Plant Stress ©2013 Global Science Books

Antioxidant Potential and Expression of *Lea* Genes under Heat Stress in Two Wheat Cultivars Differing in Heat Tolerance

Manvir Kaur • Priyanka Bhagi • Anil Kumar Gupta • Vikramjit Kaur Zhawar*

Department of Biochemistry, College of Basic Sciences and Humanities, Punjab Agricultural University, Ludhiana, 141004 India

Corresponding author: * **vikram97jit@yahoo.com**

ABSTRACT

Two wheat (*Triticum aestivum* L.) cultivars differing in heat tolerance, 'C306' (heat tolerant) and 'PBW343' (heat susceptible) were compared for their growth, antioxidant response and expression of late embryogenesis abundant (LEA) genes under heat stress (37°C) given to 4-day old seedlings. Dry biomasses and water contents in shoots and roots of 'C306' were comparatively less affected under heat stress than of 'PBW343'. Root dry mass to shoot dry mass ratios were improved by higher amount in 'C306' than in 'PBW343' under stress. Decline in proteins contents of roots under heat stress was higher in 'PBW343' than 'C306'. Ascorbate contents and ascorbate to dehydroascorbate ratios were comparatively less affected in shoots and roots of 'C306' than of 'PBW343' under stress. 'PBW343' roots showed higher levels of malondialdehyde (MDA) under prolonged stress. Dehydroascorbate and proline contents did not increase during heat stress in shoots and roots of both cultivars. Activities of antioxidant enzymes were maintained in shoots of 'C306' but affected in shoots of 'PBW343' during the stress period. In roots of both cultivars, levels of antioxidant enzymes were decreased under heat stress. Glutathione-*S*-transferase-F (GST-F, member of phi class) was induced only in C306, GST-μ (member of tau class) was not induced in both cultivars, glutathione-peroxidase (GPX) was induced by same amount in both cultivars, peroxisomal-ascorbate peroxidase was induced only in PBW343 under heat stress. Some of LEA group 2 and group 4 genes were induced while of group 3 genes were not induced under heat stress. Among group 2, *Wcor410c* was induced mainly in C306, *Td16* was induced mainly in PBW343, *Td27e* was induced higher in 'C306', *Wdhn13* was not induced in both cultivars under heat stress. LEA group4 *Td29* was induced higher in 'C306' than in 'PBW343' under heat stress.

Keywords: antioxidant, ascorbate, heat stress, LEA genes, *Triticum aestivum*
Abbreviations: DEPC, diethyl polycarbonate; **DW**, dry weight; **EST**, expressed sequence tag; **HSR**, heat stress resistance; **LEA**, Late Embryogenesis Abundant; **MDA**, malondialdehyde

INTRODUCTION

Heat stress is reported as one of the most important causes of reduction in yield and dry matter production in many crops including cereals like wheat and maize (Wahid *et al.* 2007). Multiple signalling pathways are implicated for heat stress resistance, some of which control heat shock proteins (HSPs) whereas others control the production of diverse effector components. Heat stress like other environmental stresses causes accumulation of reactive oxygen species (ROS), that could act as signal to trigger thermotolerance (Kotak *et al.* 2007; Mittler *et al.* 2012). Oxidative stress produced under heat stress is correlated with acquisition of thermotolerance (Maestri *et al.* 2002; Sairam and Tyagi 2004; Kotak *et al.* 2007).

Heat stress singly or in combination with drought is a common constraint during anthesis and grain filling stages in wheat as well as in other crops (Wahid *et al.* 2007). In wheat, both grain number and grain yield are affected by heat stress (Ferris *et al.* 1998). Other plant developmental stages are also vulnerable to high temperatures though response may vary, for example germination of cereal seeds is not thermosensitive rather thermotolerant but seedling growth and vigour are temperature sensitive. In wheat, soil temperature of up to 45°C at sowing is reported to be tolerant but mass of seedlings decline with temperatures above 20°C and this leads to reduced final yield even when rest of the season's temperatures are optimum (Maestri *et al.* 2002). Seedling development represents one of two key stages (other one is flowering) affected by heat stress and where tolerance really matters (Walbot 2011). Germinating plant seedlings constitute an excellent plant material to study the effect of not only heat stress but all environmental stresses as this stage is the most vulnerable stage to metabolic fluctuations and so highly sensitive to stresses (Rosa *et al.* 2009; Walbot 2011). Many studies have been done to compare cultivars for antioxidant capacity under heat stress in wheat so as to find marker for stress tolerance and capacity of antioxidant system has been correlated with the capacity to acquire thermotolerance as studied in leaves and flowers before and after anthesis (summarised in **Table 1**) but very rarely studied in seedlings and specially shoots and roots separately. Root systems play critical roles in whole plant adaptation to heat stress but are less studied (Huang *et al.* 2012). The present study was planned to analyse antioxidant metabolism in roots and shoots of germinating seedlings at 24, 48 and 72 h after heat stress given to 4-day old plants of two cultivars 'C306' and 'PBW343'. Purpose of this study was also to compare these two cultivars which were contrasting in drought and heat tolerance as well as in ABA sensitivity where 'C306' cultivar was ABA-higher sensitive-cum-drought and heat tolerant and 'PBW343' was ABA-lesser sensitive-cum-drought-and heat susceptible. These cultivars were already compared under ABA-supply, water stress, salt stress for same parameters in our laboratory (Bhagi 2011; Kaur *et al.* 2012), so comparing under heat stress may also indicate common pathway under all stresses or ABA-specific pathways working under heat stress. Dehydrins/LEA-proteins are reported to play important role under water stress, cold and salt stress but very rarely studied under heat stress (Wahid and Close 2007). In this study, we also studied the expression of group 2, group

Received: 2 December, 2012. Accepted: 20 December, 2012.

Original Research Paper

Table 1 Summary of important studies related to heat stress in wheat (*Triticum aestivum*).

Plant material	Cultivars	Result	Reference
Flag leaf at 8 days after anthesis (DAA) and 23 DAA in normal/late sowing.	C306, HD2285, HD2329	• Relative Water Content (RWC), ascorbate, ascorbate peroxidase (APX), catalase (CAT) were higher and malondialdehyde (MDA), H_2O_2 were lesser in resistant cultivars than susceptible cultivars under heat stress.	Sairam *et al.* 2000
Leaves of wheat seedlings at 40°C versus 25°C	HD2307, HD2327, HD2402, HD2329, HD2643, C306, PBN51, HD1553	• Photosynthesis was decreased under heat stress due to decline in photosystem (PS)-II quantum efficiency. • Evaluation of cultivars based on photosynthetic activity is HD2307, HD2327 as thermotolerant, HD2329, C306, HD2404 as moderately thermotolerant, HD1553, HD2643 as thermotolerance deficient, PBN51 as thermosensitive.	Dash and Mohanty 2001
Foliar leaves of seedlings at 30, 35, 40°C versus 25°C	C306, HD2329, HD1553, HD2307	• Heat stress during greening led to inactivation of PS-II and high turn over rate of PS-I and SOD, so produces higher level of superoxides and H_2O_2 which are dissipated by APX and CAT. • Resistant cultivars have higher APX and CAT to dissipate H_2O_2 and ameliorate photooxidative damage as compared to susceptible cultivars.	Dash and Mohanty 2002
Vegetative, anthesis and 15 DAA stages in normal/late sowing of crop.	PBW343, PBW175, HDR 77, HD2815, HD2865	• SOD, APX, CAT increased but GR and POX decreased under heat stress. • Resistant cultivars showed relative higher levels of SOD, APX, GR, CAT, POX; lesser reduction in chlorophyll and lesser membrane injury than susceptible cultivars under heat stress	Almeselmani *et al.* 2006
Leaves of plants at 22/22°C, 22/38°C, 38/38°C	Fang, Siete Cerros, Imam)	• Antioxidant enzymes were increased under heat stress. • Heat tolerant genotypes maintained higher SOD, APX, GR than susceptible genotypes under heat stress.	Badawi *et al.* 2007
Flag leaves at 7, 14, 21, 28, 35 DAA at 34/22°C, 32/24°C, 26/14°C, 24/16°C	Yangmai 9 and Xuzhou 26	• SOD and CAT first increased then declined under heat stress. • MDA increased and soluble protein, grain-filling rate and grain weight decreased under heat stress. • Response of both cultivars to heat stress appeared to be different from each other.	Zhao *et al.* 2007
35/20°C day/night during grain filling	Plainsman V, Mv Magma	• Yield, biomass, grain number, grain weight were reduced under heat stress • Glutathione-*S*-transferase (GST), APX, CAT were enhanced in Plainsman V while GST and CAT were enhanced in Mv Magma under heat stress • Heat tolerance of Mv Magma was related to higher antioxidant level in Mv Magma over Plainsman V under heat stress.	Balla *et al.* 2009
Leaves at vegetative, anthesis 7, 15 DAA at 35/25°C vs 23/18°C (day/night)	C306, PBW343	• Membrane stability index, SOD, APX, CAT, GR, POX were higher in C306 than PBW343 under heat stress. • Higher increases in APX-mRNA in C306 over PBW343 under heat stress.	Almeselmani *et al.* 2009
Vegetative, pollination, milky dough and seed hardening at 30, 35, 40°C versus 25°C	PBW343, C306	• More isoforms of APX was found in C306 than in PBW343 under heat stress.	Kumar *et al.* 2011
Flag leaves of plants with/without pre-anthesis-high-temperature-acclimation exposed to post-anthesis heat stress	Yangmai 9	• Plants with pre-anthesis-high-temperature-acclimation showed higher photosynthetic rate, cholorophyll *a*/*b* ratio, Cu/Zn SOD, MnSOD, GR than plants without such acclimation during post-anthesis heat stress.	Wang *et al.* 2011
Leaves of wheat seedlings	HW2045, WH1021, HS277, WH147	• CAT, POX, GR, APX increased under heat stress and increases were higher in tolerant cultivars over susceptible cultivars. • SOD increased only in tolerant cultivars but declined in susceptible cultivars under heat stress. • H_2O_2 and MDA related to each other and higher in susceptible cultivars over tolerant cultivars.	Mahla *et al.* 2012
Vegetative, anthesis and 15 DAA stages at 35/25°C versus 23/18°C	C306, PBW343	• Leaf chlorophyll, photosynthetic rate, RUBISCO activity were decreased in both cultivars under heat stress but reduction was more in susceptible cultivar than tolerant cultivar. • Expression of *rbcS*, *rbcL* and *HSP101* was maintained higher under heat stress in tolerant over susceptible cultivar.	Almeselmani *et al.* 2012
Leaves of seedlings at 45°C versus 25°C	Inquilab91, Sitta, Nesser, Sarsabz, Fareed, FD-83	• MDA, programmed cell death, POX, SOD, APX, protease were increased but CAT was decreased under heat stress. • CAT, APX and protease are efficient biochemical markers to assess heat tolerance in wheat genotypes.	Hameed *et al.* 2012

