Acta Agronomica Hungarica, 57(1), pp. 1–14 (2009)
DOI: 10.1556/AAgr.57.2009.1.1

HIGH TEMPERATURE STRESS TOLERANCE IN WHEAT GENOTYPES: ROLE OF ANTIOXIDANT DEFENCE ENZYMES

M. ALMESELMANI, P. S. DESHMUKH and R. K. SAIRAM

DIVISION OF PLANT PHYSIOLOGY, INDIAN AGRICULTURAL RESEARCH INSTITUTE, NEW DELHI, INDIA

Received: 28 September, 2007; accepted: 26 December, 2008

Two wheat genotypes, C 306 (tolerant) and PBW 343 (susceptible to temperature stress) were grown in growth chambers in the phytotron facility of IARI, New Delhi. The plants were maintained at 18/23°C (control) and 25/35°C (temperature stress) night/day temperatures after maximum tillering. Water potential was significantly reduced at anthesis, and at 7 and 15 days after anthesis in both genotypes in the heat stress treatment, and a greater reduction was recorded in PBW 343. The membrane stability index was also lower in the heat stress treatment in both genotypes at the vegetative stage, at anthesis and at 15 days after anthesis, and a greater reduction was observed in PBW 343 than in C 306. The hydrogen peroxide content increased as the plants advanced in age, and a higher hydrogen peroxide content was recorded in PBW 343 than in C 306 at different stages of growth in the heat stress treatment. The superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR) and peroxidase (POX) activities increased significantly at all stages of growth in C 306 in response to heat stress treatment, while PBW 343 showed a significant reduction in catalase, glutathione reductase and peroxidase activities in the high temperature treatment. Northern blot showed a significant increase in the *APX*-mRNA level under heat stress at the vegetative and anthesis stages, and the expression was greater in C 306. From the results it is apparent that the antioxidant defence mechanism plays an important role in the heat stress tolerance of wheat genotypes.

Key words: ascorbate peroxidase, catalase, gene expression, glutathione reductase, hydrogen peroxide, membrane stability index, peroxidase, superoxide dismutase, temperature stress

Introduction

High temperature limits the productivity of crops in many regions of the world (Al-Khatib and Paulsen, 1999) and continual heat stress is a problem on 7 million hectares, while terminal heat stress can be a problem on 40% of the irrigated wheat growing areas of the world (Fisher and Byerlee, 1990). Oxidative stress induced by high temperature has been reported in various higher and

0238–0161/$ 20.00

lower plants (Upadhyaya et al., 1990; Jagtap and Bhargava, 1995; Davidson et al., 1996; Sairam et al., 1997; 2000). Heat injury in cool season grasses and *Arabidopsis* has been associated with oxidative damage (Jiang and Huang, 2001; Liu and Huang, 2000; Larkindale and Knight, 2002).

Tolerance to high temperature stress in crop plants has been associated with an increase in antioxidant enzyme activity (Rui et al., 1990; Gupta et al., 1993; Badiani et al., 1994; Zhau et al., 1995; Sairam et al., 1997; 2000; Chaitanya et al., 2002; Kocsy et al., 2005). Several enzymatic and non-enzymatic antioxidant defence systems control ROS concentrations to protect cells from damage (Noctor and Foyer, 1998). Sairam et al. (2000) reported that plants protect cell and subcellular systems from the cytotoxic effects of these reactive oxygen species using antioxidant enzymes such as SOD, APX, GR, CAT and metabolites like glutathione, ascorbic acid, tocopherol and carotenoids. The activity of enzymes associated with the antioxidant defence system, especially ascorbate peroxidase, has been shown to increase rapidly under heat stress in mustard (Dat et al., 1998). The membrane stability index (MSI) is a measure of membrane integrity, which is estimated in terms of the conductivity of electrolyte leakage (Sairam et al., 1997). Sairam et al. (1998; 2000) demonstrated that drought- and heat stress-tolerant genotypes show comparatively higher MSI than susceptible genotypes.

The present work was conducted to study the physiological basis of heat stress tolerance in two wheat genotypes, C 306 (tolerant to heat and water stress) and PBW 343 (susceptible to heat stress) in relation to oxidative stress and the antioxidant defence mechanism.

Materials and methods

Plant material and growth conditions

Two wheat genotypes, namely C 306, a check variety for heat and water stress tolerance screening (Sairam, 1994; Sairam et al., 1998), and PBW 343, a heat-susceptible wheat genotype released for cultivation under irrigated, timely-sown conditions in the North-Western Plain zone of India but which suffers severely due to post-anthesis high temperatures when sown late (Almeselmani, 2006), were grown in controlled environment growth chambers at the National Phytotron Facility, Indian Agricultural Research Institute, New Delhi. The seeds were sown in pots (15 cm diameter and 30 cm height) filled with a medium consisting of coco-coir peat : vermiculite : sand in a 2:1:1 ratio. The pots were irrigated with deionized water till germination, with ¼ Hoagland solution (Hoagland and Arnon, 1950) from germination to the 2–3-leaf stage and then with full strength Hoagland solution. In the control treatment, growth chamber temperatures were maintained at 23/18°C day/night throughout the experiment, while for the high temperature (HT) stress treatment temperatures were maintained at 23/18°C day/night till the maximum tillering stage (45 days after germination), and thereafter the temperatures were raised to 35/25°C day/night. On the first day of high temperature treatment (HT), the growth chamber temperature was raised gradually at a rate of approximately 1°C h^{-1} till it attained the required temperature, and was maintained at that temperature for the rest of the treatment duration. Later the plants were directly exposed to the 35/25°C day/night cycle each day. In both treatments a photoperiod of 14 h light (250 μmol m^{-2} s^{-1}) and 10 h dark was maintained. Samples for each estimation were collected from the two genotypes under control and heat stress conditions at the vegetative stage (one week after heat stress treatment), the anthesis stage and 15 days after anthesis.

