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Temperature stress induced antioxidative and biochemical changes in wheat (*Triticum aestivum* L.) cultivars

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ABSTRACT

The aim of this study is to understand the effect of elevated temperature on wheat seedlings of four cultivars, gayetri (GY), gandhari (GN), kedar (KD), PBW343 in terms of tolerance/susceptibility, along with antioxidative and biochemical responses. For this, seedlings of four cultivars were exposed to different temperatures ranging from 25°C to 40°C for 6 h. According to heat susceptibility index calculated, GN was found to be heat tolerant, PBW343 heat sensitive, and the other two, GY and KD moderately tolerant. Exposure to high temperature led to a gradual increase in membrane lipid peroxidation and hydrogen peroxide accumulation. H_2O_2 and malonaldehyde accumulated to much higher amount in leaf tissues of PBW343 and GY than GN and KD. Among antioxidative enzymes, catalase, glutathione reductase, and ascorbate peroxidase activity initially increased at 35°C, followed by reduced activity under heat stress in all cultivars. However, peroxidase activity continued to increase under heat stress. Total chlorophyll and non-enzymatic antioxidant carotenoids, initially increased up to 30°C, following which there was a gradual decrease in these components. Compatible osmolytes - proline and total sugar level within cellular compartments were enhanced up to 35°C. Results suggested that wheat can endure heat-induced oxidative stress up to the certain period, manifested by elevation of non-enzymatic antioxidants and osmoprotectants, as well as upregulation of antioxidative enzymes.

KEY WORDS: Heat stress, *Triticum aestivum* L., heat susceptibility index, oxidative stress, nonenzymatic antioxidants, antioxidative enzymes, lipid peroxidation, hydrogen peroxide

INTRODUCTION

High temperature is one of the key abiotic stresses for hampering plant growth and yield. (Hasanuzzaman *et al.*, 2012). The most favorable temperature for wheat cultivation is between 17°C and 23°C (Porter and Gawith, 1999). High temperature up to 47-48°C negatively affect wheat plant growth and development and adversely affects productivity in many parts of the world (Hameed *et al.*, 2010). Exposure of plants to high temperature creates imbalance of physiological and biochemical activity which may have negative impact on water relations, photosynthesis, respiration and membrane stability due to changes in membrane fluidity and permeability (Mavi and Tupper, 2004) which can lead to cell damage and cell death resulting in calamitous break down in cellular organization (Schoffl *et al.*, 1999).

Like other abiotic stresses, heat stress results in production of excessive reactive oxygen species (ROS) such as superoxide radical ($O^{2\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), and singlet oxygen ($^1O_2$) causing disparity between anti and pro-oxidants (Wahid *et al.*, 2007). The hydroxyl radical OH^- can damage plant proteins, different macromolecules, DNA and lipids, chlorophyll preventing growth and yield (Sairam and Tyagi, 2004). H_2O_2 oxidizes thiol group of enzymes resulting in inactivation of enzymatic activity as well as at low concentration it acts as signaling molecule for different biotic and abiotic stresses, but at high concentration, it causes program cell death (Quan *et al.*, 2008). ROS produced within plant tissue by heat stress damages membrane by lipid peroxidation forming minute hydrocarbon fragments, like malonaldehyde (MDA), one of the important indicators of oxidative stress. As a result

of increased lipid peroxidation phospholipids easily can cross lipid bilayer and causing enhancement of membrane leakage and inactivation of receptors, ion channels, and membrane-bound enzymes (Gill and Tuneja, 2010).

Production of ROS within plants tissues turns on different enzymatic and non-enzymatic signaling pathways. Antioxidative enzymes contributing to stress signaling such as peroxidase, catalase (CAT), ascorbate peroxidase (APOX), glutathione reductase, and superoxide reductase (Lee and Lee, 2000). Superoxide dismutases are the first enzyme to initiate ROS scavenging mechanism participate in dismuting of $O^{2.-}$ radical to molecular O_2 and H_2O_2. Hydrogen peroxide is then converted to H_2O and O_2 either by CAT and peroxidase or in ascorbate-glutathione cycle by APOX and glutathione reductase help to regenerate glutathione pool resulting detoxification of H_2O_2 (Foyer and Noctor, 2003). Non-enzymatic antioxidants such as glutathione, tocopherol, ascorbic acid, and proline carotenoid play an important role in protecting the plant from stress-induced oxidative injury by performing photoprotective role and by inhibition of lipid peroxidation (Gill and Tuneja, 2010; Ashraf and Foolad, 2007).