3 and group 4 LEA genes in shoots of both cultivars at 48 h after stress.

MATERIALS AND METHODS

Chemical and plant materials

Chemicals used in this study, were purchased from different companies like acetic acid, ascorbic acid, chloroform, diethylpyrocarbonate (DEPC), dinitrophenyl hydrazine (DNPH), EDTA, ferric chloride, Folin-Ciocalteau, glutathione oxidized (GSSG), guaiacol, isopropanol, lithium chloride, NADPH, ninhydrin, polyvinylpyrrolidone (PVP), pyrogallol, potassium phosphate, potassium iodide, proline, 5-sulphosalicylic acid, sodium hydroxide, sodium carbonate, triton-X-100, thiourea, tricholoroacetic acid (TCA), toluene were purchased from SRL Pvt. Ltd. (Mumbai, India); bipyridyl, H_2SO_4, thiobarbituric acid (TBA) were purchased from Himedia Laboratories Pvt. Ltd. (Mumbai, India); hydrogen peroxide, ortho-phosphoric acid from Fisher-Scientific (Mumbai, India); Oligo$(dT)_{18}$ and agarose were purchased from G-Biosciences Pvt. Ltd. (Noida, India); TRIsoln, M-MuLV Reverse Transcriptase, Human Placental RNase Inhibitor, *Taq* DNA Polymerase, DNase I , dNTPs were purchased from GeNei Pvt. Ltd. (Banglore, India). Primers used in present study were obtained synthesized by Sigma Aldrich Chemicals Pvt. Ltd. (Banglore, India).

The experiments were conducted on roots and shoots of seedlings of wheat (*Triticum aestivum*) cultivars 'PBW343' (heat susceptible) and 'C306' (heat tolerant). Seeds were collected from Department of Plant Breeding and Genetics, P. A. U. Ludhiana (India) and sterilized in 0.1% mercuric chloride solution, washed

and incubated at 25°C overnight for stratification. Seeds were then placed over moistened filter paper in sterilized Petri dishes. Autoclaved distilled water was used to moisten filter paper. Plates were kept in the dark at 25°C for 4 days. During this period, seeds were rewatered regularly using autoclaved distilled water. Stress was applied on 4th day, where 37°C was used for heat stress samples while control seedlings were kept at 25°C. Plates were similarly incubated in the dark for another 1-3 days. Data was collected from 4 stages; 0, 24, 48, 72 h after stress treatment.

Growth measurement

Growth was measured in dry masses and lengths of shoots and roots. For dry masses, fresh roots and shoots of 25 seedlings were weighed separately in triplicates. Fresh tissues were subjected to drying for 24 h at 80°C in oven and their DW were recorded. Water contents were measured in the form of ratio by using following formula: Water contents = FW-DW/DW where FW is fresh weight and DW is dry weight. Lengths of shoots and roots were measured in cm with scale for 50 seedlings selected randomly.

Antioxidant enzymes

Antioxidant enzymes were extracted and measured as in Kaur *et al.* (2012). In brief, tissue (shoot and root) was homogenized in 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA, 2% PVP, 0.05% triton-X-100. Homogenate was centrifuged at 10000 × *g* at 4°C for 15 min. APX (ascorbate peroxidase) was assayed in reaction volume of 2.5 ml of 50 mM potassium phosphate buffer of pH 7.0, 0.1 mM EDTA, 0.3 mM ascorbate, 1 mM H_2O_2 to which enzyme extract was added. Change in absorbance was recorded at 290 nm at 25°C. Decrease in µmole of ascorbate per minute was calculated using molar extinction coefficient of ascorbate of 2.8 mM^{-1} cm^{-1}. CAT (catalase) assay was performed at 25°C in reaction volume of 2.5 ml of 50 mM potassium phosphate buffer of pH 7.0 and 25 mM H_2O_2, to which enzyme extract was added. Change in absorbance was recorded at 240 nm. Change in mmole of H_2O_2 per minute was calculated using molar extinction coefficient of H_2O_2 of 0.0394 mM^{-1} cm^{-1}. GPOX (guaiacol peroxidase) was assayed in reaction volume of 2.5 ml of 100 mM potassium phosphate buffer of pH 6.5, 50 mM guaicol, 32 mM H_2O_2, to which enzyme extract was added. Change in absorbance at 470 nm was recorded at 25°C. Change in mmole of tetraguaicol per minute was calculated using molar extinction coefficient of tetraguaicol of 26.6 mM^{-1} cm^{-1}. GR (glutathione reductase) was assayed at 25°C in a reaction volume of 3 ml containing 50 mM potassium phosphate buffer (pH 7.0), 0.7 mM GSSG, 0.07 mM NADPH and reaction was started by adding enzyme extract. Change in absorbance was recorded at 320 nm. Decrease in µmole of $NADPH+H^+$ min^{-1} was calculated using molar extinction coefficient of NADPH (6.22 mM^{-1} cm^{-1}).

All enzymes were extracted in triplicate.

Antioxidants and other related metabolites

These were extracted from roots and shoots and measured as in Kaur *et al.* (2012). H_2O_2 was extracted in 0.1% TCA and was estimated by reacting 1 ml of supernatant with 2 ml of reaction mixture of 2M potassium iodide and 50 mM potassium phosphate buffer of pH 7.0 at room temperature in dark for 1 h. Absorbance was read at 390 nm. Ascorbate was extracted in 5% TCA and estimated by reacting 0.5 ml of sample with 2 ml of mixture containing 1% H_3PO_4, 0.05% $FeCl_3$, 0.25% bipyridyl in ethanol at 37°C for 40 min. Absorbance was measured at 525 nm. Dehydroascorbate was extracted in 5% metaphosphoric acid containing 1% thiourea and estimated by incubating 1 ml of sample with 1 ml of reagent (containing 2% DNPH, 0.4% thiourea, 0.05% $CuSO_4 \cdot 5H_2O$ in 9N H_2SO_4) at 37°C for 3 h. Test tubes were incubated at room temperature for another 30 min after cooling on ice bath and adding 5 ml of 85% H_2SO_4 (cold). The content was read at 530 nm. Proline was extracted in 3% sulphosalicylic acid and estimated by incubating at 100°C for 1 h of 1 ml of sample mixed with 1 ml of acid ninhydrin reagent (0.31 g ninhydrin, 7.5 ml acetic acid, 5 ml 6M phosphoric acid) and 1 ml of glacial acetic acid. Test tubes were placed at room temperature for phase separation after cooling on ice and adding 4 ml of toluene. The pink-red colored upper layer was read at 520 nm. MDA was extracted in 0.1% TCA and estimated by mixing 1 ml of sample with 4 ml of solution containing 0.5% TBA and 20% TCA and incubating at 100°C for 30 min. Contents were read at 532 nm and 600 nm after centrifugation. Absorbance at 600 nm was subtracted from absorbance at 532 nm. The MDA content was calculated by using molar extinction coefficient of malondialdehyde of 155 mM^{-1} cm^{-1}.

Protein content was measured (Lowry *et al.* 1951) where 0.5 ml of sample was mixed and reacted to 2.5 ml of reagent C (A: B in 50 : 1 where A was 2% Na_2CO_3 in 0.1N NaOH and B was 0.5% $CuSO_4$ in 1% sodium potassium tartrate) at room temperature for 10 min then 0.25 ml of reagent D (Folin-Ciocalteau phenol reagent: water in 1:1) was added and incubated for another 30 min. Absorbance was read at 530 nm.

All contents were extracted in triplicate.

Statistical analysis

Mean ± SD was calculated. Data was analyzed by the Student's *t*-test at $P \leq 0.05$ to test for statistical differences between heat stressed sample and control.

