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Protective Role of Osmolytes and Antioxidants during High Temperature Stress in Wheat

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Abstract

Ten wheat genotypes differing thermo tolerance were exposed to gradual increase of high temperature stress from control (25 °C) to 30 °C1 h, 35 °C 1 h, 40 °C 2 h and 46 °C 3 h in order to investigate its effect on RWC, chlorophyll, osmolytes accumulation, lipid peroxidation and activity of antioxidants SOD, CAT, GPOX, APX and GR. After exposed to heat stress an increment in proline and GB content was noticed in thermo-tolerant wheat genotypes and at 40 °C 2 h and 46 °C 3 h stress treatment the levels dropped in thermo-susceptible genotypes. RWC, chlorophyll 'b' content significantly declined with steady increase in lipid peroxidation. Heat stress induces the activity of all five ROS scavenging enzymes. However, heat inactivation of all five antioxidants observed at 46 °C 3 h stress in all wheat cultivars. Wheat genotypes NIAW-34, AKAW-4627 and NIAW-917 found to be stable. Heat stress increases GB content with higher activity of all five antioxidants could probably ameliorate the adverse effect.

1. Introduction

High temperature during growth cycle of wheat is a serious abiotic constraint responsible for reduction in wheat yield (Joshi et al., 2007). High temperature causes biochemical perturbation in cereals crop plants like wheat leads to loss of cellular homeostasis accompanied by the formation of reactive oxygen species (ROS) that causes oxidative damage to membrane, lipid protein and nucleic acid (Mittler et al., 2012). The photosynthetic activity of chloroplast is considered primary target of thermal damage in plants and affects on synthesis of chlorophyll pigments (Allakhverdiev et al., 2008). The stress response involves mainly production of specific proteins (mostly heat shock protein or antioxidants) or protective compounds (osmolytes) like proline, glycinebetaine and soluble sugars and induces ROS scavenging mechanism in amelioration of high temperature stress (Wahid et al., 2007). Plant protects cell and sub-cellular systems, cytotoxic effect of the active oxygen radicals using antioxidant peroxidase such as superoxide dismutase (SOD), Guaiacol peroxidase (GPOX), Ascorbate peroxidase (APX), glutathione reductase (GR), Catalase (CAT), and metabolites like glutathione, ascorbic acid, a-tocopherol and carotenoid. These antioxidative enzymes play an important role in the protection of plant from both high and low temperature stresses (Almeselmani et al., 2009).

Therefore, improving the genetic adaptation of wheat genotype to heat stress is an important objective in wheat breeding programmes. Genotypic variations among the crop plants provide a valuable tool in the selection of genotype with desirable traits as they respond differentially to various stresses (Goyal and Asthir, 2010). The exact mechanisms underlying the thermal tolerance are not yet completely understood (Mittler et al., 2008). Some genotypes have versatile characters to cope up adverse situation, which can be exploited for further thermo tolerance study in wheat. Keeping this in view, the effect of gradual increase of high temperature stress for short period (1 to 6 h) on osmolytes accumulation, chlorophyll pigment and activity of antioxidants viz., SOD, CAT, GPOX, APX and GR with oxidative marker MDA content in ten wheat genotypes was studied.

2. Materials and Methods

2.1. Plant material, growth condition and high temperature stress treatments

Ten wheat (*Triticum aestivum* L.) genotypes including five thermo tolerant (NIAW-34, Raj-4083, NIAW-1161, HD-2932, AKAW-4627) and five thermo susceptible ones (GW-322, NIAW-301, NIAW-917, MACS-2496,HD-2189) were collected from Wheat specialist, ARS, Kundewadi, Nashik

under MPKV, Rahuri, India. The laboratory experiments were conducted in year 2012-13. The seeds were surface sterilized and then washed with distilled water thrice. The seeds were sown in plastic tray containing soil medium and was equilibrated with water as and when required and allowed to grow for10 days in a natural day light condition. After ten days, these seedlings were transferred in BOD incubator having 15 9 h light dark^{-1} cycle with 1500-lux light intensity at 25±1 °C temperature for 24 h and subsequently exposed to temperature stress at 30 °C for 1 h, 35 °C for 1 h, 40 °C for 2 h and at 46 °C for 3 h (challenge temperature). The leaves of each genotype were separately weighed and analyzed for some physiological and biochemical parameters.

2.2. Physiological traits

The % RWC was measured as per the method suggested by Weatherly (1950) using a leaf samples (0.5 g) and calculated by using the following formula:

RWC (%)=Fresh weight-dry weight turgid^{-1} weight-dry weight×100.

Leaf chlorophyll b content was determined as described by Arnon (1949). The absorbance was then read on spectronic-20 at 663 and 645 nm wavelength and the quantity of chlorophyll content were calculated and expressed as mg g^{-1} fresh weight.

2.3. Biochemical traits

2.3.1. Deterimantionof osmolytes and lipid peroxidation

Proline content was determined according to the modified method of Bates et al. (1973). Proline extracted from 0.5 g leaf sample by grinding in 5 ml of 3% (w/v) sulphosalicylic acid. The absorbance of the toluene phase was read at 520 nm.The concentration of proline was calculated from standard curve and expressed as µmoles g^{-1} fresh weight. Amount of glycine betaine was determined according to the method of Stumpf (1984) by using Dragendorff reagent with 1 g leaf samples were homogenized in 5 ml 80% ethanol. The concentration of GB was calculated by its absorbance at 467 nm using 0.49 NaIsolution as the blank and results were expressed in µg g^{-1} fresh weight from standard curve prepared for betaine aldehyde. Lipid peroxidation (MDA content) of the leaf samples was measured as the amount of thiobarbituric acid reactive substances (TBARS) determined by thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968). The concentration of MDA was calculated using its extinction coefficient of 155 m M^{-1} cm^{-1} and expressed as µmoles of MDA g^{-1} fresh weight.