Keeping in view the burning problem of global warming which may result into yield loss, negative impact on growth and photosynthetic ability, the present investigation was proposed to understand the antioxidative response of tolerant and susceptible cultivars of wheat to high-temperature stress with the main emphasis to study the role of antioxidative enzymes in imparting tolerance to wheat.

MATERIALS AND METHODS

Plant Material and Experimental Conditions

Seeds of four locally available wheat (*Triticum aestivum* L.) cultivars, namely gayetri (GY), gandhari (GN), kedar (KD), and PBW343, were chosen and collected for experimental purposes. These locally available cultivars were selected for these experiments to compare responses of different cultivars subjected to high-temperature stress. The seeds were surface sterilized with 0.1% (w/v) $HgCl_2$ solution, washed with distilled water. Then, the seeds were transferred to earthen pots containing soil with an appropriate amount of compost. The transferred seedlings in the pots were then kept in growth chamber at a favorable temperature of 20-25°C. 1 month old seedlings were selected for heat stress. Pots containing 1 month old seedlings were exposed to different temperatures (25°C, 30°C, 35°C, and 40°C) for 6 h. Leaf samples from control and heat-stressed seedlings were collected and used for analysis. The fresh weight of seedlings was taken immediately after collecting to avoid any water loss from leaf samples of both control and treated seedlings.

Relative Water Content (RWC) and Heat Susceptibility Index (HSI)

RWC was calculated by determining the fresh weight, turgid weight, and dry weight of leaf samples under treatment and using formula given by Farooqui *et al.*, 2000.

$$\mathrm{RWC}(\%) = \frac{(\text{Fresh weight} - \text{dry weight})}{(\text{Fully turgid weight} - \text{dry weight})} \times 100$$

HSI was calculated using variation in fresh weight and RWC (degree in percent decrease) using following formula (Hameed *et al.*, 2010).

$$\mathrm{HSI} = \frac{(\text{Reduction in seedling FW} + \text{\% reduction in RWC})}{2}$$

Calculated HSI values of different cultivars were compared to have an idea about degree of heat susceptibility of different cultivars.

Extraction and estimation of total sugar

Extraction of total sugar was done by following the method of Harborne (1973) by crushing of leaf samples in 95% ethanol, and the alcoholic fraction was evaporated on a boiling water bath, and the aqueous fraction was resuspended in distilled water which was then centrifuged at 5000 rpm for 10 min. The supernatant was collected for estimation of total sugar. Estimation of total sugar was done by anthrone reagent following the method of Plummer (1978).

Extraction and estimation of total chlorophyll

Chlorophyll was extracted following the method of Harborne (1973) by homogenizing leaf in 80% acetone. Estimation of chlorophyll was done by measuring the absorbance at 645 nm and 663 nm, respectively, in an ultraviolet (UV)-vis spectrophotometer and calculated using the formula as given by Arnon (1949).

Total chlorophyll = $(20.2\ A_{645} + 8.02\ A_{663})$ mg g^{-1} fresh weight.

Extraction and quantification carotenoids

Method described by Lichtenthaler (1987) was pursued for carotenoids extraction and estimation. For this, leaf samples of both control and treated plants were crushed using 100% methanol in dark and extract was filtered.

Then, the absorbance of filtrate was taken at 663,645 and 480 nm, respectively, in the UV-vis spectrophotometer.

Extraction and estimation of free proline

Extraction of free proline from the leaves was performed following the method of Caverzan *et al.* (2012). 0.5 g of leaf tissue was homogenized in 3% sulfosalicylic acid and filtered through a Whatman No. 1 filter paper. The supernatant was used for estimation. Quantification of proline was done by the mixing filtrate with 2 ml of acid ninhydrin reagent. After that, the mixture was kept for1 h in a boiling water bath and chilled rapidly. The reaction mixture was then shifted in separating funnel, and 5 ml of toluene was added and mixed vigorously. The lower colored layer was taken to measure absorbance at 520 nm in a colorimeter using toluene as blank and quantified from a standard curve of proline.

Quantification of H_2O_2

H_2O_2 within leaf tissue was measured by following the method described by Jena and Choudhuri (1981). A definite quantity of the leaf samples was taken and crushed in 50 mM potassium phosphate buffer (pH 6.5). Crushed samples were centrifuged at 2,415 g for 25 min. Whole experiment was done in minimal light conditions (Chakraborty and Pradhan, 2011). The reaction was completed as an intense yellow color started developing. This was monitored at 410 nm spectrophotometrically. The levels of H_2O_2 in the samples were determined by the use of extinction coefficient 0.28 μmol^{-1} cm^{-1} in the calculation.

