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Changes in antioxidant enzymes activity and oxidative stress by abscisic acid and salicylic acid in wheat genotypes

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Abstract

Abscisic acid (ABA) and salicylic acid (SA) were sprayed on leaves of wheat genotypes C 306 and Hira at 25 and 40 d after sowing under moderate water stress (-0.8 MPa) imposed by adding PEG-6000 in nutrient solution. ABA and SA increased the activities of superoxide dismutase, ascorbate peroxidase, glutathione reductase, and catalase in comparison to unsprayed control plants. Both ABA and SA treatments decreased the contents of hydrogen peroxide and thiobarbituric acid reactive substances, a measure of lipid peroxidation, compared to unsprayed plants. The beneficial effect of increase in antioxidant enzymes activity and decrease in oxidative stress was reflected in increase in chlorophyll and carotenoid contents, relative water content, membrane stability index, leaf area and total biomass over control plants. The lower concentrations of ABA (0.5 mM) and SA (1.0 mM) were generally more effective than higher concentrations.

Additional key words: ascorbate peroxidase, catalase, glutathione reductase, hydrogen peroxide, lipid peroxidation, membrane stability index, superoxide dismutase, *Triticum aestivum*.

Introduction

A common consequence of most abiotic and biotic stresses is an increased production of reactive oxygen species (ROS) (Polle and Rennenberg 1993). ROS such as superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$) are toxic by-products of processes such a photosynthetic or respiratory electron transport. Oxidative stress in various crop plants has been reported in response to salinity (Sairam *et al.* 2002, 2005, Sairam and Srivastava 2002), drought (Menconi *et al.* 1995, Sairam *et al.* 1998, 2001), high temperature (Davidson *et al.* 1996, Sairam *et al.* 2000) and pollutants (Ranieri *et al.* 1998). These toxic ROS causes damage to DNA, proteins, lipids, chlorophyll, *etc*. (Fridovich 1986, Davies 1987, Imlay and Linn 1988).

Plants protect cell and sub cellular systems from the cytotoxic effects of these ROS with antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APOX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), catalase (CAT) and non-enzymatic substances such as glutathione, ascorbic acid, α-tocopherol and carotenoids (Elstner 1986, Bowler *et al.* 1992, Menconi *et al.* 1995, Alscher *et al.* 1997).

Thermotolerance of mustard (*Sinapis alba* L.) seedlings could be increased by salicylic acid (SA) treatment (Dat *et al.* 1998). SA is also accumulated during ozone and UV radiation exposure (Yalpani *et al.* 1994). Rao *et al.* (1997) have reported that SA treatment increased Cu/Zn-SOD activity and also enhanced H_2O_2 content, which was independent of changes in catalase activity. In maize plants pretreatment with SA increased antioxidant enzymes activities as well as chilling tolerance (Janda *et al.* 1999).

Abscisic acid (ABA) besides regulating stomatal opening (Hartung *et al.* 1998) and root hydraulic conductivity (Hose *et al.* 2000, 2001) has also been reported to induce tolerance to different abiotic stresses including drought, salinity and low temperature (Giraudat *et al.* 1994). Chandrasekar *et al.* (2000) have reported a higher accumulation of ABA in drought tolerant wheat cultivar C 306 and HW 24 than susceptible Hira in response to water stress. Exogenous application of ABA

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Abbreviations: ABA - abscisic acid; APOX - ascorbate peroxidase; Car - carotenoids; CAT - catalase; Chl - chlorophyll; DAS - days after sowing; DMSO - dimethylsulfoxide; DTNB - 5,5-dithiobis-(2-nitrobenzoic acid); EDTA - ethylene diamine tetraacetic acid sodium salt; GR - glutathione reductase; GSSG - oxidized glutathione; MSI - membrane stability index; NBT - nitrobluetetrazolium chloride; ROS - reactive oxygen species; SA - salicylic acid; SOD - superoxide dismutase; TBARS - thiobarbituric acid reactive substances; TCA - trichloroacetic acid.

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has been reported to significantly increase the activities of SOD, CAT, APOX and GR and the contents of ascorbate, reduced glutathione, α-tocopherol and carotenoids (Jiang and Zhang 2001). Pretreatment with ABA has also been reported to reduce the content of catalytic iron and significantly increased antioxidant enzymes activity and antioxidants in maize seedlings exposed to moderate water stress (Jiang and Zhang 2002). In view of these reports the present study was undertaken to examine the role of ABA and SA on the regulation of antioxidant defence and oxidative stress in wheat genotypes.

Materials and methods

An experiment was conducted with wheat (*Triticum aestivum* L.) genotypes Hira (susceptible to water and heat stress) and C 306 (tolerant to water and heat stress) in the National Phytotronic Facility of the Institute. The temperature, humidity and light/dark periods were maintained at 25 °C, 80 % and 16/8 h, respectively. Seeds of Hira and C 306 were germinated under sterilized condition in incubator for 5 d. About 5 cm long seedlings were transplanted in pots filled with a mixture of peat and vermiculite in 1:1 ratio and four plants were maintained in each pot. For three days pots were supplied with only double distilled water till the endosperm was exhausted. After that plants were irrigated with complete Hoagland solution or double distilled water given alternatively. To study the responses of ABA or SA wheat plants were subjected to mild water stress starting on 23rd or 38th day after sowing (DAS) for a period of 7 d, by supplying Hoagland solution or distilled water containing PEG-6000 (osmotic potential -0.8 MPa). ABA (0.5 and 1.0 mM) and SA (1.0 and 2.0 mM) were sprayed on whole plant till solution started dripping 2 d after inducing stress treatment at the 2 stages. Samples for the assay of SOD, APOX, GR, CAT activities, and H_2O_2 and TBARS contents were collected from the first fully developed leaf (3rd from the top) at 30 and 45 DAS, while relative water content, membrane stability index, contents of chlorophylls and carotenoids estimated only at 45 DAS.