2.3.2. Antioxidative enzymes assay

2.3.2.1. Preparation of crude enzyme extract

For assays of SOD, CAT, APX and POX leaf samples 0.5 g were homogenized using 5 ml of an ice-cold 0.1 M potassium phosphate buffer (pH 7.5) containing 0.5 m M EDTA in prechilled moter and pestle. The homogenates was filtered and then centrifuged at 15,000 rpm for 20 min at 4°C. The supernatant was used as crude extract for enzyme assays. For estimation of ascorbate peroxidase the extraction buffer was further supplemented with 1 m M ascorbic acid and the pH was adjusted to 7.5 (Almeselmani et al., 2009).

2.3.2.2. Enzyme assays

Superoxide dismutase activity was determined by measuring its ability to inhibit the photochemical reduction of NBT using the method described by Dhindsa (1981) and Almeselmani et al. (2009). The absorbance of irradiated and non-irradiated reaction mixture were read at 560 nm using control as a blank. One unit of SOD was defined as the amount of enzyme required to cause 50% inhibition of NBT reduction min^{-1} at 560 nm.

Catalase activity was measured immediately from fresh extract and the assay employed as described by Aebi (1983). The decline in absorbance was recorded at 240 nm for three min at an interval of 30 sec. The enzyme activity was calculated by using extension coefficient (ε 39.4 m M^{-1} cm^{-1}) and expressed as µmoles H_2O_2 decomposed min^{-1} mg^{-1} protein.

Ascorbate peroxidase activity was measured the method described by Nakano and Asada (1981). The hydrogen peroxide dependent oxidation of ascorbic acid was followed by a decrease in the absorbance measured at 290 nm for three min at the interval of 30 sec. A complete reaction mixture without ascorbic acid was used as a blank. The enzyme activity was calculated using extension coefficient (ε=2.8 m M^{-1} cm^{-1}) and expressed as µmoles of ascorbate oxidized min^{-1} mg^{-1} protein.

The rate of decomposition of hydrogen peroxide by peroxidase, with guaiacol as a hydrogen donor was measured by the increase in absorbance at 470 nm min^{-1} as per the method described by Castillo et al. (1984). The increase in absorbance was recorded at 470 nm for two min at an interval of 30 sec. The enzyme activity was expressed as µmoles of tetra-guaiacol formed min^{-1}mg^{-1} protein. Glutathione reductase activity was measured as described by Smith et al. (1988). The increase in absorbance was recorded at 412 nm for three min at an interval of 30 sec. Reaction mixture without oxidized glutathione was used as a blank. The activity expressed as µmoles NADPH oxidized mg^{-1} protein min^{-1}. by using extension coefficient (ε 39.4 m M^{-1} cm^{-1}).

For estimating the antioxidative enzymes activity mg^{-1} soluble protein, the soluble protein from leaf samples at different temperature stress treatments were estimated as per the method of Lowry (1951) using bovine serum albumen

as the standard protein. The Laboratory data was analysed by using completely randomized block design with factorial concept with four replications.

3. Results and Discussion

3.1. Physiological traits

The % RWC significantly declined with increase of temperature. In thermo-susceptible group, it declined from 90.02% to 57.56% whereas in thermo-tolerant group, it declined from 89.78 to 68.74%. Among the ten wheat genotypes, a thermo-tolerant genotype NIAW-34 recorded highest RWC (78.30%) followed by tolerant AKAW-4627 (74.04%) at 46 °C 3 h temperature stress. Among susceptible ones, NIAW-917 and MACS-2496 genotypes recorded 75% RWC, while, HD-2189 recorded lowest (69.44%) values of RWC. This study revealed existence of negative correlation ($r=-0.885^{**}$) between high temperature and RWC (Table 1). In our study, RWC significantly declined due to stress treatment which indicated that water relations of all plants suffered during heat stress due to loss of turgidity and lower stomatal conductance consequently reducing leaf water potential. Similar observations were recorded earlier in broccoli and Chinese cabbage and in wheat (Lin et al., 2010; Wang et al., 2010).

Chlorophyll 'b' declined significantly in all thermo-susceptible wheat genotypes whereas it was least declined in tolerant wheat genotypes. Overall reduction in chl 'b' content ranged from 0.75 to 0.53 mg g^{-1} fresh weight in thermo-tolerant wheat genotypes and from 0.80 to 0.32 mg g^{-1} fresh weight in thermo-susceptible genotypes. Genotype HD-2189 (34.15%) recorded highest decline of chlorophyll 'b' content followed by GW-322 (23.95%), MACS-2496 (23.08%), NIAW-301 (25%) and NIAW-1161 (15.38%). On the other hand, genotype NIAW-917 showed least reduction (8.97%) in chlorophyll 'b' content. The extent of increase in chlorophyll 'a' content in wheat genotypes showed positive correlation with heat-stress (0.062) and negative correlation with chl'b' ($r-0.595^{**}$) and total chlorophyll ($r-0.321^{*}$) (Chlorophyll a and total chlorophyll data were not shown (Table 1). In present study, both group of wheat genotype showed increment in chlorophyll 'a' content but it declined at 46 °C 3 h except NIAW-34. These genotypes also showed net gain of chlorophyll 'b' content suggesting their higher capacity to acclimate at lethal temperature. Least decline also observed in other thermo-tolerant genotypes. Significantly higher decrease was observed in susceptible group of wheat genotypes suggesting that the photosynthetic activity of susceptible genotypes were more affected due to disturbances or inactivation of the activity of PS-II attributed to the inhibition of chlorophyll biosynthesis (Allakhverdiev et al., 2008). Earlier a significant correlation observed in between short term heat-induced reductions in chlo 'b' content in wheat (Almeselmani et al., 2009).