Semi-quantitative-RT-PCR

Antioxidant and LEA genes were analyzed by semi-quantitative RT-PCR. Database was searched for the availability of the gene sequences in wheat at NCBI (www.ncbi.nlm.nih.gov/). Primers were designed manually as well as with Primer-BLAST software available at NCBI. Primer sequences corresponding to each gene are given in **Table 2**.

Total RNA was extracted in TRIsoln then adding chloroform for phase separation. RNA was precipitated from aqueous phase using isopropanol and dissolved in DEPC-treated water. RNA was treated with DNase I to remove DNA and precipitated in 3.5 M LiCl, then dissolved in DEPC-treated water. RNA was estimated at 260/280 nm. For reverse transcription, total RNA along with oligodT was denatured at 72°C and then incubated at 42°C for 1 h after mixing with 500 µM of each dNTP, 20 U of Ribonuclease inhibitor, 70 U of M-MuLV Reverse Transcriptase in 1X transcription buffer. PCR was performed in 1X buffer containing 1.5 mM $MgCl_2$, 200 µM of each dNTP, 0.4% PVP, 25 ρmole of each primer and 5 U of *Taq* Polymerase using 35 cycles of 40 sec at 94°C/40 sec at 55°C/1-2 min at 72°C/final extension of 10 min. RT-PCR of cytosolic glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. PCR products were visualized and measured using Gel Doc^{TM}XR (BioRad Laboratories, Inc). Ratio for each gene in each sample was calculated by dividing intensity of band of gene by intensity of band of GAPDH.

RESULTS

Effect heat stress on growth and protein content

Dry matter was declined significantly at 48 h and 72 h of stress in shoots of 'PBW343' (**Table 3A**) from corresponding control values. In roots of 'PBW343', dry mass was not altered significantly at 24 h and 48 h but declined significantly at 72 h of stress (**Table 3A**). Dry matter was increased significantly at 24 h and then decreased non-significantly at 48 h and significantly at 72 h of stress in shoots of 'C306' (**Table 3A**). In roots of 'C306', dry matter was increased non-significantly at all three stages of stress (**Table 3A**). Ratios of root dry matter to shoot dry matter were increased significantly at 48 h and 72 h of stress in 'C306' but unaltered in 'PBW343' from their control values (**Table 3A**). Dry matter accumulation was more affected in shoots than of roots in both cultivars under heat stress.

Shoot lengths were affected significantly under heat stress in both cultivars except in 'C306' at 24 h stage where it was not decreased significantly from corresponding control value (**Table 3B**). Root lengths were not altered significantly under heat stress in both cultivars except in 'C306' at 24 h stage where it was decreased significantly from cor-

Table 2 List of genes along with their primer sequences used in the present study.

Late Embryogenesis Abundant (LEA) genes		
Gene Id (Accession No.)	**Gene name**	**Primer sequences (5' to 3')**
LEA Group 2		
AB076807	*Wdhn13*	F: CAGCACACCACTGGAATGAG R: AACGAAAACCCTCGACACAC
AL815683	99% similar to *Td27e* (X78431) of durum wheat	F: AAAGCCACAACCAAGTCCAG R: GTAGGCTCCACCAGTTCCAG
CV762802	98% similar to *Td16* (X78429) of durum wheat	F: GCCAAGTGAGCAAGACAACA R: ATGACCTTGCTGTCCGTAGG
U73211	*Wcor410c*	F: CGAGGAGGAGAAGAAAGGCT R: CTCCCACCTTGACACCAACT
LEA Group4		
GH729039	95% similar to *Td29* (AJ890139) of durum wheat	F: ATTATTACGCCGTGCACACA R: CTCGACATACCGGTGAAGGT
Antioxidant genes		
EF555121	peroxisomal ascrobate peroxidase (P-APX)	F: TAGGTCGTCCGCGATGGCGG R: CCCCTTACTTGCTCCTCTTGC
GR304984	GPX EST (cytosolic glutathione peroxidase)	F: GAATCTCGCCATGGCCGCC R: CCGATTTCTCCAGACGCTCC
AJ440796	GST-F (glutathione-*S*-transferase from phi family)	F: AGCGGCTTTACCTACCGAG R: TTCGCGACGAACGAGCACC
Constitutive gene as internal control		
EF592180	Cytosolic glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	F: GGATGCTCCCATGTTTGTCT R: CCTGTTGTCACCCTGAAAGT

Table 3 Effect of heat stress (HS) on dry weight (mg per 25 seedlings), R/S ratio (root dry weight to shoot dry weight ratio) in **A**, length (cm per seedling) and water content (in the form of ratio of FW-DW/DW where FW is fresh weight, DW is dry weight) in **B** of shoots and roots of PBW343 and C306 wheat cultivars at 0 h, 24 h, 48 h and 72 h of stress given to 4-day old seedlings. CT indicates control grown at 25ºC.

A	**PBW343**						**C306**					
	Shoot dry weight		**Root dry weight**		**R/S ratio**		**Shoot dry weight (mg)**		**Root dry weight (mg)**		**R/S ratio (dw)**	
	CT	**HS**	**CT**	**HS**	**CT**	**HS**	**CT**	**HS**	**CT**	**HS**	**CT**	**HS**
0h	96.8±3.9	96.8±3.9	37.8±2.9	37.8±2.9	0.39±0.04	0.39±0.04	84.0±4.0	84.0±4.0	47.4±4.3	47.4±4.3	0.56±0.07	0.56±0.07
24h	83.7±5.6	83.8±14.0	33.0±3.3	34.0±5.1	0.39±0.01	0.40±0.01	98.9±9.9	146.3±27.1*	44.0±5.5	55.6±14.0	0.45±0.06	0.46±0.03
48h	102.5±5.0	90.8±2.5*	32.2±3.1	29.4±2.6	0.31±0.04	0.32±0.02	120.5±27.3	98.5±12.2	40.0±5.5	44.0±2.8	0.33±0.03	0.45±0.03*
72h	118.2±9.7	96.9±6.5*	39.5±4.9	28.2±2.8*	0.33±0.03	0.29±0.04	161.2±17.1	119.4±13.6*	47.7±3.1	53.1±4.9	0.30±0.02	0.45±0.04*

B	**PBW343**				**C306**			
	Shoot length (cm)		**Root length (cm)**		**Shoot length (cm)**		**Root length (cm)**	
	CT	**HS**	**CT**	**HS**	**CT**	**HS**	**CT**	**HS**
0h	2.21±0.69	2.21±0.69	1.42±0.46	1.42±0.46	2.88±1.17	2.88±1.17	1.88±0.44	1.88±0.44
24h	2.66±0.80	2.24±0.80*	1.49±0.36	1.48±0.34	4.10±1.70	3.68±1.22	2.07±0.42	1.80±0.44*
48h	3.54±1.34	2.42±0.91*	1.52±0.44	1.42±0.42	4.93±1.95	3.24±1.54*	1.82±0.34	1.83±0.45
72h	4.63±1.85	2.50±0.93*	1.50±0.41	1.50±0.38	7.30±2.79	3.55±1.56*	1.93±0.56a	1.88±0.53
	Shoot water contents		**Root water contents**		**Shoot water contents**		**Root water contents**	
	CT	**HS**	**CT**	**HS**	**CT**	**HS**	**CT**	**HS**
0h	10.2±.006	10.2±.006	15.0±0.61	15.0±0.61	12.2±0.16	12.2±.16	14.4±2.0	14.4±2.0
24h	7.38±0.04	5.84±0.28*	6.92±0.25	5.95±0.15*	8.63±0.01	7.64±0.8	7.35±0.6	6.61±0.2
48h	7.39±0.22	5.37±0.30*	6.62±0.42	5.57±0.94	7.52±0.66	6.68±0.8	5.70±1.4	6.06±0.8
72h	7.34±0.10	5.10±0.16*	6.72±0.56	6.61±0.49	7.46±0.26	6.26±0.2*	6.68±1.0	6.50±1.2

Values for dry weights are the mean ± S.D. of triplicates with each replicate of 25 seedlings. R/S ratio is ratio of dry weights of roots to dry weights of shoots of 25 seedlings and values for R/S ratio are mean ± S.D. of three such replicates. Values for length are mean ± S.D. of 50 seedlings selected randomly. Values for water contents are the mean ± S.D. of experiment done in triplicates. * indicates significant difference as analysed by Student's t-test at $p < 0.05$ between CT and HS pair at each stage.

responding control value (**Table 3B**). Water contents were decreased significantly from corresponding controls at all three stages in shoots of 'PBW343' (**Table 3B**). In roots of 'PBW343', water contents were decreased significantly at 24 h and were not changed significantly at 48 h and 72 h of stress from corresponding control values (**Table 3B**). In 'C306', water contents were not altered significantly at any stage in both shoots and roots except in shoots at 72 h stage where water content was decreased significantly from control value (**Table 3B**).

Protein contents were decreased non-significantly at 24 h and 72 h and significantly at 48 h of stress in shoots of 'PBW343' but were almost equivalent to control (CT) throughout the stress period in shoots of 'C306' (**Fig. 1A**). In roots, these were decreased significantly at all three stages from corresponding control in 'PBW343' while in 'C306', contents were maintained equivalent to control at 24 h and decreased significantly at 48 and 72 h but these decreases were not higher as in 'PBW343' (**Fig. 1B**).