Leaf relative water content (RWC) was estimated gravimetrically according to the method of Weatherley (1950). Membrane stability index (MSI) was estimated by taking 200 mg leaf material, in two sets, in test tubes containing 10 cm^3 of double distilled water (Sairam 1994). One set was heated at 40 °C for 30 min in a water bath, and the electrical conductivity of the solution recorded on a conductivity bridge (C_1). Second set was boiled at 100 °C on a boiling water bath for 10 min, and its conductivity was measured on a conductivity bridge (C_2). MSI was calculated as $MSI = [1 - (C_1/C_2)] \times 100$.

Chlorophyll and carotenoid contents were estimated by extracting 0.05 g of the leaf material in 10 cm^3 dimethylsulfoxide (DMSO) (Hiscox and Israelstam 1979). Samples were heated in an incubator at 65 °C for 4 h and than after cooling to room temperature, the absorbances of extracts were recorded at 665, 645 and 470 nm, and chlorophylls (Arnon 1949) and carotenoid contents (Lichtenthaler and Wellburn 1983) were calculated.

Hydrogen peroxide was estimated by forming titanium-hydro peroxide complex (Rao *et al*. 1997). Leaf material (1 g) was grinded with liquid nitrogen and the fine powdered material was mixed with 10 cm^3 cooled acetone in a cold room (10 °C). Mixture was filtered with *Whatman No. 1* filter paper followed by the addition of 4 cm^3 titanium reagent and 5 cm^3 ammonium solution to precipitate the titanium-hydrogen peroxide complex. Reaction mixture was centrifuged at 10 000 *g* for 10 min in the centrifuge (*J2–21*, *Beckman*, Geneva, Switzerland). Precipitate was dissolved in 10 cm^3 2 M H_2SO_4 and then recentrifuged. Supernatant was read at 415 nm against reagent blank in UV-visible spectrophotometer (Model *M 36*, *Beckman*, CA, USA). H_2O_2 contents were calculated by comparing with a standard curve drawn with known H_2O_2 concentrations. The lipid peroxidation was measured in terms of thiobarbituric acid reactive substances (TBARS) content (Heath and Packer 1968). Leaf sample (0.5 g) was homogenized in 10 cm^3 0.1 % trichloroacetic acid (TCA). The homogenate was centrifuged at 15 000 *g* for 15 min. To 1.0 cm^3 aliquot of the supernatant 4.0 cm^3 of 0.5% thiobarbituric acid (TBA) in 20 % TCA was added. The mixture was heated at 95 °C for 30 min in the laboratory electric oven (*Scientific*, New Delhi, India) and than cooled in an ice bath. After centrifugation at 10 000 *g* for 10 min the absorbance of the supernatant was recorded at 532 nm. The TBARS content was calculated according to its coefficient of absorbance (155 mM^{-1} cm^{-1}). The values for non-specific absorbance at 600 nm were subtracted.

Enzyme extract for superoxide dismutase, ascorbate peroxidase, glutathione reductase and catalase was prepared by first freezing the weighed amount of leaf samples (1 g) in liquid nitrogen to prevent proteolytic activity followed by grinding with 10 cm^3 extraction buffer [0.1 M phosphate buffer, pH 7.5, containing 0.5 mM di-sodium ethylene diaminetetraacetic acid (EDTA)] and 1 mM ascorbic acid. Brie was passed through 4 layers of cheesecloth and filtrate was centrifuged for 20 min at 15 000 *g*, and the supernatant was used.

Superoxide dismutase (SOD) activity was assayed by recording the enzyme mediated decrease in absorbance of the formazon made by superoxide radical and nitroblue tetrazolium dye (Dhindsa *et al*. 1981). The 3 cm^3 of reaction mixture contained, 13.33 mM methionine, 75 µM nitroblue tetrazolium chloride (NBT), 0.1 mM EDTA, 50 mM phosphate buffer (pH 7.8), 50 mM

sodium carbonate, 0.05 to 0.1 cm^3 enzyme, 0.9 to 0.95 cm^3 of water. Reaction was started by adding 2 μM riboflavin and placing the tubes under two 15 W fluorescent lamps for 15 min. A complete reaction mixture with out enzyme, which gave the maximal colour, served as control. Switching off the light and putting the tubes into dark stopped the reaction. A non-irradiated complete reaction mixture served as a blank. The absorbance was recorded at 560 nm, and one unit of enzyme activity was taken as that amount of enzyme, which reduced the absorbance reading to 50 % in comparison with tubes lacking enzyme.