3.2. Biochemical traits

Oxidative stress marker (MDA content) significantly increased and reached peak as temperature stress increased from 30 °C 1 h to 46 °C 3 h (Table 1). However, the level of MDA content recorded higher in thermo-susceptible group as compared to thermo-tolerant group. Lipid peroxidation increased in the range of 1.26 to 8.65 (µmoles of MDA g^{-1} fresh weight) in thermo-susceptible genotypes whereas it displayed lower range 1.35 to 6.19 µmoles of MDA g^{-1} fresh weight in tolerant group of wheat genotypes. Among the ten wheat genotypes, lower lipid peroxidation rate of 4.10 µmoles MDA g^{-1} fresh weight was recorded in NIAW-34. While, among thermo-susceptible genotypes, HD-2189 recorded highest rate of lipid peroxidation (10.20 µmoles MDA g^{-1} fresh weight). The results indicated a positive correlation ($r=0.902^{**}$) between LPO and temperature stress (Table 1). Lipid peroxidation is always linked to electrolyte leakage through plasma lemma. Increased MDA might limit CO_2 exchange and metabolic fixation that results in excess excitation of energy from chloroplast and that might divert to activate ROS causing lipid peroxidation (Halliwell and Chirico, 1993). In our study, higher MDA might relate to reduction of chlorophyll b and mitochondrial activity in plant cell. Reduction in RWC and with higher LPO at 40 °C 2 h and 46 °C 3 h stress (Table 1) is directly or indirectly might be elicited by membrane damage (Kangasjarvi et al., 2008). Earler reports showed that lower levels of lipid peroxidation in wheat and correlated with tolerance to high temperature (Sairam et al., 2000; Almeselmani et al., 2009).

Accumulation of proline increased significantly in all ten wheat genotypes when exposed 30 °C for 1 h and 35 °C for 1 h to heat stress however, at 40 °C (2 h) and at 46 °C (3 h) stress treatment proline declined in all wheat genotypes (Table 3). At control stage, a proline level was also higher in tolerant group 0.72 µmoles g^{-1} fresh weight (1.50 fold) as against 0.52 µmoles g^{-1} fresh weight (1.0 fold) in susceptible group of genotypes. After acclimation to 30 °C 1 h, the levels of proline accumulation increased 2.88 fold in thermo-tolerant and 2.39 fold in thermo-susceptible wheat genotypes at 35 °C 1 h stress. The genotypic variation in the percent increase or decrease over control was highly significant among all the variety and treatment combinations and showed positive correlation ($r=0.523^{**}$) with high temperature treatments (Table 2).

GB content increased significantly in all wheat genotypes as temperature and duration of exposure to heat stress increased from 30 °C for 1 h to 46 °C for 3 h (Table 2). Increase of GB at 30 °C for 1 h stress was not so high, however, at 35 °C for 1 h stress treatments, increase in GB was significant in all ten wheat genotypes (0.43 to 1.50 µg g^{-1} fresh weight). At 46 °C 3 h stress, wheat genotype AKAW-4627(6.43 fold) and NIAW-

34 (5.04 fold) showed net gain of GB content. However, at 46 °C 3 h, the increment in content of GB showed to be slightly declined in HD-2932 and higher in NIAW-301, HD-2189 and MACS-2496. Among thermo-susceptible genotypes NIAW-917 reported 3.97 fold (1.39 µg g^{-1} fresh weight) accumulation of GB. A positive correlation ($r=0.771^{**}$) in between of GB and heat stress was observed (Table 1).

Osmolytes accumulation is an important factor in determining abiotic tolerance that can have a major role in osmotic adjustment and play number of other protective roles under abiotic stress. Under heat stress, accumulation of proline in wheat has been reported by Ahmed and Hassan (2011). On the contrary to this, decrease of proline has also been recorded in wheat by Kumar et al. (2012); in Arabidopsis by Wei-Tao et al. (2011). In the present study, the proline levels increased at 30 °C 1 h and 35 °C 1 h temperature stress over control might be due to activation of ABA protein kinases SnRKs 2 but then gradually declined in all wheat genotypes (Table 2). The reduction in proline content in all susceptible genotypes and to some extent at 46 °C 3 h in tolerant genotypes may probably be due to increased rate of proline utilization over synthesis to defend oxidative burst by ROS (Raymond and Smirnoff, 2002). The negative correlation between proline and heat stress even for short term heat stress observed in this study suggest that the levels of proline therefore are not always correlated with osmotolerance (Miller et al., 2009) and higher proline might be inhibitory (Rizhsky et al., 2004) even for a short period.

Increased of GB concentration in cell (0.50 to 1.82 µg g^{-1} fresh weight) with higher content of chlorophyll and lower the levels of MDA in thermo-tolerant wheat cultivars probably that GB might help to maintain integrity by inducing activity of all five antioxidative enzymes. It has been reported that GB protect the inactivation of Rubisco by sequestering Rubiscoactivase in chloroplast by preventing its thermal inactivation (Yang et al., 2007). In wheat, it has been reported that the accumulation of GB in cell enhance thermotolerance under adverse conditions (Wang et al., 2010).

Increment in proline levels in wheat seedlings probably due to heat induced GB can enhance the water status into plant cells or might due to activation of heat stock transcription factors (HSF) which might regulates heat stress response genes and enhanced thermo-tolerance as suggested by Nishizawa et al. (2008). Similar observation were recorded in wheat by Wang et al. (2010) under heat stress and suggested that over accumulated GB is related to the accumulation of other metabolites. It has been reviewed that accumulation of GB and other metabolite like sugars might enhance cellular reducing power though maintenance of higher NADPH concentration and involved in the detoxification of ROS by activation of oxidative pentose pathway to enhance NADPH production (Couee et al., 2006).