Determination of lipid peroxidation

MDA is an indicator of lipid peroxidation within the plant. The presence of MDA was measured by following the method described by Heath and Packer (1968). It was measured by thiobarbiturate reaction where leaf tissue was homogenized in 0.1% (w/v) trichloroacetic acid. Absorbance of MDA content was then measured at 600 and 532 nm and MDA was quantified using extinction coefficient of 155 mM^{-1} cm^{-1}.

Extraction and Estimation of Antioxidative Enzymes

Extraction

Leaf samples were homogenized in 50 mM sodium phosphate buffer (For peroxidase and CAT [pH 6.8]; and ascorbate peroxidase (APOX) [pH 7.2]) and 100 mM potassium phosphate buffer pH 7.6 (For glutathione reductase and superoxide dismutase) using PVP under ice cold conditions. The homogenates were then centrifuged at 10,000 rpm for 10 min at 4°C. Supernatants were used as crude enzyme extracts.

Estimation of antioxidative enzymes

Glutathione reductase (GR, EC 1.6.4.2)

GR activity was evaluated by quantifying the oxidation of NADPH at 340 nm as described by Lee and Lee (2000). The reaction mixture consisted of 1 ml of 0.1 M potassium buffer (pH 7.6), 0.2 ml of 0.1 M ethylenediaminetetraacetic acid, 0.1 ml of freshly prepared 6 mM glutathione, 0.2 ml of 0.1 mM NADPH, and 0.2 ml of enzyme extract. Change in absorbance was measured instantly at 340 nm in the UV-VIS spectrophotometer.

APOX, EC 1.11.1.11

APOX activity was measured by quantifying oxidation of ascorbate in the UV-vis spectrophotometer following the method of Asada and Takahashi (1987). Enzyme activity was expressed as mmol ascorbate mg $protein^{-1}$ min^{-1}.

Peroxidase (POX, EC 1.11.17)

POX activity was assayed by monitoring the oxidation of o-dianisidine in the presence of H_2O_2 at 460 nm following the method of Chakraborty *et al*. (1993). The change in absorbance was noted down immediately at specific intervals up to 3 min in the UV-vis spectrophotometer. The POX activity was expressed as mmol O-dianisidine mg $protein^{-1}$ min^{-1}.

CAT (CAT, EC 1.11.1.6)

CAT activity was assayed following the method of Chance and Maehly (1955) by estimating the decomposition of H_2O_2 at 240 nm in the UV-VIS spectrophotometer. The enzyme activity was expressed as mol H_2O_2 mg $protein^{-1}$ min^{-1}.

Polyacrylamide gel electrophoresis (PAGE) analysis of peroxidase isozymes

PAGE was performed for isozyme analyses of peroxidase and CAT enzymes by following the method given by Davis (1964) and Reddy and Gasber (1971).

Statistical Analysis

Data were investigated statistically by the least significance test difference at $P = 0.05$ probability level.

RESULTS

It is necessary to recognize different biochemical and physiological changes proceeding within plant tissue to develop heat tolerant wheat cultivars. The objective of this study is to evaluate different biochemical parameters within four wheat cultivars subjected to temperature-induced oxidative stress. Findings regarding tolerance response under heat stress in terms of antioxidative and biochemical response are given below.

Evaluation of Heat Susceptibility of Cultivars

Elevated temperature significantly decreased fresh weight of seedlings ($P = 0.05$) in all four wheat cultivars. Reduction in fresh weight ranges from 21.97% highest in PBW343 to 4.02% lowest in GN at 40°C. FW in the leaf of all the cultivars gradually reduced with elevated temperature stress (Figure 1). RWC change (Figure 2) was lesser in case of GN and KD at 40°C when compared to their respective controls (20.60% and 30.98%, respectively); however, the heat-induced reduction in RWC was much more in GY and PBW343 in respect to their controls (36.48% and 42.63%, respectively). HSI of GN, KD, GY, and PBW343 are 11.65, 19.73, 25.48, 30.90, respectively. Maximum and minimum values were noticed in case of PBW343 and GN, making these two heat sensitive and heat tolerant cultivars, respectively, and the other two cultivars KD and GY were moderately heat tolerant and moderately heat sensitive among four cultivars (Table 1).