Ascorbate peroxidase (APOX) was assayed by recording the decrease in absorbance due to ascorbic acid at 290 nm (Nakano and Asada 1981). The 3 cm^3 reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM EDTA, 0.1 mM H_2O_2 and 0.1 cm^3 enzyme. The reaction was started by the addition of 0.1 mM H_2O_2. Decrease in absorbance for a period of 30 s was measured at 290 nm. Activity was expressed by calculating the decrease in ascorbic acid content by comparing with a standard curve drawn with known concentrations of ascorbic acid.

Glutathione reductase (GR) was assayed as per the method of Smith *et al.* (1988). The reaction mixture contained, 66.67 mM potassium phosphate buffer (pH 7.5), 0.33 mM EDTA, 0.5 mM 5,5-dithiobis-(2-nitro-benzoic acid) in 0.01 M potassium phosphate buffer (pH 7.5), 66.67 μM NADPH, 666.67 μM oxidized glutathione (GSSG), 0.1 cm^3 enzyme extract. Adding GSSG started reaction. The increase in absorbance at 412 nm was recorded spectrophotometrically. The activity is expressed as total absorbance (A_{412}) per mg protein per min.

Catalase (CAT) was assayed by monitoring the decrease in absorbance due to hydrogen peroxide at 240 nm as described by Aebi (1984). The 3.0 cm^3 reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.0), 12.5 mM H_2O_2, 0.05 cm^3 enzyme and water to make up the volume to 3.0 cm^3. Adding H_2O_2 started reaction and decrease in absorbance was recorded for 1 min. Enzyme activity was computed by calculating the amount of H_2O_2 decomposed by referring to a standard curve of known concentrations of hydrogen peroxide.

Soluble protein was estimated according to the method of Bradford (1976). For leaf area and biomass estimation plants were harvested from 4 pots separately for each treatment at 45 DAS. Leaves were separated and their area was estimated using leaf area meter (*LiCOR-1600*, Lincoln, USA). For biomass estimation samples were first dried at 100 °C for 1 h followed by heating at 65 °C in a hot air oven till constant mass were achieved.

Samples for biochemical estimations were collected in 4 replicates (from 4 pots) and each replicate/sample was assayed twice (n = 8). Samples for biomass and leaf area were collected in quadruplicate (n = 4). The design of the experiment was CRD, and data was analyzed for analysis of variance (*ANOVA*). Standard error of mean was also calculated.

Results

ABA at 0.5 mM concentration increased leaf area in C 306 over control, while slight decrease was observed in Hira. However, at 1.0 mM ABA both the genotypes recorded a significant decrease in leaf area compared to control. Leaf area increased at both the concentrations of SA over untreated control in Hira, though the response was more at higher concentration. In case of C 306 the positive response was observed only at 1.0 mM SA (Table 1) and higher concentration was inhibitory. Both the concentrations of ABA showed increase in biomass over control in the 2 genotypes, though lower concentration (0.5 mM) was more responsive in case of C 306, while Hira showed more response at higher concentration (1.0 mM). Biomass also increased at both the concentrations of SA in C 306 and Hira; however, lower concentration of SA (1.0 mM) resulted in higher total biomass in C 306, while in Hira 2.0 mM SA was more effective (Table 2).

Relative water content (RWC) increased in both the genotypes in response to ABA and SA spray over untreated control. Lower concentration of ABA (0.5 mM) was comparatively more beneficial in increasing RWC

Table 1. Effect of abscisic acid and salicylic acid on leaf area [cm^2 $plant^{-1}$] of wheat genotypes. Means ± SE (n = 4). LSD at P = 0.05.

Genotypes	ABA [mM]			SA [mM]		
	control	0.5	1.0	control	1.0	2.0
C 306	155.41 ± 4.09	174.32 ± 15.69	130.92 ± 15.28	191.62 ± 9.02	203.04 ± 10.78	181.19 ± 7.99
Hira	156.76 ± 5.81	151.48 ± 5.01	104.46 ± 8.72	157.38 ± 8.70	169.91 ± 11.32	173.99 ± 7.11
Genotypes (G)	10.29			9.20		
Treatment (T)	12.60			11.27		
G × T	NS			15.94		

Table 2. Effect of abscisic acid and salicylic acid on biomass [g plant^{-1}] of wheat genotypes. Means ± SE (n = 4). LSD at P = 0.05.

Genotypes	ABA [mM]			SA [mM]		
	control	0.5	1.0	control	1.0	2.0
C 306	2.90 ± 0.21	3.69 ± 0.12	3.53 ± 0.10	3.23 ± 0.26	3.97 ± 0.12	3.33 ± 0.20
Hira	3.13 ± 0.09	3.70 ± 0.19	4.35 ± 0.15	2.70 ± 0.19	2.88 ± 0.13	2.97 ± 0.08
Genotypes (G)	0.16			0.18		
Treatment (T)	0.19			0.22		
G × T	0.27			0.31		