3.3. Antioxidative enzymes

Increment in SOD activity was recorded from control (25 °C) to 40 °C 2 h heat stress. However, when exposed 46 °C 3 h, it declined in thermo-tolerant Raj 4083 and NIAW-1161 and HD-2932 except NIAW-34 and AKAW-4627 (Figure 1a). In thermo-susceptible genotypes, SOD induced at 30 °C 1 h and 35 °C 1 h temperature stress but there after declined at higher temperature stress of 40 °C 2 h and 46 °C 3 h except NIAW-917 and MACS-2496 where it showed considerable increase. The mean varietal values indicated that NIAW-34 and AKAW-4627 recorded highest SOD activity 5.24 and 4.18 U mg^{-1} proteins. Over all, SOD activity increase by 198.61% in thermo-tolerant whereas susceptible displayed least increase by 48%. A positive correlation ($r=0.602^{**}$) noticed during this study in between SOD and heat stress (Table 1).

Heat stress induced peroxidase activity and it ranged between 0.64 to1.16 µmoles of tetra guaiacol min^{-1} mg^{-1} protein (10 to 84.13%) in thermo-tolerant whereas in thermo susceptible it was ranged from 0.57 to 0.78 µmoles of tetra guaiacol min^{-1} mg^{-1} protein (0 to 20.59%) over control (Figure 1b). As compared to control and other genotypes thermo-tolerant NIAW-34 significantly recorded higher activity in terms of percentage (84.13%) and also in terms of magnitude (1.16 µmoles of tetra guaiacol min^{-1} mg^{-1} protein). GPOX activity was drastically decreased in HD-2189 from 30 °C 1 h to 46 °C 3 h heat stress treatment and came below control levels 0.88 to 0.23 µmoles of tetra guaiacol min^{-1} mg^{-1} protein (4.39% to 0%). A significant positive correlation ($r=0.439^{**}$) was observed in case of peroxidase activity (Table 1).

From Figure 1c, it is clear that heat induces CAT activity up to 40 °C 2 h in thermo-tolerant wheat genotypes. But, it declined at 46 °C 3 h in both groups of wheat genotypes whereas net gain in NIAW-34 (43%) and AKAW-4627 (69%) was recorded. On the other hand, the catalase activity also decreased in thermo-tolerant HD-2932, Raj-4083, NIAW-1161 genotypes but percent increase over control was higher. In susceptible group,catalase activity was significantly induced in all five thermo-susceptible wheat genotypes by 33.90% in GW-322, 138% in NIAW-917, 45.58% in MACS-2496 and 33.33% in HD-2189 and least in NIAW-301 (24.89%). Further, the enzyme activity declined at 40 °C 2 h and 46 °C 3 h heat stress except NIAW-917 where it was showed to be increased by 18.14%.

APX activity significantly differed in the all ten genotypes at different time point under heat stress (Figure 1d). Similar to catalase APX activity showed to be induced from 35 °C 1 h to 40 °C 2 h heat stress treatments. Further, at 46 °C 3 h temperature stress, it declined in thermo-tolerant group except

NIAW-34 and NIAW-1161 showed to be increased by 6 fold and 4 fold. The mean APX activity increased in the range of 0.83 to 3.46 µmole g^{-1} fresh weight i.e., 73 to 256% increase in thermo-tolerant whereas it was increased in the range of 0.84 to 1.68 µmoles of ascorbate oxidized min^{-1} mg^{-1} protein (4.28 to 100%) in susceptible over control. The activity declined at 46 °C for 3 h stress in tolerant cultivars Raj-4083, NIAW-1161, HD-2932, and AKAW-4627. In susceptible wheat cultivars, GW-322, NIAW-301, and HD-2189 APX activity increased around 2 fold up to 35 °C 1 h heat stress thereafter it declined. Among susceptible wheat NIAW-917 genotype recorded 3.79 fold higher activities over control. A positive correlation with increasing temperature ($r=0.455^{**}$) was observed during this investigation (Table 1).

The enzymatic activity of glutathione reductase increased steadily from 30 °C 1 h to 46 °C 3 h in all thermo-tolerant genotype (Figure 1e). Whereas in susceptible the GR activity increased at 35 °C 1 h stress but further, it was decreased and came to below control level at 46 °C 3 h. Among susceptibles, NIAW-917 showed increasing trend at all temperature levels. The results showed that heat induced the activity of GR but the magnitude of expression was very marginal in all contrast wheat genotype when exposed to heat stress. Among the tolerant genotypes, NIAW34 showed highest and steady increase of glutathione activity (0.85) by followed by AKAW-4627 (0.72), HD-2932 (0.61), Raj-4083 (0.62) and least in NIAW-1161 0.59 µmoles of NADH oxidized$min^{-1}mg^{-1}$ protein, respectively as compared to control. Whereas, among thermo-susceptible genotypes, NIAW-917 (0.42 µmoles of NADH oxidized$min^{-1}mg^{-1}$ protein) recorded higher activity and cultivar NIAW-301 and HD-1289 showed drastic reduction as compared to other wheat genotypes. Our results showed positive correlation ($r=0.456^{**}$) in between high temperature stress with glutathione reductase activity as compared to control (Table 1).

Table 1: Effect high temperature stress on relative water content, chlorophyll 'b' lipid peroxidation and correlation coefficient between physiological and biochemical variables measured in ten wheat genotypes differing in thermotolrance (significant at $^{*}0.05$ and **at 0.01 probabilities)