Effect of heat stress on non-enzymatic antioxidants and other contents

In shoots of 'PBW343', ascorbate contents increased significantly at 24 h only while at later stages (48 h and 72 h), its levels decreased significantly from corresponding control values (**Fig. 2A**). Dehydroascorbate contents were decreased throughout the stress period from corresponding control values (**Fig. 2E**) in shoots of 'PBW343'. Ratio of ascorbate to dehydroascorbate was higher from control value at 24 h but thereafter ratio was decreased at 48 h and 72 h from corresponding controls in shoots of 'PBW343' (**Fig. 2I**). In shoots of 'C306', ascorbate contents were maintained equivalent to control at 24 h, were decreased sig-

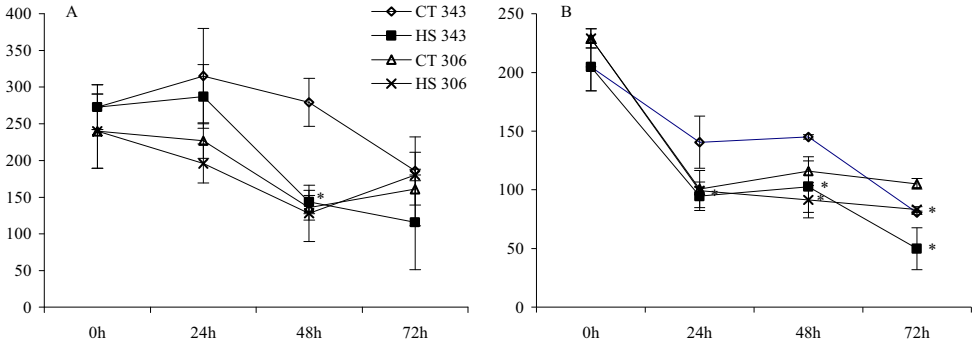

Fig. 1 Effect of heat stress (HS, 37°C) on protein content (mg g^{-1}DW) in shoots (**A**) and roots (**B**) of 'PBW343' and 'C306' at 0, 24, 48, 72 h of stress given to 4-day old seedlings. CT is control at 25°C. * indicates significant difference between stressed and control sample in each cultivar as analysed by Student's *t*-test ($P < 0.05$).

nificantly at 48 h but increased significantly at 72 h of stress (**Fig. 2B**) from corresponding control values. Dehydroascorbate contents were decreased or unaltered throughout the stress period in shoots of 'C306' (**Fig. 2F**). Ratios of ascorbate to dehydroascorbate (**Fig. 2J**) were higher than controls throughout the stress period in shoots of 'C306'.

In roots of 'PBW343', ascorbate contents were decreased significantly from control values throughout the stress period (**Fig. 2C**). Dehydroascorbate contents were also decreased significantly throughout the stress period in roots of 'PBW343' (**Fig. 2G**). Ratios of ascorbate to dehydroascorbate were maintained almost equivalent to control at 24 h, decreased at 48 h but increased at 72 h from corresponding control values in roots of 'PBW343' (**Fig. 2K**). In roots of 'C306', ascorbate contents were not altered significantly from control values throughout the stress period (**Fig. 2D**) and dehydroascorbate contents were decreased significantly or non-significantly from control values throughout the stress period (**Fig. 2H**). Ratios of ascorbate to dehydroascorbate were higher at 24 h and 48 h of stress but decreased at 72 h of stress in roots of 'C306' (**Fig. 2L**).

H_2O_2 contents were decreased non-significantly at 24 h and significantly at 48 h of stress but contents were increased significantly at 72 h of stress from their corresponding control values in shoots of 'PBW343' (**Fig. 3A**). In shoots of 'C306', H_2O_2 contents were decreased non-significantly at all three stages from their corresponding control values (**Fig. 3B**). In roots of 'PBW343', H_2O_2 contents were decreased significantly or non-significantly and were not increased at any stage from control values (**Fig. 3C**). In roots of 'C306', H_2O_2 contents were increased significantly at 24 h of stress but then decreased significantly at 48 h and 72 h of stress from corresponding control values (**Fig. 3D**).

Proline contents were decreased significantly at 24 h but remained unaltered at 48 h and 72 h of stress from corresponding control values in shoots of 'PBW343' (**Fig. 3E**). Proline contents were not altered significantly at any stage from corresponding control values in shoots of 'C306' (**Fig. 3F**). Proline contents were decreased significantly at 24 h and 48 h of stress and were unaltered at 72 h of stress from corresponding control in roots of 'PBW343' (**Fig. 3G**) as well as 'C306' (**Fig. 3H**).

MDA contents were increased significantly at 24 h of heat stress in shoots of both cultivars (**Figs. 3I, 3J**) otherwise MDA contents were either decreased or unaltered from control at other stages in shoots of both cultivars. MDA contents were increased significantly in roots of 'PBW343' at 72 h stage (**Fig. 3K**) and were not increased in roots of 'C306' at all three stages (**Fig. 3L**).

Effect of heat stress on antioxidant enzymes

APX activities were unaltered in shoots (**Fig. 4A**) throughout the stress period and were decreased significantly in roots (**Fig. 4B**) at 24 h and 48 h of heat stress in 'PBW343'. APX activities were not changed significantly at 24 h and 48 h but decreased significantly at 72 h of stress in shoots of 'C306' (**Fig. 4C**). APX activities were increased significantly at 24 h of stress then decreased significantly at 48 h and non-significantly at 72 h of stress in roots of 'C306' (**Fig. 4D**).

CAT activities were decreased significantly at 24 h and non-significantly 48 h of stress but increased non-significantly at 72 h of stress in shoots of 'PBW343' (**Fig. 4E**). CAT activities were unaltered at 24 h but were decreased significantly at 48h and 72 h of stress in roots of 'PBW343' (**Fig. 4F**). CAT activities were maintained almost equivalent to control values during stress period in shoots of 'C306' (**Fig. 4G**) and were decreased significantly or non-significantly in roots of 'C306' (**Fig. 4H**) during stress period.

GPOX activities were decreased under heat stress significantly at 24 h and 72 h and non-significantly at 48 h of stress in shoots of 'PBW343' (**Fig. 5A**). In roots of 'PBW343' (**Fig. 5B**), GPOX activities were decreased non-significantly at 24 h and significantly at 48 h and 72 h of stress. In shoots of 'C306', GPOX activities were maintained almost equivalent to control values throughout the heat stress period (**Fig. 5C**). In roots of 'C306' (**Fig. 5D**), GPOX activities were decreased significantly throughout the stress period.

GR activities were decreased non-significantly at 24 h and significantly at 48 h but unaltered at 72 h of stress from corresponding control values in shoots of 'PBW343' (**Fig. 5E**). GR activities were decreased non-significantly at 24 h and 48 h and significantly at 72 h of stress in roots of 'PBW343' (**Fig. 5F**). GR activities were maintained almost equivalent to control values at 24 h and 72 h of stress and were increased significantly at 48 h of stress in shoots of 'C306' (**Fig. 5G**). GR activities were decreased significantly at 24 h of stress only and were not altered significantly at 48 h and 72 h of heat stress in roots of 'C306' (**Fig. 5H**).

Effect of heat stress on antioxidant and LEA genes

Four antioxidant genes; peroxisomal ascorbate peroxidase (P-APX), GST-F, GPX EST (belonging to cytosolic GPX) and ten LEA genes belonging to group2 group3 and group4 (*Wrab15*, *Wrab17*, *Wrab18*, *Wrab19*, Ta-LEA3-like from group 3; *Wdhn13*, *Td16*, *Td27e*, *Wcor410c* from group 2 and *Td29* from group 4) were used to check their expression and induction under heat stress in shoots of both cultivars at 48 h of stress (**Fig. 6**). GPX-EST was induced under heat stress in shoots of both cultivars and induction level was almost same in both cultivars. GST-F was induced only in 'C306' but not induced in 'PBW343' while other GST-u (GST from tau-class, AJ414697) was used but was not