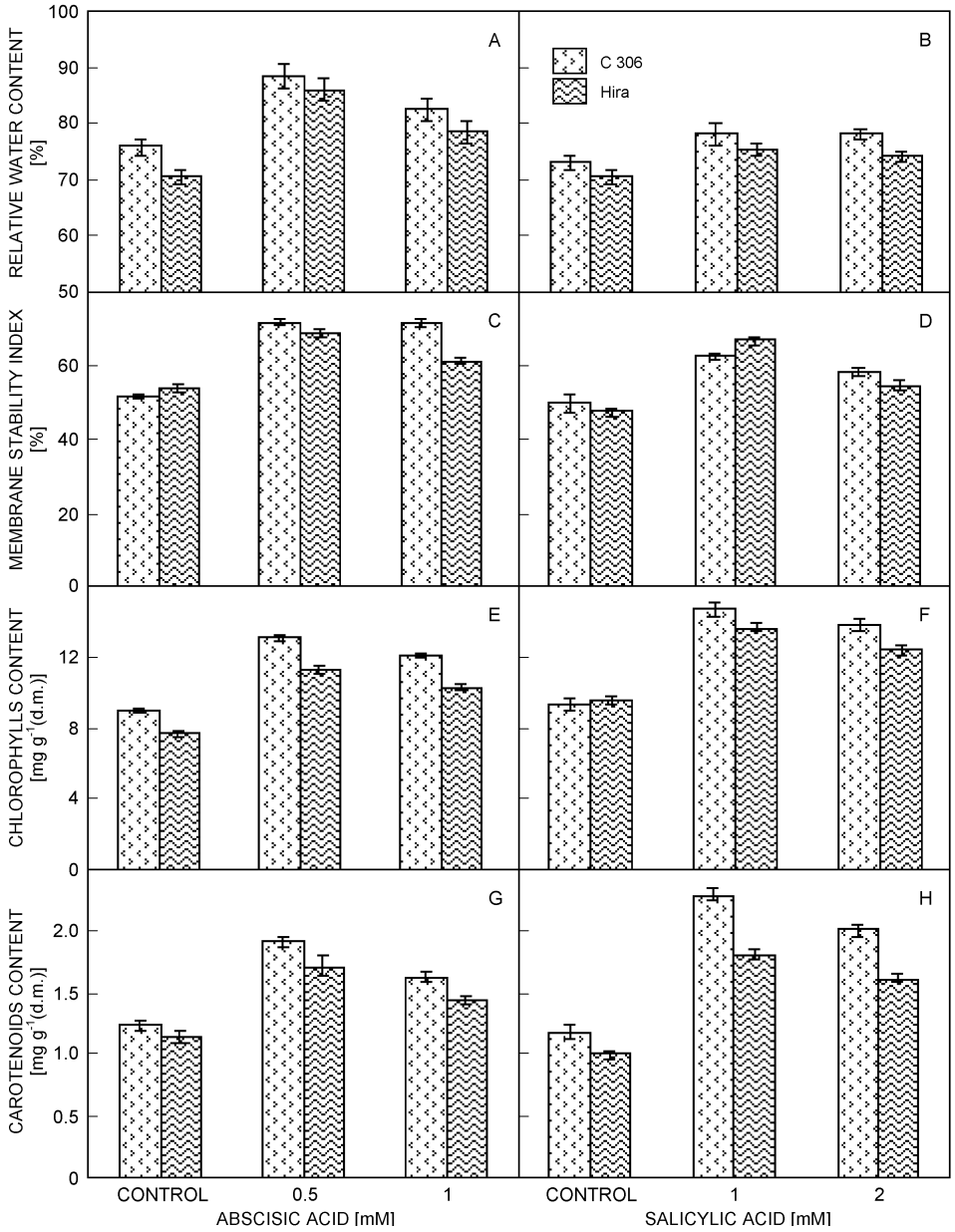

Fig. 1. Effect of abscisic acid and salicylic acid spray on relative water content (*A*, *B*), membrane stability index (*C*, *D*), contents of chlorophylls (*E*, *F*) and carotenoids (*G*, *H*) at 45 DAS in wheat genotypes. *Vertical bars* show SE (n = 8). Differences for genotypes (G), treatments (T) and G × T interactions were significant (P = 0.05).

than higher concentration of ABA (1.0 mM). Both the concentrations of SA invoked similar response on RWC. However, the increase in RWC over control plants was higher in case of ABA application than SA. Genotype C 306 generally showed more response to the ABA and SA application than Hira (Figs. 1*A*,*B*).

ABA and SA have positive affect on membrane stability index (MSI) (Fig. 1*C*,*D*). Treatment with 0.5 mM ABA and 1.0 mM SA had maximum effect in both the genotypes, and higher concentrations of ABA and SA did not cause further increase in MSI. The response to ABA was more in C 306 than in Hira.

Chlorophyll contents increased with ABA treatment (0.5 and 1.0 mM) compared to untreated control in both the genotypes. However, ABA induced increase in Chl was higher in C 306 (Fig. 1*E*) than Hira. Carotenoid contents also increased at 0.5 and 1.0 mM ABA. Both the genotypes showed decrease in Car content at 1.0 mM ABA over 0.5 mM ABA, but the values remained significantly higher than in control plants (Fig. 1*G*). Chl and Car contents also increased with SA treatment (1.0 and 2.0 mM) over untreated control (Fig. 1*F*,*H*). The increase in Car content was higher at 1.0 mM SA, though values at 2.0 mM were also higher than control plants.