The experiment was laid out in a completely randomized block design with four replications. The data were analysed by analysis of variance, and least significant differences (LSD) were calculated. The bar in the diagrams represents ± standard deviation. Each data point represents the mean of four replicates analysed twice, and each value is the mean of eight estimations (n=8).

Water potential estimation

Leaf water potential was measured on leaf samples in a pressure chamber (S-pms Instruments, New Delhi, India) following the method of Scholander et al. (1964).

Membrane stability index

The membrane stability index was determined by recording the electrical conductivity of leaf leachates in double distilled water at 40 and 100°C (Sairam et al., 1997). Leaf samples (0.1 g) were cut into discs of uniform size and placed in test tubes containing 10 ml of double distilled water in two sets. One set was kept at 40°C for 30 minutes and another set at 100°C in a boiling water bath for 15 minutes and their respective electric conductivities, C_1 and C_2, were measured with a Conductivity Meter (Century Instruments, Chandigarh, India). The membrane stability index (MSI) was calculated using the equation:

$$MSI = [1–(C_1/C_2)] \times 100$$

Hydrogen peroxide estimation

Hydrogen peroxide was estimated by forming a titanium-hydroperoxide complex (Rao et al., 1997). One g root material was ground with liquid nitrogen and the fine powdered material was mixed with 10 cm^3 cooled acetone in a cold room (10°C). The mixture was filtered through 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-hydroperoxide complex. The reaction mixture was centrifuged at 10,000 rpm for 10 min in a Sigma refrigerated centrifuge (model 3K 30, Osterode, Germany). The precipitate was dissolved in 10 cm^3 2 M H_2SO_4 and then recentrifuged. The supernatant was read at 415 nm against a blank in a UV-visible spectrophotometer (model Specord Bio-200, Analytik Jena, Germany). Hydrogen peroxide contents were calculated by comparison with a standard curve drawn with known hydrogen peroxide concentrations.

Antioxidant enzyme assay

The enzyme extract for SOD, CAT, GR and POX was prepared by grinding 0.5 g leaf sample in the presence of liquid nitrogen and 10 ml of 0.1 M potassium phosphate buffer, pH 7.5, containing 0.5 mM EDTA. For the estimation of ascorbate peroxidase, the extraction buffer was further supplemented with 1 mM ascorbic acid and the pH was adjusted to 7.5. The extract was centrifuged at 15,000 rpm at 4°C for 20 min and the supernatant was used as enzyme.

The reaction mixture for superoxide dismutase estimation consisted of 20 mM methionine, 2.25 mM nitro-blue tetrazolium chloride, 3 mM EDTA, 60 µM riboflavin, 50 mM sodium carbonate, 100 mM phosphate buffer, pH 7.8, and 100 µl enzyme extract. The tubes containing the reaction mixture were kept under two 15 W fluorescent lamps for 15 min. Reaction mixture without the enzyme was used as a control. A sample containing the complete reaction mixture kept in the dark was used as a blank. Absorbance was recorded at 560 nm (Dhindsa et al., 1981). One unit of enzyme activity was taken as the amount of enzyme that reduced the absorbance reading to 50% in comparison with tubes lacking the enzyme.

Ascorbate peroxidase (APX) activity was assayed in a reaction mixture containing 3 mM ascorbic acid, 3 mM EDTA, 3 mM H_2O_2 and 33.33 mM phosphate buffer, pH 7.0. The reaction was started by the addition of H_2O_2, and the decrease in absorbance was recorded for a period of 1 min in a UV-visible spectrophotometer at 290 nm. A complete reaction mixture without ascorbic acid was used as a blank (Nakano and Asada; 1981).

Glutathione reductase activity was estimated by recording the increase in absorbance for 2 min at 412 nm. The reaction mixture contained 30 mM 5,5-dithiobis (2-nitrobenzoic acid), 20 mM oxidized glutathione, 2 mM NADPH, 20 mM phosphate buffer, pH 7.5, and 50 µl enzyme extract. Reaction mixture without oxidized glutathione was used as a blank (Smith et al., 1988).

Catalase (CAT) was assayed by monitoring the decrease in absorbance due to hydrogen peroxide at 240 nm (Aebi, 1984). The reaction mixture consisted of 50 mM potassium phosphate buffer, 12.5 mM hydrogen peroxide, 0.05 ml enzyme, and water to make up the volume to 3.0 ml. Adding H_2O_2 started the reaction and the 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.

Peroxidase was assayed as per Castillo et al. (1984). The reaction mixture contained 10 mM phosphate buffer, pH 6.1, 12 mM hydrogen peroxide, 96 mM guaiacol and 50 µl enzyme extract. The blank contained the complete reaction mixture without H_2O_2. The increase in absorbance was recorded for 2 min at 470 nm.