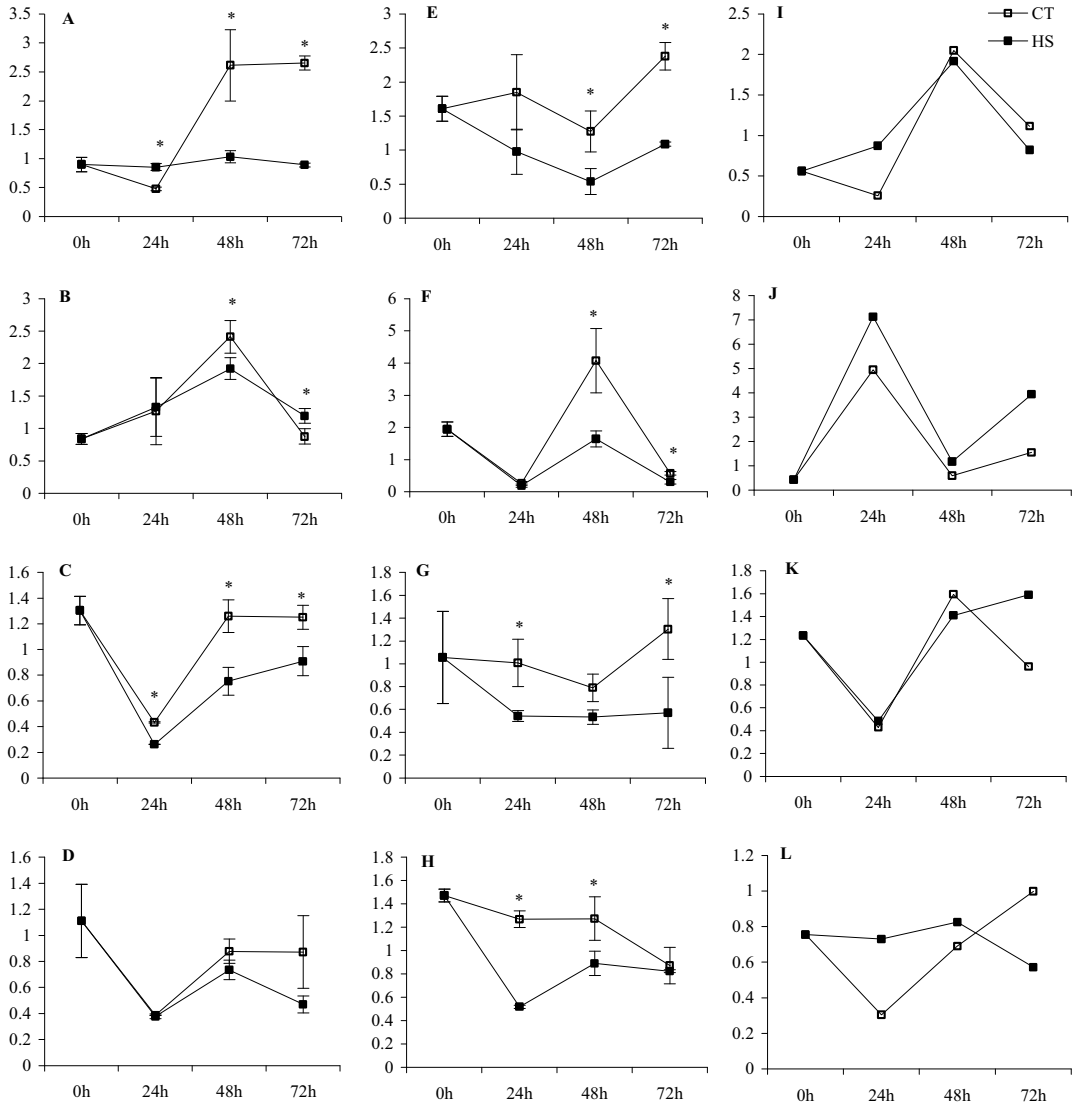

Fig. 2 Effect of heat stress (HS, 37°C) on ascorbate (mg g^{-1} DW) in 'PBW343' shoots (**A**), 'C306' shoots (**B**), 'PBW343' roots (**C**), 'C306' roots (**D**); dehydroascorbate (mg g^{-1} DW) in 'PBW343' shoots (**E**), 'C306' shoots (**F**), 'PBW343' roots (**G**), 'C306' roots (**H**); ascorbate to dehydroascorbate ratio in 'PBW343' shoots (**I**), 'C306' shoots (**J**), 'PBW343' roots (**K**), 'C306' roots (**L**) at 0, 24, 48, 72 h of stress given to 4-day old seedlings. CT is control at 25°C. * indicates significant difference between stressed and control samples as analysed by Student's *t*-test ($P < 0.05$).

found to be induced under heat stress in both cultivars (data not shown). P-APX was induced only in 'PBW343'.

Among the LEA genes, all five LEA genes of group 3 i.e. *Wrab19* (AF255052), *Wrab18* (AB115914), *Wrab17* (AF255053), *Wrab15* (AB115913), LEA-group3-like gene (AY148490) were not found to be induced under heat stress in both cultivars (data not shown). Among four LEA genes of group 2, only three; *Td27e Td16, Wcor410c* were induced under heat stress but their induction status varied between cultivars however *Wdhn13* was not induced under heat stress in both cultivars. *Td16* was induced mainly in 'PBW343' while *Wcor410c* was induced mainly in 'C306'. *Td27e* was induced in both cultivars. *Td29* belonging to LEA group 4 was induced in both cultivars. The level of induction of both *Td27e* and *Td29* was comparatively higher in 'C306' than in 'PBW343' (**Fig. 6**).

DISCUSSION

Heat stress is reported as one of the most important causes of reduction of dry matter production in many crops including cereals (Wahid *et al.* 2007). We found dry matter of wheat plant affected under heat stress in both cultivars where shoot growth was affected more than root growth (**Table 3**). This might be due to as plant adapts to stresses by partitioning more biomass towards roots than towards shoots (Shao *et al.* 2008). Comparing cultivars in the present study, 'C306' cultivar was observed to maintain dry masses of shoot and root better under heat stress than 'PBW343' and accompanied with improved root dry mass to shoot dry mass ratio (**Table 3**). Heat tolerant cultivars were reported to maintain or increase root growth under heat stress over heat susceptible cultivars as roots are more susceptible to heat stress than shoots (Huang *et al.* 2012). Improving root to shoot ratio by 'C306' under heat stress might be the part of its heat tolerance mechanism. Higher rates of respiration and lower rate of photosynthesis in 'PBW343' over 'C306' under heat stress were found and related to low productivity and heat-susceptibility of 'PBW343' over 'C306' (Almeselmani *et al.* 2012).

Heat stress is frequently associated with reduced water availability as it affects root hydraulic conductance (Wahid and Close 2007; Wahid *et al.* 2007). In the present study, water contents were observed to be decreased in both shoots and roots of 'PBW343' but were not altered in 'C306' under heat stress (**Table 3B**). Maintaining water contents under heat stress by 'C306' can be related to its heat tolerance over 'PBW343'.

Though there are multiple signalling pathways implicated for HSR (heat stress resistance), there is emerging evidence that heat stress is accompanied with some degree of oxidative stress (Kotak *et al.* 2007). Higher ascorbate contents along with higher levels of antioxidant enzymes and lesser oxidative damage are correlated with the capacity

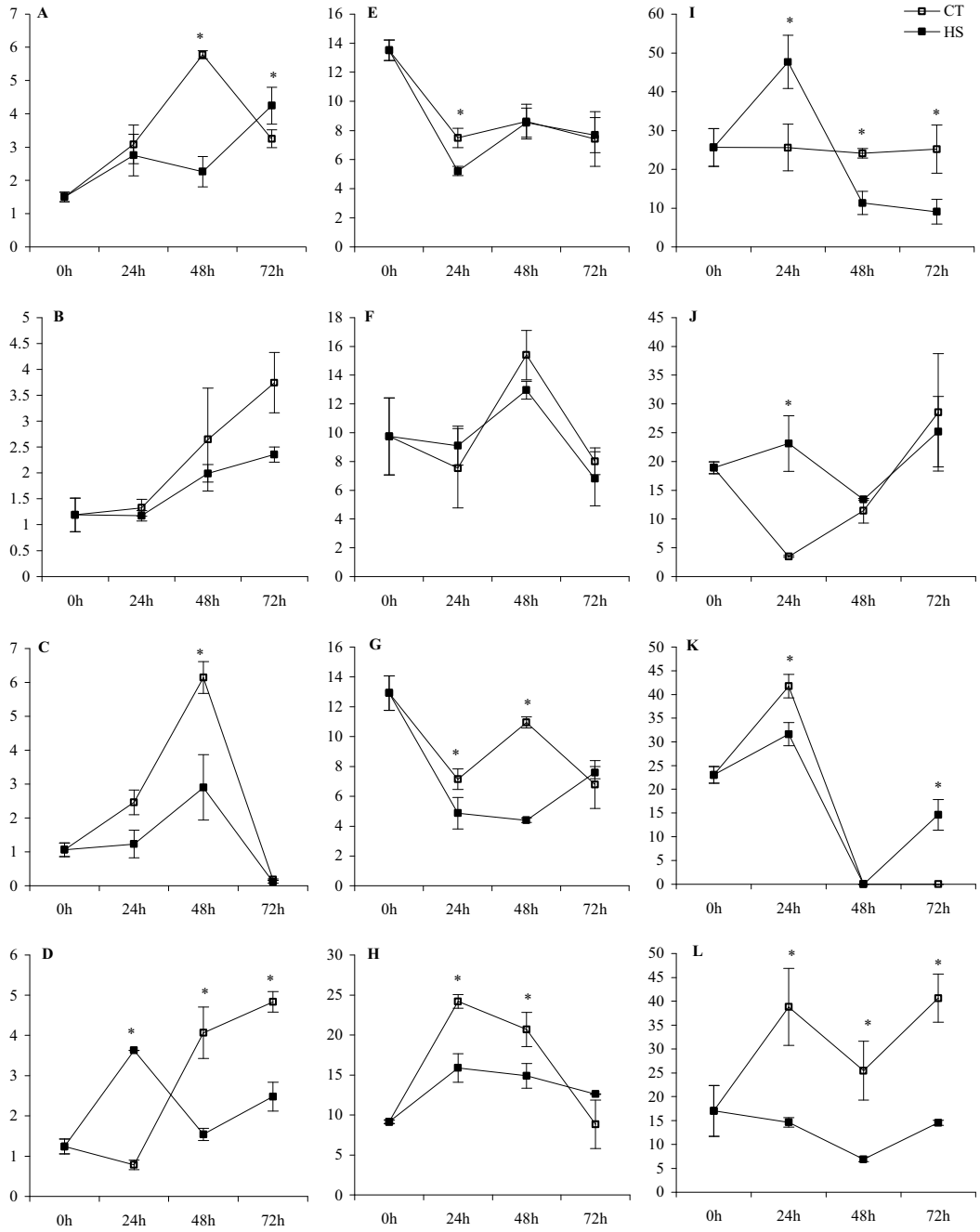

Fig. 3 Effect of heat stress (HS, 37°C) on H_2O_2 (µmole g^{-1} DW) in 'PBW343' shoots (**A**), 'C306' shoots (**B**), 'PBW343' roots (**C**), 'C306' roots (**D**); proline (µmole g^{-1}DW) in 'PBW343' shoots (**E**), 'C306' shoots (**F**), 'PBW343' roots (**G**), 'C306' roots (**H**); malondialdehyde (MDA) (ηmole g^{-1} DW) in 'PBW343' shoots (**I**), 'C306' shoots (**J**), 'PBW343' roots (**K**), 'C306' roots (**L**) at 0, 24, 48, 72 h of stress given to 4-day old seedlings. CT is control at 25°C. * indicates significant difference between stressed and control samples as analysed by Student's *t*-test ($P < 0.05$).