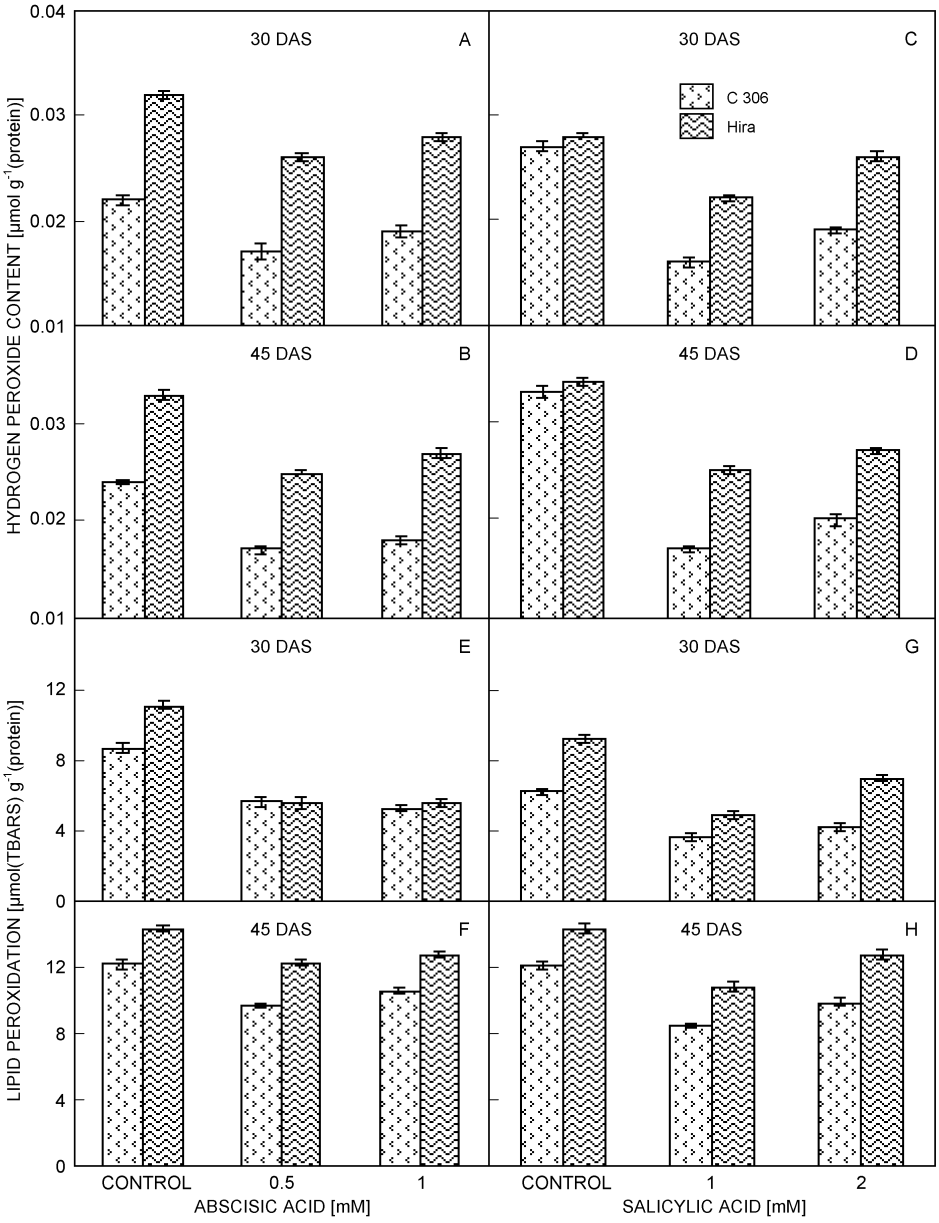

Fig. 2. Effect of abscisic acid and salicylic acid foliar spray on hydrogen peroxide content (*A*, *B*, *C*, *D*) and lipid peroxidation (*E*, *F*, *G*, *H*) at 30 and 45 DAS in wheat genotypes. *Vertical bars* show SE (n = 8). Differences for genotypes (G), treatments (T) and G × T interactions were significant (P = 0.05).

Response to SA (1.0 and 2.0 mM) was more marked in C 306 than in Hira.

H_2O_2 content decreased in ABA treated plants compared to control plants and the reduction was highest at 0.5 mM ABA in both the genotypes at the second stage. Hira showed higher H_2O_2 accumulation than C 306 at both the stages (Fig. 2*A*,*B*). At 1.0 mM ABA, H_2O_2 accumulation was slightly higher than at 0.5 mM ABA, but the values were lower than in control plants. Similar pattern was observed at 45 DAS. Lipid peroxidation increased with age and Hira showed higher lipid peroxidation than C 306. The TBARS contents were higher in control plants and lowest in 0.5 mM ABA treated C 306 and Hira. Higher dose of ABA (1.0 mM) was less effective in decreasing lipid peroxidation (Fig. 2*E*,*F*). SA (1.0 mM) caused decrease in H_2O_2 and TBARS contents at 30 and 45 DAS in both genotypes compared to control plants, and the values were lower in C 306 at both the stages. At higher concentration (2.0 mM) values of H_2O_2 and TBARS contents were marginally higher than at 1.0 mM SA, but were lower than in control plants (Fig. 2*C*,*D*,*G*,*H*).