Genotypes	RWC		LPO		Chlo 'b'	
	(%)		µmoles of MDA g^{-1} fr.wt		mg g^{-1} fr.wt	
	25 °C Control	After stress	25 °C Control	After stress	25 °C Control	After stress
Thermo-tolerant						
Raj-4083	91.53	64.56	1.36	6.66	0.71	0.28
NIAW-34	89.12	78.30	1.52	4.10	0.67	0.98
NIAW-1161	85.85	62.50	1.29	7.01	0.78	0.39
HD-2932	89.87	64.28	1.12	7.48	0.80	0.48
AKAW-4627	92.51	74.04	1.45	5.69	0.78	0.51
Tolerant mean	89.78	68.74	1.35	6.19	0.75	0.53
Thermo-susceptible						
GW-322	91.90	62.03	1.29	9.22	0.81	0.35
NIAW-301	90.27	52.90	1.17	8.92	0.80	0.35
NIAW-917	88.27	62.22	1.37	6.22	0.78	0.49
MACS-2496	91.00	58.16	1.02	7.11	0.78	0.23
HD-2189	88.67	52.51	1.43	10.20	0.82	0.20
Susceptible mean	90.02	57.56	1.26	8.65	0.80	0.32
	SEm±	CD (p=0.05)	SEm±	CD (p=0.05)	SEm±	CD (p=0.05)
Treatment	0.370	1.039	0.015	0.042	0.003	0.005
Variety	0.524	1.469	0.021	0.059	0.003	0.008
TXV	1.171	3.286	0.047	0.132	0.006	0.017
Correlation coefficient with high temperature						
Parameter	RWC	LPO	Chlo'b'	Proline	GB	
Correlation	-0.885^{**}	0.902^{**}	-0.595^{**}	0.523^{**}	0.771^{**}	
Parameter	SOD	GPOX	CAT	APX	GR	
Correlation	0.602^{**}	0.439^{**}	0.104	0.455^{**}	0.456^{**}	

Table 2: Effect of high temperature stress treatments on osmolytes accumulation in wheat genotypes differing in thermo-tolerance

Genotypes	Proline content (µmoles g^{-1} fresh weight)					Glycine betaine content (µg g^{-1} fresh weight)				
	25 °C	30 °C 1 h	35 °C 1 h	40 °C 2 h	46 °C 3 h	25 °C	30 °C 1 h	35 °C 1 h	40 °C 2 h	46 °C 3 h
Thermo-tolerant										
Raj-4083	0.72	1.3	1.49	1.42	1.37	0.46	0.55	0.93	1.42	1.66
NIAW-34	0.75	1.56	2.45	1.9	1.66	0.43	0.46	0.97	1.84	2.16
NIAW-1161	0.74	1.30	1.74	2.02	1.74	0.46	0.56	1.40	1.52	1.7
HD-2932	0.66	1.32	2.15	1.72	1.23	0.48	0.46	1.00	2.23	1.99
AKAW-4627	0.74	1.39	2.56	2.48	1.81	0.39	0.47	0.78	1.66	2.51
T. Mean	0.72	1.37	2.08	1.91	1.57	0.44	0.50	1.01	1.75	2.00
Thermo-susceptible										
GW-322	0.50	0.89	1.41	1.28	1.11	0.49	0.69	1.50	0.92	0.92
NIAW-301	0.59	0.82	1.10	1.21	0.99	0.36	0.55	1.15	1.06	0.79
NIAW-917	0.60	0.8	1.45	1.99	1.66	0.35	0.34	0.72	1.53	1.39
MACS-2496	0.40	1.12	1.6	1.02	0.96	0.37	0.5	1.21	1.16	1.09
HD-2189	0.59	0.80	1.55	1.14	1.10	0.47	0.54	1.11	0.80	0.78
S.Mean	0.54	0.89	1.42	1.33	1.16	0.41	0.53	1.14	1.10	1.00
	SEm±			CD (p=0.05)		SEm±			CD (p=0.05)	
Treatment	0.005			0.013		0.002			0.007	
Variety	0.007			0.019		0.002			0.005	
TXV	0.015			0.041		0.005			0.014	