RNA isolation and gene expression analysis

Total RNA was extracted from the frozen leaf tissue using TRIZOL reagent (Invitrogen). Leaf sample (1 g) was ground in liquid nitrogen, and the powder was added to 10 mL of TRIZOL reagent in a RNAse-free centrifuge tube and incubated at room temperature for 5 min with intermittent vortexing. After adding 2 mL of chloroform, the tubes were shaken for 15 s. After 5 min of incubation at room temperature, the tubes were centrifuged at 12,000 rpm at 4°C for 15 min. The resulting upper aqueous colourless phase was transferred to a new tube, to which 5 ml of isopropanol was added. The contents of the tubes were mixed well, incubated for 10 min at room temperature and then centrifuged at 12,000 rpm at 4°C for 30 min. The resulting pellet was washed with 75% (v/v) ethanol, and the tubes were respun at 10,000 rpm at 4°C for 5 min. After removing the ethanol, the pellet was air-dried for 10 to 15 min at room temperature. The RNA was resuspended in DEPC-treated water. To eliminate DNA from the aqueous RNA extractions, samples of isolated nucleic acid were treated with 10 units of RNase-free DNase I (Qiagen, USA). Total RNA was quantified spectrophotometrically by measuring the absorbance at 260 nm. RNA was fractionated on 1% agarose gel to check the quantity and integrity.

Probes for the gene coding thylakoid-bound *APX* were amplified from wheat var. C 306 by PCR with gene-specific primers. The following gene-specific primers were designed based on the GenBank accession number AF532973:

APX – Forward Primer: GGC ATG ATT CGG GTA CAT ATG

APX – Reverse Primer: CCT GGT CCT CTG CGT ACT TC

For Northern blot analysis equal amounts of total RNA (20 µg) from normal and HT-treated plants of each genotype were fractionated on a 1.2% (w/v) denaturing formaldehyde-agarose gel as described by Sambrook et al. (1989). After electrophoresis, the RNA was capillary-blotted to nylon membranes (Hybond-N, Amersham, Arlington Heights, IL) overnight using 10× SSC. The membranes were UV-crosslinked using a Stratalinker (Stratagene) and prehybridized in 200 mM Na_2PO_4, pH 7.2, 5% (v/v) SDS, 1 mM EDTA, 10 mg/mL bovine serum albumin and 0.1 mg/mL sheared salmon sperm DNA for 4 h at 65°C. Radiolabelled probes were prepared using the HexaLabel™ DNA labelling kit (MBI Fermentas). Blots were probed with denatured ^{32}P-labelled probes added directly to the prehybridization solution at 65°C for 16 h. The blots were washed twice for 15 min at 65°C in 40 mM Na_2PO_4, pH 7.2, 5% (v/v) SDS and 1 mM EDTA, washed again for 15 min at 65°C in 40 mM Na_2PO_4, pH 7.2, 1% (v/v) SDS and 1 mM EDTA and signals were detected by exposure to Kodak X-ray films (Sigma).

Results

Leaf water potential

Water potential (ψ_w) decreased with age in both the genotypes under the two treatments, with the greatest value being recorded at the anthesis stage and the lowest 15 days after anthesis. Water potential also decreased under heat stress compared to the control treatment in both genotypes at all three stages, and the decline in ψ_w was greater in PBW 343 than in C 306 (Table 1).

Table 1
Water potential (MPa) at anthesis and 7 and 15 days after anthesis, in a heat stress-tolerant wheat genotype (C 306) and a heat stress-susceptible wheat genotype (PBW 343) grown under control (23/18°C day/night throughout the experiment) and heat stress conditions (temperature was raised to 35/25°C day/night after the maximum tillering stage)

Treatments	Genotypes	Anthesis	Anthesis+7	Anthesis+15
Control	C306	–1.88	–2.09	–2.31
	PBW 343	–2.18	–2.21	–2.42
Temp. stress	C 306	–2.08	–2.17	–2.37
	PBW 343	–2.57	–2.50	–2.84
CD at 5%	Treatment	0.033	0.038	0.066
	Genotypes	0.041	0.057	0.084
	Treatment × genotypes	0.068	0.084	0.154

Membrane stability index

The membrane stability index (MSI) decreased under heat stress in both the genotypes at all stages of growth, and a greater reduction was observed at the vegetative stage. C 306 maintained higher MSI at all the stages under heat stress compared to PBW 343 (Fig. 1). The percentage reduction in MSI under heat stress compared to normal temperature was 13, 13 and 10% at the vegetative stage, anthesis and 15 days after anthesis in C 306 and 35, 33 and 29% in PBW 343, respectively.

Hydrogen peroxide content

A significant increase in hydrogen peroxide (H_2O_2) content was recorded in both the genotypes in the heat stress treatment at all stages of growth, and the highest content in both genotypes was recorded 15 days after anthesis. However, a greater increase in H_2O_2 content was observed in the heat stress treatment in PBW 343 than in C 306 at all the stages of growth (Fig. 2). The percentage increase in H_2O_2 content under heat stress compared to normal temperature was 10, 5 and 14% at the vegetative stage, anthesis and 15 days after anthesis in C 306 and 25, 37 and 27% in PBW 343, respectively.

Antioxidant enzyme activities

There was a significant increase in all the antioxidant enzyme activities in both genotypes under heat stress conditions at all the stages of growth, but a greater increase was recorded in C 306 compared to PBW 343. Superoxide dismutase (SOD) activity increased significantly in both the genotypes under heat stress at all the growth stages and the highest SOD activity was recorded 15 days after anthesis in C 306 (Fig. 3). C 306 showed 13, 20 and 25% increases in SOD activity at the vegetative stage, anthesis and 15 days after anthesis, respectively, while PBW 343 showed 4, 7 and 11% increases over the control.

