and heat stress

(Horváth et al. 2007). However, if ROS accumulation induced by SA were excessive, serious oxidative stress can occur as well as unrecoverable membrane damage (Rao et al. 1997). The observed declines in the protection of stressed photosynthesis and recovery after stress in 0.5 mM SA treatments might be the result of excessive production of ROS.

Moreover, SOD, CAT, and APX are three of the main enzymes involved in the water–water cycle, which is regarded as one of the safe dissipation pathways of excess photon energy under environmental stress (Asada 1999). SOD is the most effective in preventing cellular damage by converting superoxide anion to H_2O_2. CAT takes the responsibility for removing the bulk of H_2O_2 generated in photorespiration, and APX is the isoenzymic profile of a major enzyme that decomposes H_2O_2 to H_2O (Sharma and Dubey 2007). The slight increase in activity of SOD and significant decline in activity of CAT in heat and high light stressed wheat plants was (Fig. 5a, b), however, accompanied by a significant $O_2^{\cdot-}$ production rate (Fig. 4a), which may explain the higher H_2O_2 accumulation and greater oxidative stress. This agreed with the observed changes of H_2O_2 and MDA content (Fig. 4b, c). In the present study, foliar application of SA on the grain filling wheat plants could enhance the activities of SOD, CAT, and APX not only during heat and high light stress exposure, but also during the recovery stage after stress (Fig. 5). The higher anti-oxidative enzyme activity in SA-treated wheat plants may suggest a more effective operation of the water–water cycle, thus, more excess energy may be dissipated. However, it is unclear how SA maintains the anti-oxidative enzyme activities, which might be responsible for the activation of some common transcription factor associated with SOD, APX, and CAT (Agarwal et al. 2005). Furthermore, genetic studies using various *Arabidopsis thaliana* mutants have demonstrated that SA exerts its role in a variety of plant developmental processes through coordinating interactions with gibberellins (GAs), abscisic acid (ABA), jasmonic acid (JA), and ethylene (Santner et al. 2009). The potential interactions with other phytohormones in the protective effect of SA against photoinhibition of wheat also need to be further examined.

In summary, the present study showed that foliar application of 0.3 mM SA was able to decrease the susceptibility of the PSII in grain filling wheat leaves to photoinhibition that is caused by heat stress combined with high irradiance; this is mainly through accelerating the turnover of D1 protein in PSII by the inducement of the *psbA* gene transcription, and through maintaining the anti-oxidative enzyme activities to avoid or mitigate photooxidation.

Acknowledgments This research was supported by the National Natural Science Foundation of China (Grant No. 31000688) and the Education Department of Henan Province (Grant No. 13A210487).

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