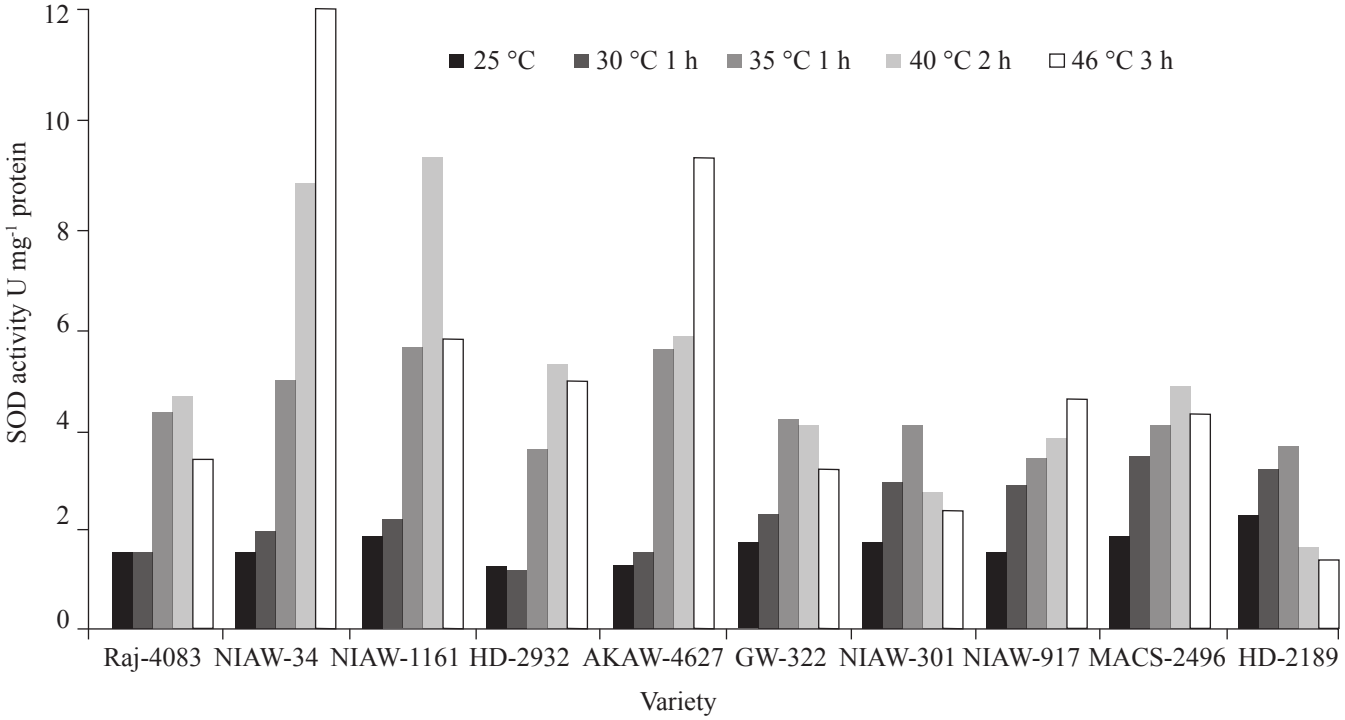

Figure 1a: Activity of superoxide dismutase

Temperature stress accelerates the generation of ROS in different compartments of plant cell. These ROS are necessary for inter and intracellular signaling, but under stress conditions, they seriously disrupt the normal metabolism of plants through oxidation of membrane lipids, protein and nucleic acids in the absence of protective mechanisms (Wahid et al., 2007). In our study, significantly higher SOD activity is consistent with lowest oxidative stress in thermo-tolerant

wheat genotypes especially in NIAW-34 and AKAW-4627. Lower catalase and peroxidase in susceptible cultivars could also be consequence of reported decreased activation of these enzymes and induction of their capacity and or synthesis of enzyme protein by H_2O_2 (Sairam et al., 2000). Here, susceptible cultivar NIAW-917 has reported considerable higher levels of antioxidative enzymes at 40 °C 2 h heat stress considered their involvement Ascorbate peroxidase- glutathione reductase system to scavenge H_2O_2 accumulation formed by superoxide radicals. In this susceptible wheat cultivars, the concentration of GB is also higher that indicates GB help to stabilize the proteins of antioxidants. Subsequently increased of soluble sugar might also help to stabilize membrane or acts as signaling molecules to activate defense cascade thus maintain lower rate of lipid peroxidation as compared to other susceptible wheat cultivars. Higher SOD activity during heat stress might