Table 1: HSI of four wheat cultivars

Cultivars	HSI
GN	11.65
KD	19.73
GY	25.48
PBW343	30.9

Calculated on the basis of 3 replicate sets of 10 seedlings each.
GN: Gandhari, KD: Kedar, GY: Gayetri, HSI: Heat susceptibility index

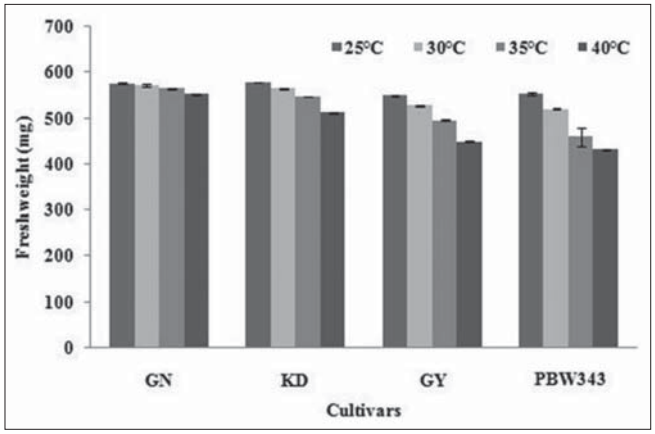

Figure 1: Fresh weight of leaf of four wheat cultivars exposed to heat stress. Results are articulated as the mean±standard deviation. *n*=4. Least significance test difference (*P*=0.05) within cultivars=162.9 and within treatments=155.50

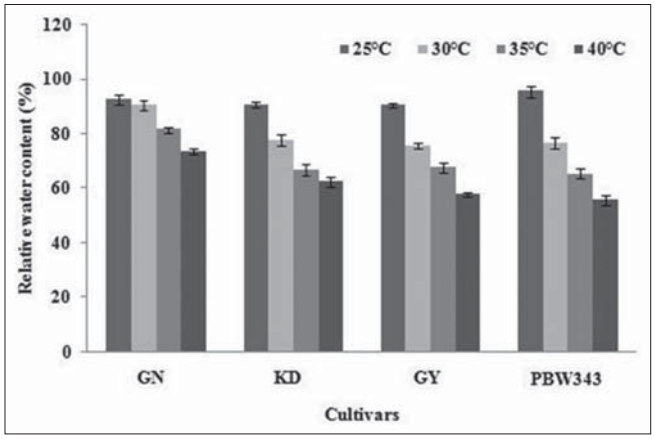

Figure 2: Relative water content of four cultivars exposed to heat stress. Results are articulated as the mean±standard deviation. *n*=4. Least significance test difference (*P*=0.05) within cultivars=25.31 and within treatments=14.19

Biochemical Response to Elevated Temperature

Lipid peroxidation and H_2O_2 content

Temperature stress enhanced membrane peroxidation as reflected by the elevated level of MDA and H_20_2 (Table 2). In all four cultivars, MDA content gradually increased with elevated temperature. Accumulation of MDA with elevated temperature in relation to control was highest in heat sensitive PBW343 (3.55-fold higher than control) and minimum in heat tolerant GN (0.61-fold higher than control).

Similarly, maximum accumulation of H_2O_2 was noticed within leaf samples of heat sensitive PBW343 and minimum in GN.

Antioxidative Response

Activities of antioxidative enzymes in both control and treated seedlings were analyzed. Among four antioxidative enzymes, APOX (Figure 3a) and glutathione reductase (Figure 3b) activity enhanced up to 30°C. Similarly, CAT activity (Figure 4a) gradually increased up to 35°C in GN, while it decreased after 30° in all other cultivars. Enhancement of CAT was maximum in case of GN (1.02-fold higher in relation to control) and minimum in PBW343 (0.07-fold higher in relation to control) (Figure 4a). The highest reduction at 40° was noticed in PBW343 and lowest in GN, which was in conformity with results from HSI.

On the other hand, peroxidase activity continued to increase with elevated temperature in all four cultivars in relation to control. The highest increase was noticed in GN at 40°C (3.58-fold), and lowest was obtained in PBW343 (0.78-fold) (Figure 4b).

Peroxidase isozyme analysis of four cultivars was performed. Isozyme analysis showed an increase in band number and color intensity with elevated temperature stress in all four cultivars. In heat tolerant cultivar, GN color of bands was much more intense than heat susceptible cultivars (Figure 5).