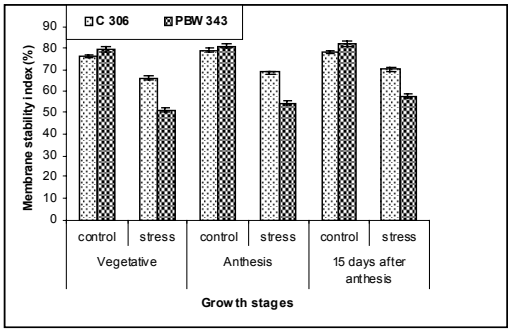

Fig. 1. Membrane stability index (%) at the vegetative stage, anthesis and 15 days after anthesis in heat stress-tolerant (C 306) and susceptible (PBW 343) wheat genotypes grown under control and heat stress conditions (control: temperature 23/18°C day/night throughout the experiment; heat stress: temperature was raised to 35/25°C day/night after the maximum tillering stage). (LSD values at $P \leq 5\%$: treatment = 6.647, genotypes = 3.713, treatment × genotypes = 12.430)

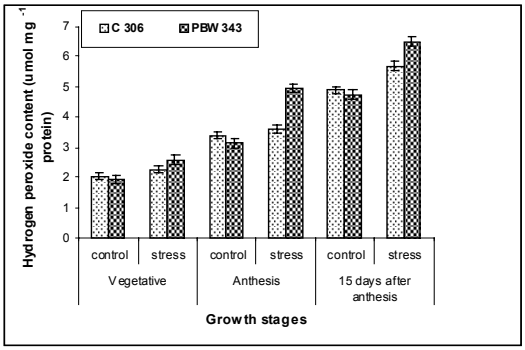

Fig. 2. Hydrogen peroxide content at the vegetative stage, anthesis and 15 days after anthesis in heat stress-tolerant (C 306) and susceptible (PBW 343) wheat genotypes grown under control and heat stress conditions (control: temperature 23/18°C day/night throughout the experiment; heat stress: temperature was raised to 35/25°C day/night after the maximum tillering stage). (LSD values at $P \leq 5\%$: treatment = 0.645, genotypes = 0.846, treatment × genotypes = 1.321)

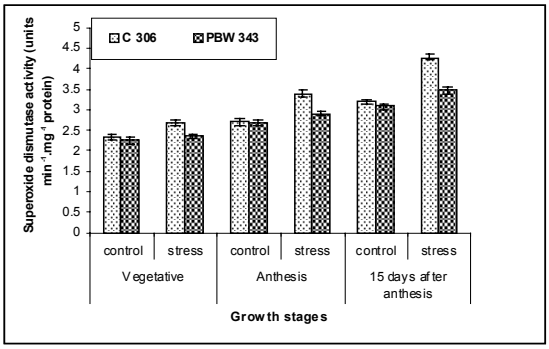

Fig. 3. Superoxide dismutase activity at the vegetative stage, anthesis and 15 days after anthesis in heat stress-tolerant (C 306) and susceptible (PBW 343) wheat genotypes grown under control and heat stress conditions (control: temperature 23/18°C day/night throughout the experiment; heat stress: temperature was raised to 35/25°C day/night after maximum tillering stage). (LSD values at $P \leq 5\%$: treatment = 0.306, genotypes = 0.571, treatment × genotypes = 0.842)

Ascorbate peroxidase (APX) activity also increased under temperature stress in both the genotypes at all stages of growth, and the values were higher in C 306 than in PBW 343 (Fig. 4). Greater APX activity was recorded 15 days after anthesis in both the genotypes. APX activity increased by 26, 35 and 30 % in C 306 at the vegetative stage, anthesis, and 15 days after anthesis, and by 14 and 16% in PBW 343 at the vegetative stage and 15 days after anthesis, respectively, while it decreased in PBW 343 at the anthesis stage by 2% under heat stress compared to control conditions.

There was a significant increase in the glutathione reductase (GR) activity in C 306 under heat stress at all stages of growth, with increases of 41, 35 and 37%, respectively, over the control. The highest GR activity in C 306 was recorded in both treatments 15 days after anthesis (Fig. 5). PBW 343 showed a reduction in the enzyme activity under heat stress compared to normal temperature at all stages of growth, and a greater reduction in enzyme activity was recorded 15 days after anthesis.

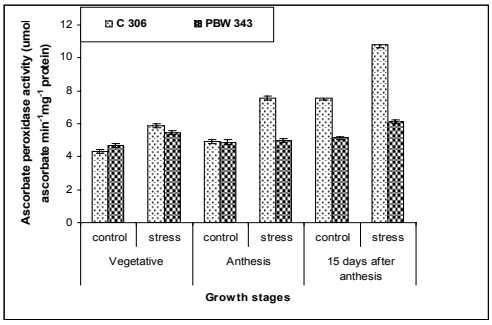

Fig. 4. Ascorbate peroxidase activity at the vegetative stage, anthesis and 15 days after anthesis in heat stress-tolerant (C 306) and susceptible (PBW 343) wheat genotypes grown under control and heat stress conditions (control: temperature 23/18°C day/night throughout the experiment; heat stress: temperature was raised to 35/25°C day/night after the maximum tillering stage). (LSD values at P ≤ 5%: treatment = 0.11, genotypes = 0.328, treatment × genotypes = 0.568)

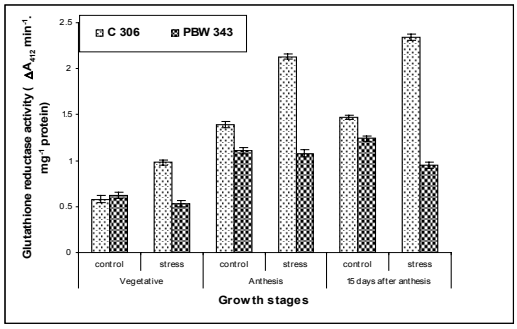

Fig. 5. Glutathione reductase activity at the vegetative stage, anthesis and 15 days after anthesis in heat-stress tolerant (C 306) and susceptible (PBW 343) wheat genotypes grown under control and heat stress conditions (control: temperature 23/18°C day/night throughout the experiment; heat stress: temperature was raised to 35/25 °C day/night after the maximum tillering stage). (LSD values at P ≤ 5%: treatment = 0.132, genotypes = 0.231, treatment × genotypes = 0.421)