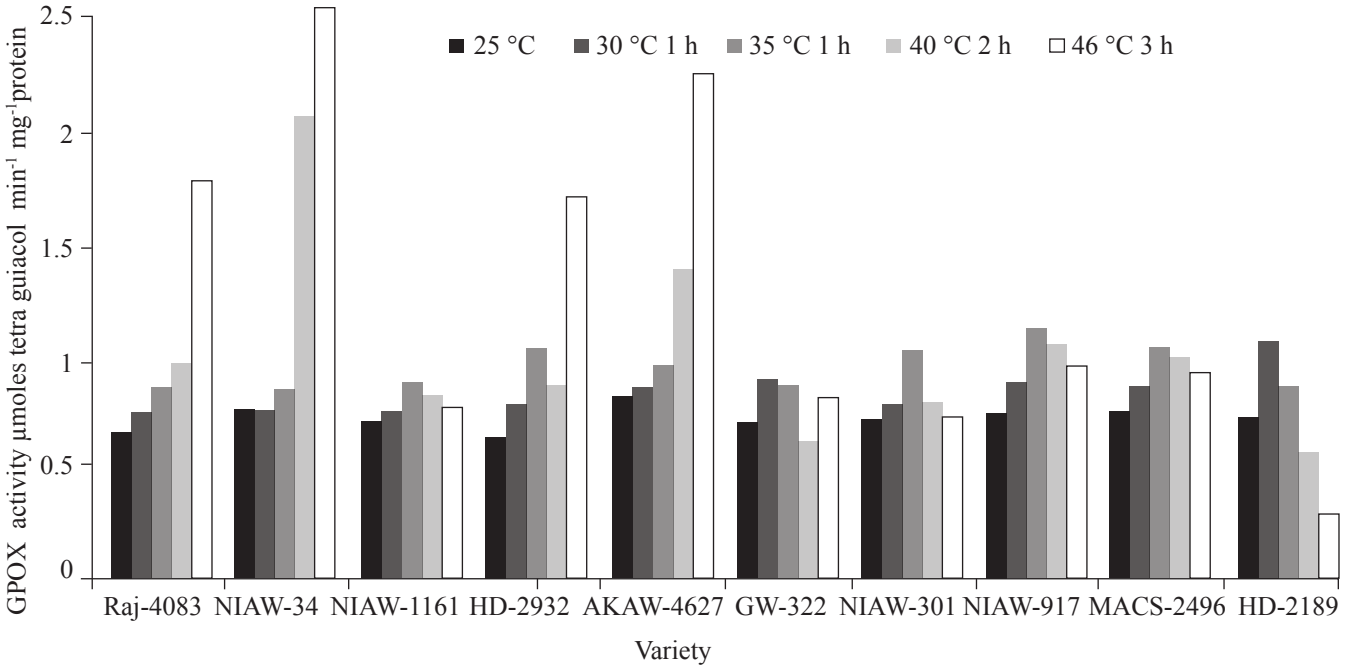

Figure 1b: Activity of guaiacol peroxidase

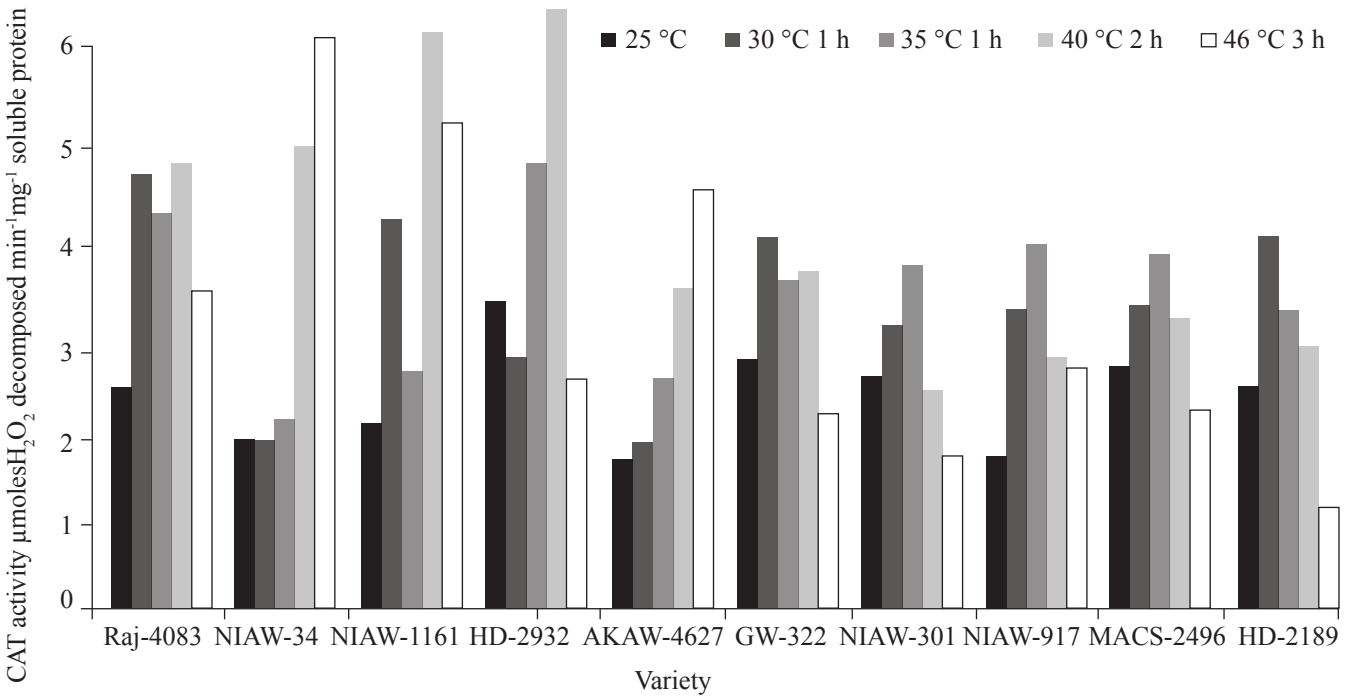

Figure 1c: Activity of catalase

be due to increased levels of the cytosolic and chloroplastic SOD activity. Whereas decrease in SOD activity at sublethal (40 °C 2 h) and lethal temperature at 46 °C 3 h suggest that a lesser O^{2-} (ROS) scavenging and dis-mutating capacity in heat thermo-susceptible genotypes and signifies a possible involvement of this enzymes in heat tolerance. Earlier reports in wheat showed higher SOD activities with improved tolerance to temperature stresses (Almeselmani et al., 2009; Mahla et al., 2011). The least pronounced changes in GPOX activity which thereafter tended to decrease indicates that this enzyme was degraded to a great extent by heat stress. Enhanced activity of these enzymes could contribute to the development of various stress factors and enzyme remain active at 30 °C 1 h and 35 °C 1 h and became inactive when temperature increased from