SOD activity increased significantly at 0.5 mM ABA at 30 and 45 DAS. At 1.0 mM ABA, though the SOD activity increased over control plants, the increase was less than at 0.5 mM ABA. C 306 showed higher activity than Hira at both the stages and under all the treatments (Fig. 3*A*,*B*). SA (1.0 and 2.0 mM) also significantly increased SOD activity. However, activity was higher at 1.0 mM SA in both the genotypes compared to 2.0 mM.

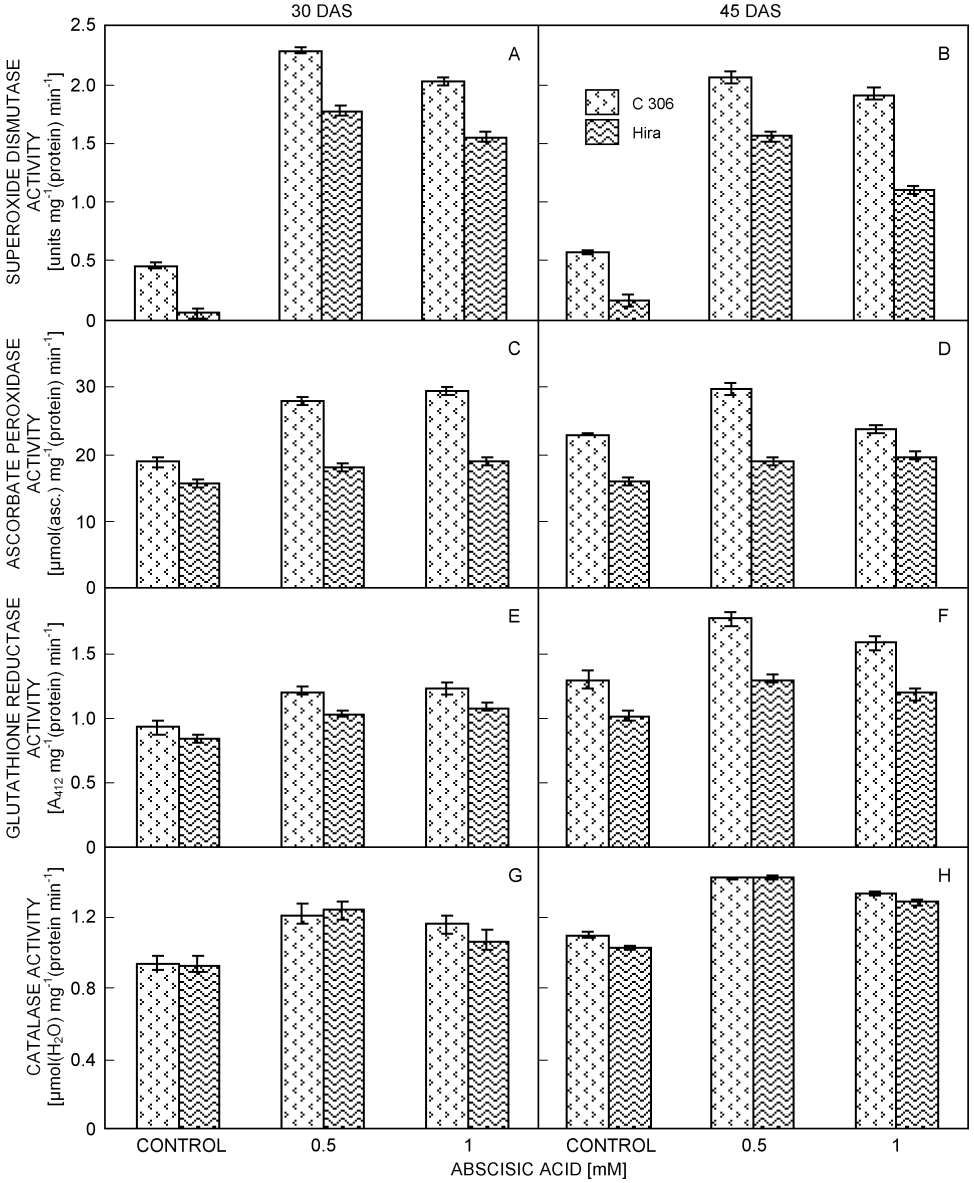

Fig. 3. Effect of abscisic acid spray on the activity of superoxide dismutase (*A*, *B*), ascorbate peroxidase (*C*, *D*), glutathione reductase (*E*, *F*) and catalase (*G*, *H*) at 30 and 45 DAS in wheat genotypes. *Vertical bars* show SE (n = 8). Differences for genotypes (G), treatments (T) and G × T interactions were significant (P = 0.05).

C 306 showed higher values than Hira at both stages and treatments (Fig. 4*A*,*B*).