Catalase activity also increased significantly in C 306 at all stages of growth under heat stress, the increase being 21, 24 and 24% at the vegetative stage, anthesis and 15 days after anthesis, respectively, over the control, the maximum activity being recorded 15 days after anthesis (Fig. 6). PBW 343 only showed a significant increase in catalase activity at the vegetative stage (8%), while during anthesis and 15 days after anthesis there was a 23 and 11% decline in catalase activity. In PBW 343 under both normal and heat stress conditions comparatively greater catalase activity was recorded at the vegetative and anthesis stages.

Peroxidase activity increased significantly under heat stress in C 306 at all stages of growth, and the maximum activity was observed 15 days after anthesis (Fig. 7). The percentage increase in the enzyme activity under heat stress over the control was 21, 25 and 30%, respectively. Though PBW 343 also showed the highest catalase activity 15 days after anthesis in both the treatments, heat stress only induced an increase in activity over the control at the vegetative stage (1%), while at anthesis and 15 days after anthesis there was a 17 and 24% reduction in the enzyme activity, respectively.

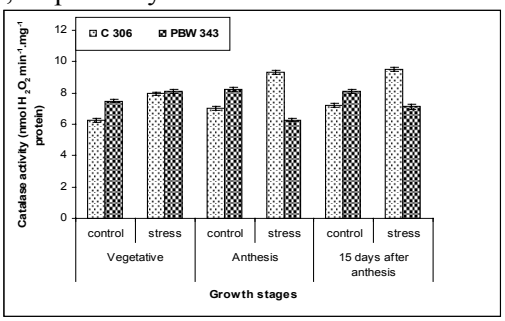

Fig. 6. Catalase activity at the vegetative stage, anthesis and 15 days after anthesis in heat stress-tolerant (C 306) and susceptible (PBW 343) wheat genotypes grown under control and heat stress conditions (control: temperature 23/18°C day/night throughout the experiment; heat stress: temperature was raised to 35/25°C day/night after the maximum tillering stage). (LSD values at $P \leq 5\%$: treatment = 0.432, genotypes = 0.893, treatment × genotypes = 1.012)

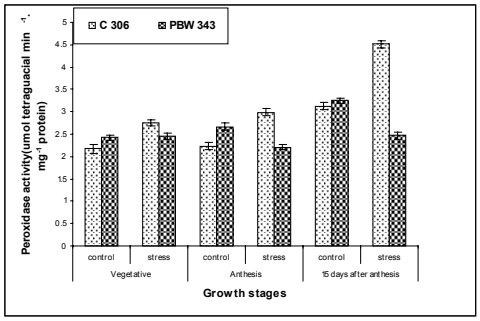

Fig. 7. Peroxidase activity at the vegetative stage, anthesis and 15 days after anthesis in heat stress-tolerant (C 306) and susceptible (PBW 343) wheat genotypes grown under control and heat stress conditions (control: temperature 23/18°C day/night throughout the experiment; heat stress: temperature was raised after maximum tillering stage to 35/25°C day/night). (LSD values at $P \leq 5\%$: treatment = 0.207, genotypes = 0.361, treatment × genotypes = 0.546)

Northern blot analysis

The *APX*-mRNA expression level increased in both the genotypes at the vegetative and anthesis stages in the heat stress treatment, but no significant differences were detected 15 days after anthesis (Fig. 8).

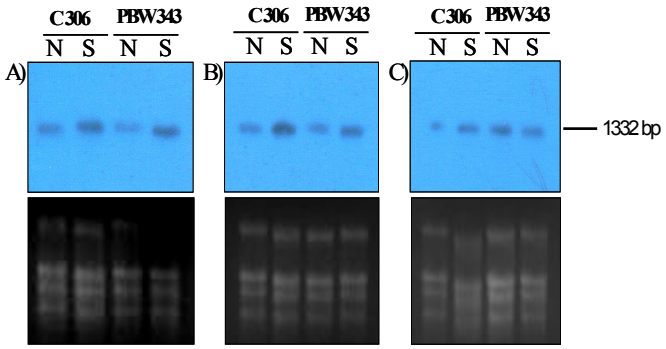

Fig 8. *APX* expression in response to high temperature stress in wheat genotypes differing in their heat tolerance. N: normal temperature (25/18°C day/night), S: high temperature (35/25°C day/night). Plants were exposed to high temperature stress from 45 days after sowing till maturity. Leaf samples from control and high temperature-treated plants were collected at A) vegetative stage (50 days); B) anthesis and C) anthesis+15 days. RNA was isolated and subjected to Northern blot hybridization analysis. The lower panel shows the ethidium bromide stained rRNA bands of the gel used to prepare the above RNA blot

Discussion

Crop plants experience various stresses during their life cycle, such as high or low temperature, drought and salinity, which result in the formation of various reactive oxygen species (ROS) (Sairam et al., 2000). Under unfavourable conditions 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 (Asada, 1996; Singh and Singhal, 2001). In the present study heat stress resulted in a significant increase in hydrogen peroxide content and a decline in water potential and MSI in both the genotypes at all stages. The increase in hydrogen peroxide content and the decline in water potential and MSI were greater in the susceptible genotype PBW 343. Smirnoff and Colombe (1988) suggested that the enhanced rate of hydrogen peroxide formation indicates a decrease in the capacity of the hydrogen peroxide scavenging system. Hydrogen peroxide is a toxic compound produced as a result of scavenging the superoxide radical, and its higher concentration is injurious to the cell/plant, resulting in lipid peroxidation and membrane injury (Pastori and Trippi, 1992; Baisak et al., 1994; Menconi et al., 1995). The peroxidation of membrane lipids has been observed at high temperatures (Mishra and Singhal,

1992; Upadhyaya et al., 1990), which is a symptom of cellular injury. The greater increase in H_2O_2 under high temperature stress in PBW 343 could also be the reason for higher membrane damage (low MSI). Membrane disruption may alter water, ion and organic solute movement, photosynthesis and respiration (Christiansen, 1978).