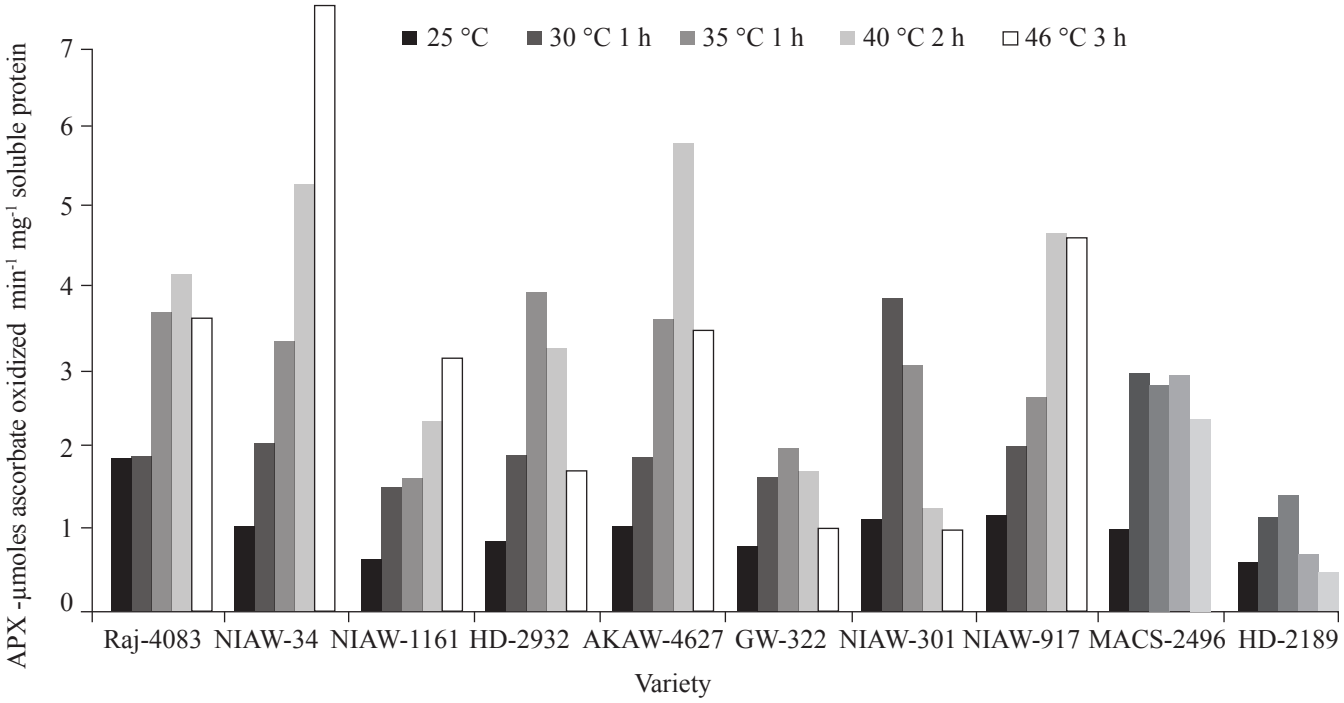

Figure 1d: Activity of guaiacol peroxidase

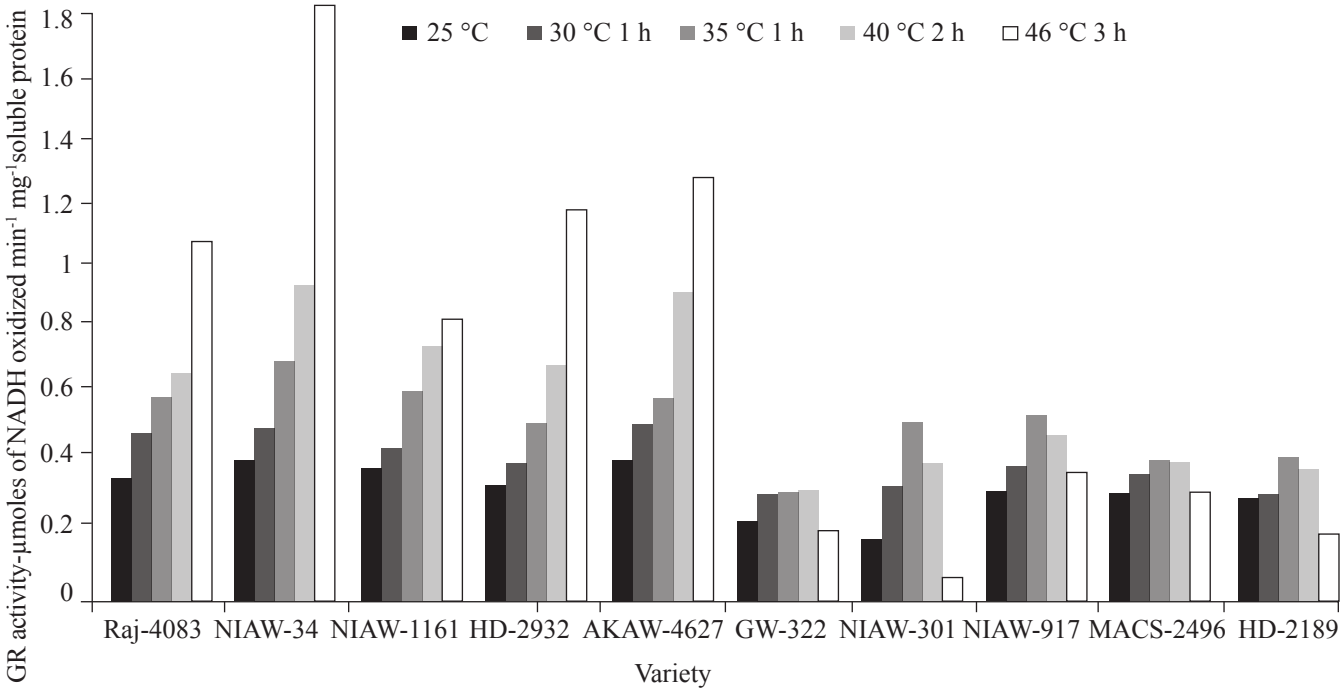

Figure 1e: Activity of Glutathione reductase

40 °C 2 h and 46 °C 3 h as reported by many other authors in wheat seedlings exposed to heat stress (Balla et al., 2013).

In this study, high temperature induced the reduction in catalase activity was compensated for parallel increase in peroxidase activity. It means detoxification of hydrogen peroxidase under heat stress is due to participation of peroxidase enzymes with other enzymes like SOD, APX, and GR. The results of present investigation indicates that catalase is very sensitive to high temperature, thus declined activity in thermo-susceptible genotypes. Our results of catalase activity are in agreement with the results recorded in wheat by Balla et al. (2013). Ascorbate peroxidase is a major enzyme responsible for elimination of H_2O_2. One such system is ascorbate peroxidase and glutathione reductase has been reported in tolerant wheat cultivar (Almeselmani et al., 2009). In wheat, the activities of APX and GR were maintained at higher level at 40 °C 2 h and 46 °C 3 h in thermo-tolerantgenotypes suggest that ascorbate glutathione cycle play a crucial role in mitigating the accumulation of H_2O_2 in wheat under gradual increase of heat stress. There are in agreement with the earlier results of Almeselmani et al. (2009) in wheat and Lin et al. (2010) in broccoli and Chinese cabbage. The declined in the GR activity at 38-39 °C for 2 h followed by 46-48 °C for 8 h resulted 50% reduction in catalase and glutathione reductase activity has been reported by Babu and Devaraj (2008). Increased levels of glutathione reductase under abiotic stresses including heat stress has been reported in wheat (Sairam et al., 2000; Almeselmani et al., 2009) and in other crops like bent grass and Lily (Xu et al., 2006; Yin et al., 2008).

4. Conclusion

A significant increase in activities of ROS scavenging enzymesin wheat seedlings from 35 °C 1 h to 46 °C 3 h heat stress showed their involvement in removing ROS during oxidative stress. Among thermo-tolerant NIAW-34 and AKAW-4627 genotype and thermo-susceptible NIAW-917 genotype showed better adaptability at higher temperature stress. GB and proline increased in thermo-tolerant group of wheat genotypes with higher content of Chlorophyll 'b' and lower LPO indicates their involvement in maintaining membrane integrity and in activation of ROS scavenging mechanism. Proline levels showed more fluctuation and need further clarification. It is concluded that significantly higher levels of osmolytes like Glycinebetaine with relative water content and chlorophyll content and higher activities of enzymes SOD, GPOX, CAT, APX, and GR can be used efficiently for assessing thermo-tolerant in wheat breeding.

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