ABA at 0.5 mM significantly increased APOX activity in both the genotypes at 30 and 45 DAS. In case of C 306 there was further slight increase in APOX activity at 1.0 mM ABA over 0.5 mM ABA at 30 DAS, while, the activity decreased and reached to control level at 45 DAS. In case of Hira, there was marginal increase in APOX activity at 30 and 45 DAS by 1.0 mM ABA spray over control and 0.5 mM ABA treatments (Fig. 3*C*,*D*). Both the concentrations of SA caused significant increase in APOX activity over control plants at both the stages. However, 1.0 mM SA was more effective compared to 2.0 mM SA. C 306 generally showed higher APOX activity than Hira at both the levels of SA and all the stages (Fig. 4*C*,*D*). GR activity increased with age. ABA (0.5 mM) significantly increased the activity in both the cultivars and stages. GR activity decreased at 1.0 mM ABA compared to 0.5 mM ABA, but values remained higher than control in both the genotypes. Higher GR activity was detected in C 306 than in Hira (Fig. 3*E*,*F*). SA (1.0 and 2.0 mM) increased GR activity over untreated control in both the genotypes and stages. GR activity was higher in C 306 than in Hira. At 30 DAS, C 306 showed increasing response with increasing SA concentration, while Hira at both the

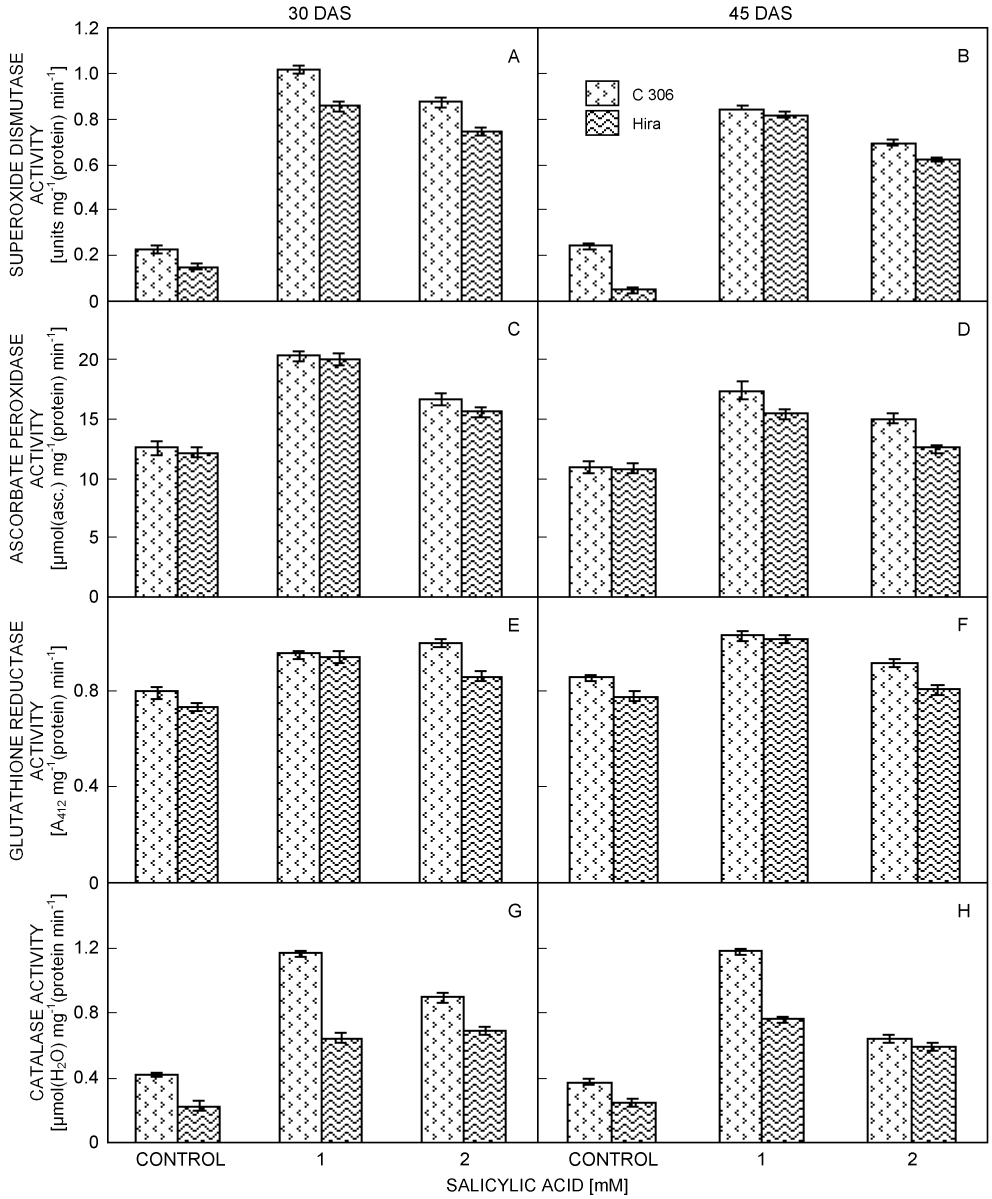

Fig. 4. Effect of salicylic acid spray on the activity of superoxide dismutase (*A*, *B*), ascorbate peroxidase (*C*, *D*), glutathione reductase (*E*, *F*) and catalase (*G*, H) at 30 and 45 DAS in wheat genotypes. *Vertical bars* show SE (n = 8). Differences for genotypes (G), treatments (T) and G × T interactions were significant (P = 0.05).

stages and C 306 at 45 DAS showed maximum response to 1.0 mM SA (Fig. 4*E*,*F*).