Hydrogen peroxide, though an injurious oxidant, also serves as a secondary messenger in the stress-induced, ABA-mediated signalling pathway. The role of hydrogen peroxide signalling in the induction of transcription factors associated with the induction of genes coding antioxidant enzymes has been reported by various workers (Pastori and Foyer, 2002; Agarwal et al., 2005). However, it is obvious that the greater stress-induced accumulation of H_2O_2 in PBW 343 has a more inhibitory effect, as redox signalling requires only a micromolar concentration of H_2O_2. Larkindale and Huang (2004) reported the variable influence of putative signalling components such as salicylic acid, abscisic acid, calcium, hydrogen peroxide and ethylene on antioxidant enzyme activity in *Agrostis stolonifera* var. *palustris* under heat stress.

Plants have developed enzymatic and non-enzymatic scavenging systems to quench ROS. When plants are subjected to stresses such as high temperatures, the scavenging system, in terms of antioxidant enzymes and metabolites, is not able to cope with the excess levels of ROS production, resulting in an imbalance in the production and quenching of ROS and consequently in oxidative damage (Price et al., 1989, Bowler et al., 1992; Zhang and Kirkham, 1994).

In the present study both the wheat genotypes showed increases in SOD and APX activity at all stages of growth under heat stress. The increase in SOD activity under heat stress indicates the role of SOD in the scavenging of O_2^- and the protection of the photosynthetic apparatus and demonstrates the crop/genotype's ability to tolerate stress conditions (Foster and Hess, 1982; Smirnoff, 1993). The H_2O_2 scavenging enzyme, APX, removes H_2O_2 efficiently, especially in the chloroplast, where CAT is absent (Groden and Beck, 1979). While the tolerant genotype C 306 showed a significant increase in APX activity under heat stress conditions over the control at all stages of growth, with a maximum 15 days after anthesis, PBW 343 showed only a slight increase in APX activity at the vegetative stage and 15 days after anthesis, and the level of activity was always lower than in C 306.

APX gene expression increased under heat stress conditions at the vegetative and anthesis stages in both the genotypes, though under temperature stress C 306 showed a slight increase in *APX*-gene expression 15 days after anthesis, while PBW 343 showed a decrease in gene expression in spite of the slight temperature-induced increase in APX activity level, which could be due to post-transcriptional changes in APX, resulting in a marginal increase in APX activity 15 days after anthesis.

C 306 showed an increase in the activity of GR, CAT and POX, while, PBW 343 showed a significant reduction in the activities of these three enzymes,

especially in the reproductive stages (anthesis and 15 days after anthesis). This reduction in the activity of enzymes involved in H_2O_2 scavenging could be the reason for the heat stress susceptibility of PBW 343 and could explain the significant damage experienced by the plant under high temperature conditions. GR is an important enzyme as it provides reduced glutathione and thus helps to regenerate ascorbic acid and consequently to continue the Halliwell-Asada Pathway. The decline in GR activity in PBW 343 under temperature stress ultimately affected the Halliwell-Asada Pathway, and thus adversely affected the H_2O_2 scavenging activity. The increase in GR in C 306 could protect its chloroplastic component against oxidation by H_2O_2 and minimize the potential inactivation of SOD within the chloroplasts (Foster and Hess, 1980). Catalase breaks down and detoxifies the H_2O_2 produced in mitochondria and peroxisomes. A reduction in CAT activity has been reported during short-time heat shock (Willekens et al., 1995; Foyer et al., 1997).

Comparatively lower activities of all the antioxidant enzymes in PBW 343 and the temperature-induced decline in the activities of GR, CAT and POX at anthesis and 15 days after anthesis could be attributed to the heat inactivation of these enzymes (Feierabend and Engel, 1986;Polle, 1997) as well as the failure to induce gene expression or enzyme protein synthesis (Lokhande et al., 2003) under stress conditions, which resulted in the accumulation of H_2O_2, leading to greater damage to cell membranes (Dhindsa et al., 1981), as manifested by the lower MSI in PBW 343. Chaitanya et al. (2002) also reported variations in heat stress-induced antioxidant enzyme activities between three mulberry cultivars. The greater heat inactivation of the antioxidant enzyme in the susceptible genotype PBW 343 could also be due to a deficiency in other associated mechanisms, such as osmolytes/compatible solutes, which provide protection to proteins under stress conditions (Chen and Murata, 2002).

On the other hand, the significant increase in all the antioxidant enzymes in C 306 at all stages of growth resulted in reduced oxidative damage to cell membranes under heat stress, which was further reflected in the lower hydrogen peroxide content and the less pronounced decline in MSI.

Finally, it can be concluded that the antioxidant defence mechanism plays an important role in the heat stress tolerance of wheat genotypes. The susceptibility of PBW 343 can be attributed to the lower activity of antioxidant enzymes in general and to the heat stress-induced decrease in GR, CAT and POX activities, resulting in enhanced oxidative stress, leading to damage to membranes and cellular structures, and consequently to plant growth.