to acquire thermotolerance as studied in a set of wheat genotypes (Sairam *et al.* 2000; Sairam and Tyagi 2004). In this study, ascorbate contents were comparatively lesser affected and were accompanied with higher ascorbate to dehydroascorbate ratios in both shoots and roots of 'C306' than of 'PBW343' (**Fig. 2**) under heat stress. Higher levels of antioxidant enzymes were also found in shoots of 'C306' than of 'PBW343' under heat stress (**Figs. 4, 5**). Roots of 'PBW343' showed higher levels of MDA under prolonged heat stress which were not observed in 'C306' (**Figs. 3K, 3L**). Antioxidant mechanism contributing lesser oxidative damage by activating or maintaining higher levels of antioxidants under heat stress is a part of heat tolerance mechanism which seems to be working better in 'C306' than in 'PBW343' under heat stress. Comparing heat stress with ABA-supply, water stress (Kaur *et al.* 2012), salt stress (Bhagi 2011) in same two cultivars showed that higher level of antioxidant potential (higher ascorbate to dehydroascorbate ratio and higher levels of antioxidant enzymes) may be a ABA-regulated process (as maintained higher in ABA-higher sensitive cultivar, 'C306' than ABA-lesser sensitive cultivar, 'PBW343' under water and heat stress, secondly this level was increased in PBW343 by supplying ABA under water stress than under water stress alone) and may also be a part of cross-tolerance mechanism as this was observed under all three stresses and under ABA-supply in 'C306' (Bhagi 2011; Kaur *et al.* 2012, in this study).

Proline contents were unaltered or decreased under heat stress in both cultivars (**Figs. 3E to 3H**) but these contents were increased under water stress and salt stress in same two cultivars (Bhagi 2011; Kaur *et al.* 2012). Accumulation of proline was usually not reported under heat stress as reported under other abiotic stresses in literature rather its accumulation was found to be decreased by heat stress as reported in polar extracts of *Arabidopsis thaliana* subjected to drought, heat, both (drought and heat combined) where proline accumulated under drought but not under heat and combined drought-heat treatment (Rizhsky *et al.* 2004), its

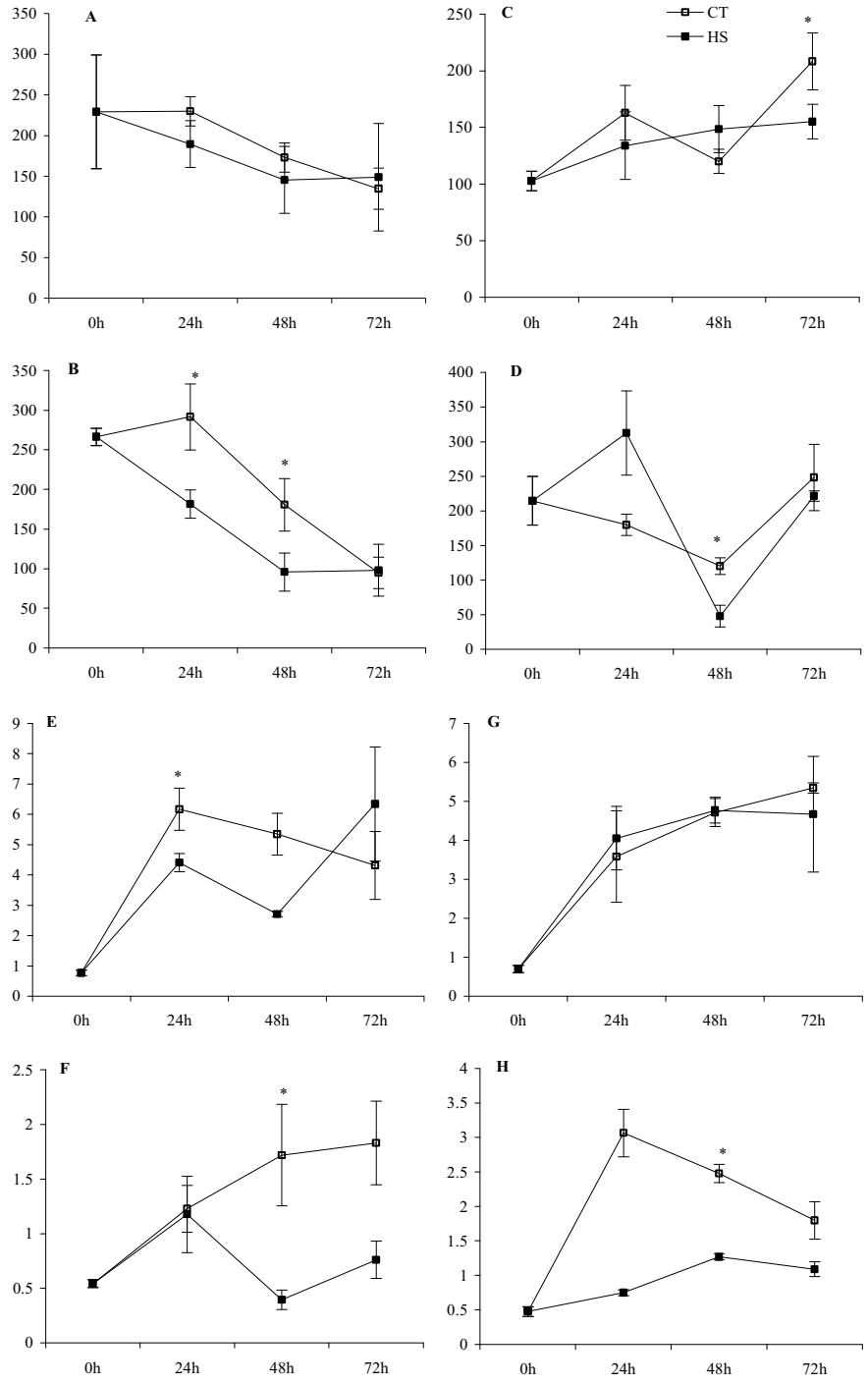

Fig. 4 Effect of heat stress (HS, 37°C) on ascorbate peroxidase (APX) activity (µmole of ascorbate changed min^{-1} g^{-1} DW) in 'PBW343' shoots (**A**), 'PBW343' roots (**B**), 'C306' shoots (**C**), 'C306' roots (**D**); catalase (CAT) activity (mmole of H_2O_2 changed min^{-1} g^{-1} DW) in 'PBW343' shoots (**E**), 'PBW343' roots (**F**), 'C306' shoots (**G**), 'C306' roots (**H**) at 0, 24, 48, 72 h of stress given to 4-day old seedlings. CT is control at 25°C. * indicates significant difference between stressed and control samples as analysed by Student's *t*-test ($P < 0.05$).

accumulation under heat stress was also reported to be toxic as observed in proline-overproducing transgenic of *Arabidopsis thaliana* under heat stress (Lv *et al.* 2011), lesser proline was also observed in acclimated plants than non-acclimated plants of *A. thaliana* under heat stress (Larkindale and Vierling 2008). Proline might be inhibiting ABA and ethylene biosynthesis and producing ROS at elevated temperature (Lv *et al.* 2011).

Maintenance of higher growth, higher amount of proteins in roots of 'C306' than in roots of 'PBW343' (**Table 3; Fig. 1**) and induction of GST only in 'C306' (**Fig. 6**) under heat stress resembled to earlier such observation in two species of *Agrostis* where *Agrostis scabra* (heat resistant, can survive high soil temperature of 45°C) maintained higher root growth and higher proteins contents in roots and GST induction occurred only in this species under heat stress as compared to *Agrostis stolonifera* (heat susceptible) (Huang *et al.* 2012). Maintenance of higher protein levels in 'C306' showed either more thermostable proteins or lower proteolytic activities in this cultivar under heat stress.