CAT activity increased in ABA treated plants compared to control plants and also with plant age. The increase in CAT activity was higher at 0.5 mM ABA than at 1.0 mM ABA at both the stages and in both genotypes (Fig. 3*G*,*H*). Highest values of CAT activity were observed at 1.0 mM SA in C 306 and Hira at both stages. At 2.0 mM SA CAT activity was higher than in control plants, but lower than at 1.0 mM SA (Fig. 4*G*,*H*). C 306 showed significantly higher CAT activity than Hira under all treatment and both stages.

Discussion

ABA at concentration 0.5 mM was comparatively more effective than at 1.0 mM in increasing the activities of SOD, APOX, GR and CAT. Among various antioxidant enzymes SOD showed maximum response to ABA. Positive effects of ABA in triggering adaptive responses have been reported by Chandler and Robertson (1994) and Giraudat *et al.* (1994). ABA induced increase in SOD, CAT, APOX and GR activities and other non-enzymatic antioxidant such as glutathione and ascorbate have also been reported by Jiang and Zhang (2001, 2002).

ABA treatment (0.5 and 1.0 mM) decreased oxidative stress (H_2O_2 and TBARS contents) in both the cultivars. However, the response was higher in tolerant cultivars C 306 and the lower concentration was more effective in both the cultivars. From the results, it is clear that ABA increased the activity of antioxidant enzymes, which caused a decrease in oxidative stress as shown by a decrease in H_2O_2 and TBARS contents. Gong *et al.* (1998) reported a decrease in lipid peroxidation in ABA treated heat stressed maize seedlings compared to control seedlings. A decrease in oxidative stress under mild stresses by ABA pre-treatment has also been reported by Jiang and Zhang (2002). ABA spray was also effective in increasing RWC, membrane stability, chlorophylls and carotenoids content over untreated plants. The response was more considerable in C 306 than in Hira. Decreases in electrolyte leakage and membrane permeability by ABA treatment have also been reported by Gong *et al.* (1998). It is apparent that ABA induced decrease in oxidative stress was responsible for increase in membrane stability and consequently higher chlorophyll and carotenoids contents. The positive effect of ABA induced antioxidant activity and reduction in oxidative stress was further manifested by an increase in RWC and plant growth. The ABA-induced increase in total biomass was observed at both concentrations in spite of decrease in leaf area at 1.0 mM ABA in C 306 and at both concentrations in Hira. It was probably due to more numbers of tillers in ABA treated plants (results not reported).

SA content is reported to increase in plant tissue both under biotic and abiotic stresses. Molina *et al.* (2002) reported 51 and 35 % increase in free and conjugated SA after 4-h NaCl treatment in tomato cell suspension. So, it is pertinent to examine the role of SA in regulating antioxidant activity under non-pathogenic conditions. Foliar spray of SA to wheat plants caused a very significant increase in SOD and CAT activity in both the cultivars at both the stages. An increase in activity of APOX and GR was also observed, though not as marked as for SOD. Unlike, the results of Chen *et al.* (1993) and Conrath *et al.* (1995), we did not observe any SA induced decrease in CAT activity. On the contrary, there was 3 times increase in CAT activity by SA treatment. Salicylic acid induced increases in CAT, GR and SOD activity have also been reported by Clark *et al.* (2002) and Molina *et al.* (2002). Tenhaken and Rubel (1997) and Rao *et al.* (1997) have reported that though SA was required in hypersensitive cell death, but there was no inhibition of CAT or APOX, suggesting that SA induced hypersensitive reaction or enhanced H_2O_2 production leading to cell death was not associated with the inhibition of these H_2O_2 scavenging enzymes. Similarly, there was no inhibition of APOX activity by SA, thus confirming the results reported by Durner and Klessig (1995). It is apparent that SA induced redox signal (H_2O_2) leading to increase in antioxidant activity is not linked to inhibition of CAT and APOX. The redox signal required for antioxidant enzyme induction is most probably generated by some other process such as plasma membrane linked NADPH oxidase as suggested by Kauss and Jeblick (1996), which may be the cause of H_2O_2 required for redox signaling for antioxidant enzyme induction. However, this H_2O_2, which is the part of SA or ABA induced redox signaling mechanism must be required in very low concentration for induction of antioxidant activity, as the plants sprayed with SA or ABA showed a decrease in both H_2O_2 and TBARS content compared to control plants. The decrease in H_2O_2 and TBARS content in spray experiment is a reflection of increase in antioxidant activity, which signifies an increased defense mechanism. The beneficial effect of SA was also reflected on membrane stability index, chlorophylls and carotenoids contents. Similarly, SA also promoted growth (increase in total biomass and leaf area). SA induced thermotolerance has also been reported by Dat *et al.* (1998) and Larkindale and Knight (2002). Kang and Saltveit (2002) have reported increased chilling tolerance by SA in rice seedlings as reflected by decrease in electrolyte leakage. They further reported SA induced chilling tolerance in maize and cucumber was associated with increase in GR and POX activity.

From the aforesaid discussion it is evident that foliar spray of ABA and SA increase antioxidant enzymes activity (SOD, APOX, GR, CAT), which was further reflected in decrease in oxidative stress (H_2O_2 and TBARS content) and increase in membrane stability,

chlorophylls and carotenoids contents and growth parameter. Further it may be assumed that SA induced redox signal may not be associated with the inhibition of CAT or APOX, as both the enzymes showed significant increase in activity by SA treatment.

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