References

Aebi, H. (1984): Catalase *in vitro*. *Method. Enzymol.*, **105**, 121–126.

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

Al-Khatib, K., Paulsen, G. M. (1999): High temperature effects on photosynthetic processes in temperate and tropical cereals. *Crop Sci*., **39**, 119–125.

Almeselmani, M. (2006): *Studies on the mechanism of high temperature stress tolerance in wheat* (*Triticum aestivum* L.) *genotypes*. Ph.D. Thesis, Indian Agricultural Research Institute, New Delhi, India.

Asada, K. (1996): Radical production and scavenging in chloroplasts. pp. 123–150. In: Baker, N. (ed.), *Photosynthesis and the Environment*. Kluwer Academy Press, Dordrecht.

Badiani, M., Schenone, G., Paolacci, A., Artemi, F. (1994): Daily fluctuations of antioxidant in bean (*Phaseolus vulgaris* L.) leaves. The influence of climatic factors. *Agrochimica*, **38**, 25–36.

Baisak, R., Rana, D., Acharya, P. B. B., Kar, M. (1994): Alteration in the activities of active oxygen scavenging enzymes of wheat leaves subjected to water stress. *Plant Cell Physiol.*, **35**, 489–495.

Bowler, C., Van Montagu, M., Inze, D. (1992): Superoxide dismutase and stress tolerance. *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, **43**, 83–116.

Castillo, F. I., Penel, I., Greppin, H. (1984): Peroxidase release induced by ozone in *Sedum album* leaves. *Plant Physiol.*, **74**, 846–851.

Chaitanya, K. V., Sundar, D., Masilamani, S., Ramachandra Reddy, A. (2002): Variation in heat stress-induced antioxidant enzyme activities among three mulberry cultivars. *Plant Growth Regul.*, **36**, 175–180.

Chen, T. H. H., Murata, N. (2002): Enhancement of tolerance of abiotic stress by metabolic engineering of betaines and other compatible solutes. *Curr. Opin. Plant Biol*., **5**, 250–257.

Christiansen, M. N. (1978): The physiology of plant tolerance to temperature extremes. pp. 173–191. In: Jung, G. A. (ed.), *Crop Tolerance to Suboptimal Land Conditions*. American Soc. Agron., Wisconsin.

Dat, J. F., Lopez-Delgado, H., Foyer, C. H., Scott, I. M. (1998): Parallel changes in H_2O_2 and catalase during thermotolerance induced by salicylic acid or heat-acclimation in mustard seedlings. *Plant Physiol.*, **116**, 1351–1357.

Davidson, J. E., Whyte, B., Bissinger, P. H., Schiestl, R. H. (1996): Oxidative stress is involved in heat induced cell death in *Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA*, **93**, 5116–5121.

Dhindsa, R. S., Dhindsa, P. P., Thorpe, T. A. (1981): Leaf senescence: Correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot.*, **32**, 93–101.

Feierabend, J., Engel, S. (1986): Photo-inactivation of catalase *in vitro* and in leaves. *Arch. Biochem Biophys*., **251**, 567–576.

Fisher, R. A., Byerlee, D. R. (1990): Trends of wheat production in the warmer areas: Major issues and economic considerations. pp. 3–27. In: Saunders, D. A. (ed.), *Wheat for Nontraditional, Warm Areas*. CIMMYT, Mexico, DF.

Foyer, C. H., Lopez-Delgado, H., Dat, J. F., Scott, I. M. (1997): Hydrogen peroxide- and glutathione-associated mechanisms of acclimatory stress tolerance and signaling. *Physiol. Plant*., **100**, 241–254.

Foster, J. G., Hess, J. L. (1980): Responses of superoxide dismutase and glutathione reductase activities in cotton leaf tissue exposed to an atmosphere of enriched oxygen. *Plant Physiol.,* **66**, 482–487.

Foster, J. G., Hess, J. L. (1982): Oxygen effects on maize leaf superoxide dismutase and glutathione reductase. *Phytochemistry*, **21**, 1527–1532.

Groden, D., Beck, E. (1979): H_2O_2 destruction by ascorbate-dependent system from chloroplast. *Bichim. Biophys. Acta*, **546**, 426–435.

Gupta, A. S., Webb, R. P., Holaday, A. S., Allen, R. D. (1993): Overexpression of superoxide dismutase protects plants from oxidative stress. (Induction of ascorbate peroxidase in superoxide dismutase overexpressing plants). *Plant Physiol.*, **103**, 1067–1073.

Hoagland, C. R., Arnon, D. I. (1950): The solution-culture method for growing plants without soil. *Calif. Agric. Exp. Circ.,* **347**, 1036–1043.

Jagtap, V., Bhargava, S. (1995): Variation in the antioxidant metabolism of drought tolerant and drought susceptible varieties of *Sorghum bicolor* (L.) Moench. exposed to high light, less water and high temperature stress. *J. Plant Physiol*., **145**, 195–197.

Jiang, Y., Huang, B. (2001): Effects of calcium on antioxidant activities and water relations associated with heat tolerance in two cool season grasses. *J. Exp. Bot*., **52**, 341–349.

Kocsy, G., Laurie, R., Szalai, G., Szilágyi, V., Simon-Sarkadi, L., Galiba, G., de Ronde, J. A. (2005): Genetic manipulation of proline levels affects antioxidants in soybean subjected to simultaneous drought and heat stresses. *Physiol. Plant*., **124**, 227–235.