H_2O_2 contents were either unaltered or decreased under heat stress in both roots and shoots of both cultivars except it increased at 72 h of stress in shoots of 'PBW343' and at 24 h of stress in roots of 'C306' (**Figs. 3A, 3D**). These increases were accompanied with corresponding increases in antioxidant enzymes like catalase at 72 h in shoots of 'PBW343' (**Fig. 4E**) and APX in roots of 'C306' at 24 h (**Fig. 4D**). MDA contents were also not found to be related to H_2O_2 contents (**Fig. 3**). Many reports observed no correlation between these contents (Triantaphylides *et al.* 2008; Jubany-Mari *et al.* 2010). H_2O_2 is recently considered as non-toxic ROS molecule among other ROS species, which

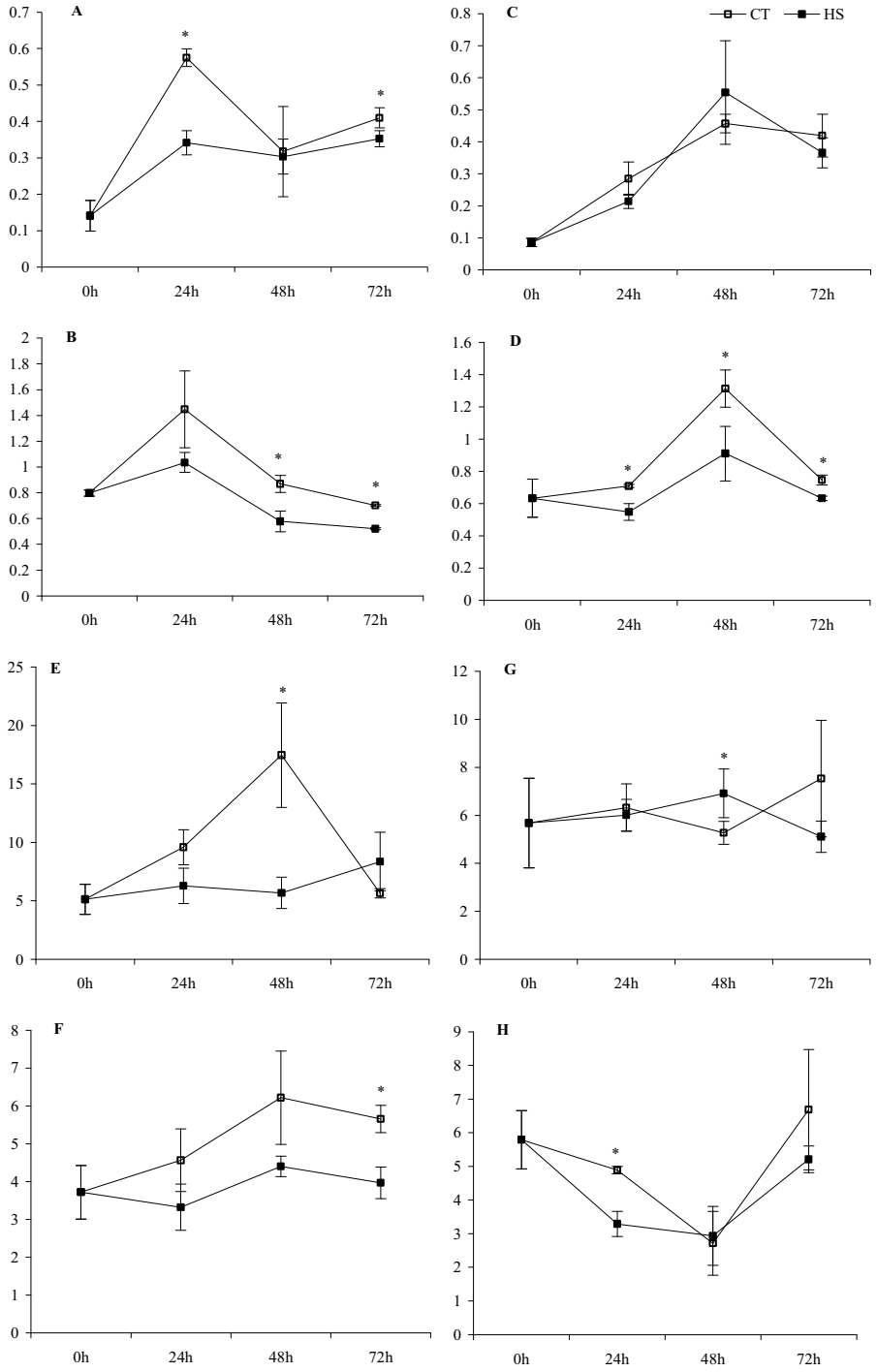

Fig. 5 Effect of heat stress (HS, 37°C) on guaiacol peroxidase (GPOX) activity (mmole of tetraguaiacol changed min^{-1} g^{-1} DW) in 'PBW343' shoots (**A**), 'PBW343' roots (**B**), 'C306' shoots (**C**), 'C306' roots (**D**); glutathione reductase (GR) activity (µmole of NADPH2 changed min^{-1} g^{-1} DW) in 'PBW343' shoots (**E**), 'PBW343' roots (**F**), 'C306' shoots (**G**), 'C306' roots (**H**) at 0, 24, 48, 72 h of stress given to 4-day old seedlings. CT is control at 25°C. * indicates significant difference between stressed and control samples as analysed by Student's *t*-test ($P < 0.05$).

has important functions to play in cells.

Expression of LEA genes is induced under stresses notably water stress, salt stress, cold stress, but very rarely studied under heat stress. LEA genes are involved in heat stress resistance (Wahid *et al.* 2007; Wahid and Close 2007). In present study, we studied four dehydrin genes (*Wcor410c*, *Td27e*, *Td16*, *Wdhn13*), out of which three were induced under heat stress though their induction status varied between cultivars (**Fig. 6**). Fourth dehydrin (*Wdhn13*) was not induced in both cultivars under heat stress (**Fig. 6**) though it was reported to be induced under ABA-supply and under low temperature in seedling leaves of common wheat (Kobayashi *et al.* 2006), was induced higher in freezing tolerant over freezing susceptible cultivar of common wheat under low temperature (Kobayashi *et al.* 2004), was induced higher in ABA-higher sensitive-cum-drought tolerant accessions than ABA-lesser sensitive-cum-drought sensitive accessions of *Aegilposis tauschii* under ABA and under drought (Kurahashi *et al.* 2009), was induced under ABA-supply and water stress in 'C306' not in 'PBW343' (Kaur *et al.* 2012) and induced under salt stress in both 'C306' and 'PBW343' (Bhagi 2011). *Wcor410c* was induced by higher amounts in 'C306' and *Td16* was induced by higher amount in 'PBW343'. *Wcor410c* (98% similar to *Td11*) is reported to be expressed in vegetative tissue, not induced under water stress and late in embryogenesis while *Td16*, *Td27e*, *Td29b* were induced under dehydration as well as late in embryogenesis of wheat seeds (Ali-Benali *et al.* 2005). *Wcor410c* belongs to COR proteins family and is reported to be expressed only in freezing tolerant cultivar of wheat (Danyluk *et al.* 1998). LEA group 3 genes were reported to be up-regulated under ABA and under cold stress (Kobaya-

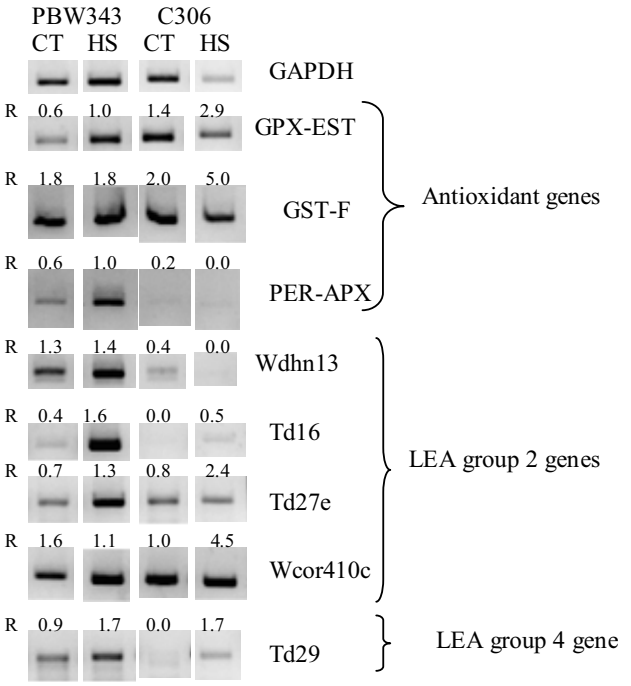

Fig. 6 Effect of heat stress (HS, 37°C) on expression level of antioxidant and LEA genes in shoots of 'PBW343' and 'C306' at 48 h of stress given to 4-day old seedlings where CT is control at 25°C. Antioxidant genes are glutathione peroxidase (GPX) EST (belonging to cytosolic GPX), glutathione-*S*-transferase from phi-family (GST-F), peroxisomal ascorbate peroxidase (P-APX). LEA genes are *Wdhn13*, *Td16*, *Td27e*, *Wcor410c* from LEA group 2 and *Td29* from LEA group 4. R value is the ratio of intensity of band of gene to intensity of band of GAPDH (glyceraldehyde-3-phosphate dehydrogenase) gene used as an internal control.

shi *et al.* 2004) but were not induced under heat stress in this study (not shown), though were induced under ABA treatment, water stress (Kaur *et al.* 2012) and salt stress (Bhagi 2011) in same two cultivars. Comparing LEA genes expression under heat stress with other stresses given to same cultivars in our previous analyses (Bhagi 2011; Kaur *et al.* 2012), levels and number of LEA genes induction was higher under water and salt stress than under heat stress in both cultivars. This could be due to the involvements of dehydrins in dehydration stress than in heat stress where heat shock proteins might be more involved in stabilizing proteins denatured at higher temperature. In another study (Rizhsky *et al.* 2004), expression of dehydrins and heat shock factors (HSFs) were compared under drought, heat stress and combined drought and heat stress in *Arabidopsis*, dehydrins genes were induced mainly under drought while HSFs genes were induced mainly under heat stress as well as under combined heat and drought stress.

CONCLUSIONS

From the results obtained in our work, it can be concluded that heat tolerance feature of 'C306' involves maintenance of higher root growth, higher root to shoot growth ratio, retaining higher amount of water in both roots and shoots, lesser degradation of proteins specially in roots, lesser oxidative toxicity in roots, higher ascorbate and ascorbate to dehydroascorbate ratio in both roots and shoots, higher antioxidant enzymes (APX, CAT, GR, GPOX) in shoots, higher expression of *GST-F*, *Wcor410c*, *Td27e* and *Td29* genes in shoots as compared to 'PBW343' under heat stress. Secondly, maintaining higher antioxidant potential specially higher ascorbate to dehydroascorbate ratio under stress may be a ABA-regulated process and a part of cross-tolerance mechanism as observed under ABA, water stress, salt stress, ABA plus water stress and salt plus water stress (Bhagi 2011; Kaur *et al.* 2012) and heat stress (in this study) in 'C306' cultivar.

REFERENCES

Ali-Benali MA, Alary R, Joudrier P, Gautier MF (2005) Comparative expression of five *Lea* genes during wheat seed development and in response to abiotic stresses by real-time quantitative RT-PCR. *Biochimica et Biophysica Acta* **1730**, 56-65

Almeselmani M, Deshmukh PS, Chinnusamy V (2012) Effects of prolonged high temperature stress on respiration, photosynthesis and gene expression in wheat (*Triticum aestivum* L.) varieties differing in their thermotolerance. *Plant Stress* **6**, 25-32

Almeselmani M, Deshmukh PS, Sairam RK (2009) High temperature stress tolerance in wheat genotypes: Role of antioxidant defence enzymes. *Acta Agronomica Hungarica* **57**, 1-14

Almeselmani M, Deshmukh PS, Sairam RK, Kushwaha SR, Singh TP (2006) Protective role of antioxidant enzymes under high temperature stress. *Plant Science* **171**, 382-388

Badawi GH, Tahir ISA, Nakata N, Tanaka K (2007) Induction of some antioxidant enzymes in selected wheat genotypes. *African Crop Science Conference Proceedings* **8**, 841-848

Balla K, Bencze S, Janda T, Veisz O (2009) Analysis of heat stress tolerance in winter wheat. *Acta Agronomica Hungarica* **57**, 437-444

Bhagi P (2011) Role of antioxidant- and *lea* genes in wheat (*Triticum aestivum* L.) seedlings under different abiotic stresses. MSc thesis, P. A. U. Ludhiana, India

Danyluk J, Perron A, Houde M, Limin A, Fowler B, Benhamou N, Sarhan F (1998) Accumulation of an acidic dehydrin in the vicinity of the plasma membrane during cold acclimation of wheat. *Plant Cell* **10**, 623-638

Dash S, Mohanty N (2001) Evaluation of assays for the analysis of thermotolerance and recovery potentials of seedlings of wheat (*Triticum aestivum* L.) cultivars. *Journal of Plant Physiology* **158**, 1153-1165

Dash S, Mohanty N (2002) Response of seedlings to heat-stress in cultivars of wheat: Growth temperature-dependent differential modulation of photosystem 1 and 2 activity, and foliar antioxidant defense capacity. *Journal of Plant Physiology* **159**, 49-59

Ferris R, Ellis RH, Wheeeler TR, Hadley P (1998) Effect of high temperature stress at anthesis on grain yield and biomass of field grown crops of wheat. *Plant Cell and Environment* **34**, 67-78

Hameed A, Goher M, Iqbal N (2012) Heat stress-induced cell death, changes in antioxidants, lipid peroxidation, and protease activity in wheat leaves. *Journal of Plant Growth Regulation* **31**, 283-291

Huang B, Rachmilevitch S, Xu J (2012) Root carbon and protein metabolism associated with heat tolerance. *Journal of Experimental Botany* **63**, 3455-3465

Jubany-Mari T, Munne-Bosch S, Alegre L (2010) Redox regulation of water stress responses in field-grown plants. Role of hydrogen peroxide and ascorbate. *Plant Physiology and Biochemistry* **48**, 351-358

Kaur M, Gupta AK, Zhawar VK (2012) Antioxidant response and *Lea* genes expression under exogenous ABA and water deficit stress in wheat cultivars contrasting in drought tolerance. *Journal of Plant Biochemistry and Biotechnology* in press

Kobayashi F, Takumi S, Egawa C, Ishibashi M, Nakamura C (2006) Expression patterns of the low temperature responsive genes in a dominant ABA-less-sensitive mutant of common wheat. *Physiologia Plantarum* **127**, 612-623

Kobayashi F, Takumi S, Nakata M, Ohno R, Nakamura T, Nakamura C (2004) Comparative study of the expression profiles of the Cor/Lea gene family in two wheat cultivars with contrasting levels of freezing. *Physiologia Plantarum* **120**, 585-594

Kotak S, Larkindale J, Lee U, von Koskull-Doring P, Vierling E, Scharf KD (2007) Complexity of the heat stress response in plants. *Current Opinion in Plant Biology* **10**, 310-316

Kumar RR, Goswami S, Kumar N, Pandey SK, Pandey VC, Sharma SK, Pathak H, Rai RD (2011) Expression of novel ascorbate peroxidase isoenzymes of wheat (*Triticum aestivum* L.) in response to heat stress. *International Journal of Plant Physiology and Biochemistry* **3**, 188-194

Kurahashi Y, Terashima A, Takumi S (2009) Variation in dehydration tolerance, ABA sensitivity and related gene expression patterns in D-genome progenitor and synthetic hexaploid wheat lines. *International Journal of Molecular Science* **10**, 2733-2751

Larkindale J, Vierling E (2008) Core genome responses involved in acclimation to high temperature. *Plant Physiology* **146**, 748-761

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with folin phenol reagent. *Journal of Biological Chemistry* **193**, 265-268

Lv WT, Lin B, Zhang M, Hua XJ (2011) Proline accumulation is inhibitory to Arabidopsis seedlings during heat stress. *Plant Physiology* **156**, 1921-1933

Maestri E, Klueva N, Perrotta C, Gulli M, Nguyen HT, Marmiroli N (2002) Molecular genetics of heat tolerance and heat shock proteins in cereals. *Plant Molecular Biology* **48**, 667-681

Mahla R, Madan S, Munjal R, Behl RK (2012) Heat-induced oxidative stress and changes in protein profile in wheat cultivars. *Quality Assurance and Safety of Crops and Foods* **4**, 137-138

Mittler R, Finka A, Goloubinoff P (2012) How do plants feel the heat? *Trends*

in Biochemical Sciences **37**, 118-125

Rizhsky L, Liang H, Shuman J, Shulaev V, Devletova S (2004) When defense pathways collide. The response of Arabidopsis to a combination of drought and heat stress. *Plant Physiology* **134**, 1683-1696

Rosa M, Prado C, Podazza G, Interdonato R, Gonzalez JA, Hilal M, Prado FE (2009) Soluble sugars - Metabolism, sensing and abiotic stress. *Plant Signaling and Behavior* **4**, 388-393

Sairam RK, Srivastava GC, Saxena DC (2000) Increased antioxidant activity under elevated temperature: A mechanism of heat stress tolerance in wheat genotypes. *Biologia Plantarum* **43**, 245-251

Sairam RK, Tyagi A (2004) Physiology and molecular biology of salinity tolerance in plants. *Current Science* **86**, 407-421

Shao HB, Chu LY, Jaleel CA, Zhao CX (2008) Water-deficit stress-induced anatomical changes in higher plants. *Comptes Rendus Biologies* **331**, 215-225

Triantaphylides C, Krischke M, Hoeberichts FA, Ksas B, Gresser G, Havaux M, Breusegem Van, Mueller MJ (2008) Singlet oxygen is the major reactive oxygen species involved in photo-oxidative damage to plants. *Plant Physiology* **148**, 960-968

Wahid A, Close TJ (2007) Expression of dehydrins under heat stress and their relationship with water relations of sugarcane leaves. *Biologia Plantarum* **51**, 104-109

Wahid A, Gelani S, Ashraf M, Foolad MR (2007) Heat tolerance in plants: an overview. *Environmental and Experimental Botany* **61**, 199-223

Walbot V (2011) How plants cope with temperature stress. *BMC Biology* **9**, 79-82

Wang X, Cai J, Jiang D, Liu F, Dai T, Cao W (2011) Pre-anthesis high temperature acclimation alleviates damage to the flag leaf caused by post-anthesis heat stress in wheat. *Journal of Plant Physiology* **168**, 585-593

Zhao H, Dai T, Jing Q, Jiang D, Cao W (2007) Leaf senescence and grain filling affected by post-anthesis high temperatures in two different wheat cultivars. *Plant Growth Regulation* **51**, 149-158