Larkindale, J., Knight, M. R. (2002): Protection against heat stress-induced oxidative damage in *Arabidopsis* involves calcium, abscisic acid, ethylene, and salicylic acid. *Plant Physiol*., **128**, 682–695.

Larkindale, J., Huang, B. (2004): Thermotolerance and antioxidant systems in *Agrostis stolonifera*: Involvement of salicylic acid, abscisic acid, calcium, hydrogen peroxide, and ethylene. *J. Plant Physiol.*, **161**, 405–413.

Liu, X., Huang, B. (2000): Heat stress injury in relation to membrane lipid peroxidation in creeping bentgrass. *Crop Sci*., **40**, 503–510.

Lokhande, S. D., Ogawa, K., Tanaka1, A., Hara, T. (2003): Effect of temperature on ascorbate peroxidase activity and flowering of *Arabidopsis thaliana* ecotypes under different light conditions. *J. Plant Physiol.*, **160**, 57–64.

Menconi, M., Sgherri, C. L. M., Pinzino, C., Navari-Izzo, F. (1995): Activated oxygen production and detoxification in wheat plants subjected to a water deficit programme. *J. Exp. Bot.*, **46**, 1123–1130.

Mishra, R. K., Singhal, G. S. (1992): Function of photosynthetic apparatus of intact wheat leaves under high light and heat stress and its relationship with thylakoid lipids. *Plant Physiol*., **98**, 1–6.

Nakano, Y., Asada, K. (1981): Hydrogen peroxide is scavenged by ascorbate specific peroxides in spinach chloroplasts. *Plant Cell Physiol*., **22**, 867–880.

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

Pastori, G. M., Trippi, V. S. (1992): Oxidative stress induces high rate of glutathione reductase synthesis in a drought resistant maize strain. *Plant Cell Physiol.*, **33**, 957–961.

Pastori, G. M., Foyer, C. H.(2002): Common components, networks, and pathways of cross-tolerance to stress. The central role of 'redox' and abscisic acid-mediated controls. *Plant Physiol.*, **129**, 7460–7468.

Polle, A. (1997): Defense against photo-oxidative damage in plants. pp. 785–813. In: Scandalios, J. (ed.), *Oxidative Stress and The Molecular Biology of Antioxidant Defence*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Price, A. H., Atherton, N. M., Hendry, G. A. F. (1989): Plants under drought-stress generated activated oxygen. *Free Radical Res. Commun*., **8**, 61–66.

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

Rui, R. L., Nie, Y. Q., Tong, H. Y. (1990): SOD activity as a parameter for screening stress tolerant germplasm resources in sweet potato (*Ipomoea batatas* L.). *Jiangsu J. Agr. Sci.*, **6**, 52–56.

Sairam, R. K. (1994): Effect of moisture stress on physiological activities of two contrasting wheat genotypes. *Indian J. Expt. Biol.*, **32**, 594–597.

Sairam, R. K., Deshmukh, P. S., Shukla, D. S. (1997): Tolerance of drought and temperature stress in relation to increased antioxidant enzyme activity in wheat. *J. Agron. Crop Sci.*, **178**, 171–178.

Sairam, R. K., Deshmukh, P. S., Saxena, D. C. (1998): Role of antioxidant systems in wheat genotypes tolerance to water stress. *Biol. Plant.*, **41**, 387–394.

Sairam, R. K., Srivastava, G. C., Saxena, D. C. (2000): Increased antioxidant activity under elevated temperature: a mechanism of heat stress tolerance in wheat genotypes. *Biol. Plant.*, **43**, 245–251.

Sambrook, J., Fritsch, E. F., Maniatis, T. (1989): *Molecular Cloning: A Laboratory Manual*, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Scholander, P. L., Hammel, H. T., Bradstreet, E. D., Hemmingsen, E. Q. (1964): Hydrostatic pressure and osmotic potential in leaves of mangroves and some other plants. *Proc. Nat. Acad. Sci. USA*, **52**, 119–125.

Singh, A. K., Singhal, G. S. (2001): Effect of irradiance on the thermal stability of thylakoid membrane isolated from acclimated wheat leaves. *Photosynthetica*, **39**, 23–27.

Smirnoff, N. (1993): The role of active oxygen in the responses of plants to water deficit and desiccation. *New Phytol.*, **125**, 27–58.

Smirnoff, N., Colombe, S. V. (1988): Drought influences the activity of enzymes of the chloroplast hydrogen peroxide scavenging system. *J. Exp. Bot.*, **39**, 1097–1108.

Smith, I. K., Vierheller, T. L., Thorne, C. A. (1988): Assay of glutathione reductase in crude tissue homogenates using 5,5'-dithiobis (2-nitrobenzoic acid). *Anal. Biochem.*, **175**, 408–413.

Upadhyaya, A., Davis, T. D., Larsen, N. H., Walser, R. H., Sankhla, N. (1990): Uniconazole-induced thermotolerance in soybean seedling root tissue. *Physiol. Plant.*, **79**, 78–84.

Willekens, H., Inze, D., Van Montagu, M., Van Camp, W. (1995): Catalase in plants. *Mol. Breeding*, **1**, 207–228.

Zhang, J. X., Kirkham, M. B. (1994): Drought-stress-induced changes in activities of superoxide dismutase, catalase, and peroxidase in wheat species. *Plant Cell Physiol.*, **35**, 785–791.

Zhau, R. G., Fan, Z. H., Li, X. Z., Wang, Z. W., Han, W. (1995): The effect of heat acclimation on membrane thermo-stability and reactive enzyme activity. *Acta Agron. Sin.*, **21**, 568–572.

Corresponding author: R. K. Sairam
E-mail: rks_ppl@yahoo.co.uk