Table 2: Effect of elevated temperatures on lipid peroxidation and H_2O_2 content

Cultivars	MDA Content (μ mol g tissue^{-1})				H_2O_2 Content (μ mol g tissue^{-1})			
	25°C	30°C	35°C	40°C	25°C	30°C	35°C	40°C
GN	0.49±0.03	0.66±0.01	0.75±0.02	0.79±0.02	157.36±0.80	193.53±0.87	198.86±1.03	200.10±1.63
KD	0.60±0.03	1.19±0.07	1.18±0.09	1.34±0.06	130.45±0.87	183.3±1.06	197.34±1.23	210.19±1.01
GY	0.54±0.06	1.23±0.10	1.86±0.03	1.97±0.03	151.27±0.76	195.65±1.06	237.45±1.01	240.22±1.26
PBW343	0.43±0.06	1.12±0.01	1.75±0.05	1.96±0.06	126.79±1.14	185.45±1.19	245.30±0.95	250.38±01.41

Mean±SD; *n*=4; LSD (*P*=0.05) within cultivars=1.48 and within treatments=2.03 (MDA Content), LDS value (*P*=0.05) within cultivars=54.51 and within treatments=54.5.5 (H_2O_2 content). GN: Gandhari, KD: Kedar, GY: Gayetri, HSI: Heat susceptibility index, SD: Standard deviation, LSD: Least significance test difference, MDA: Malonaldehyde

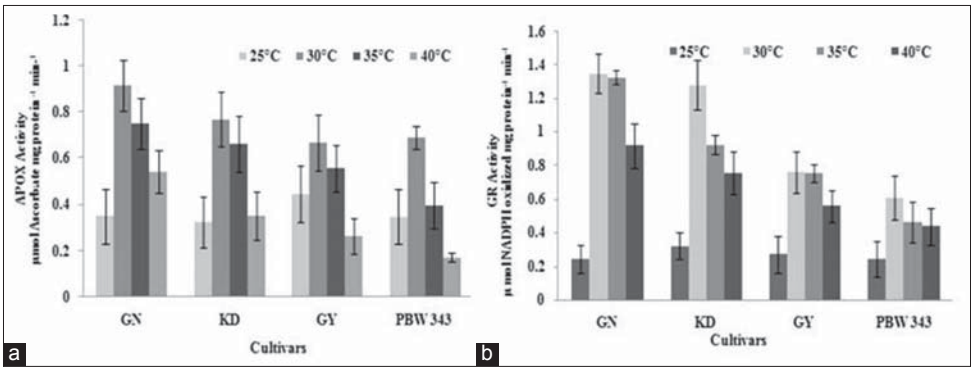

Figure 3: Effect of elevated temperature on ascorbate peroxidase and glutathione reductase activities in the four cultivars exposed to heat stress. Results are expressed as mean±standard deviation. *n*=4. (a) Least significance test difference (LSD) (*P*=0.05) within cultivars=0.443 and within treatments=0.904, (b) LSD (*P*=0.05) within cultivars=1.01 and within treatments=1.432

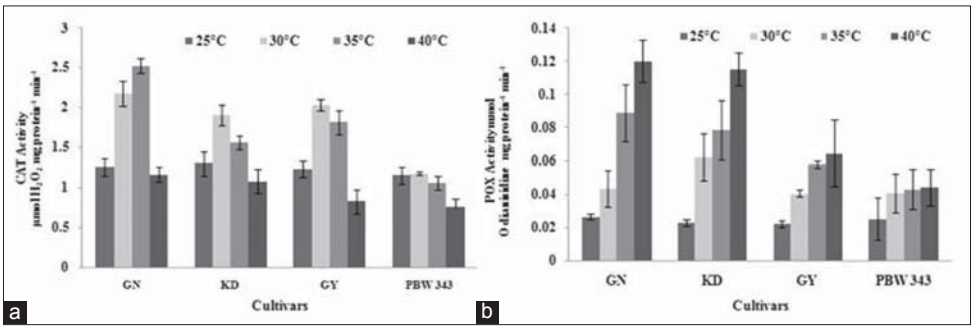

Figure 4: Effect of elevated temperature on catalase and peroxidase activities in four wheat cultivars exposed to heat stress. Results are expressed as mean±standard deviation. *n*=4. (a) Least significance test difference (LSD) (*P*=0.05) within cultivars=1.37 and within treatments=1.86, (b) LSD (*P*=0.05) within cultivars=0.07 and within treatments=0.01

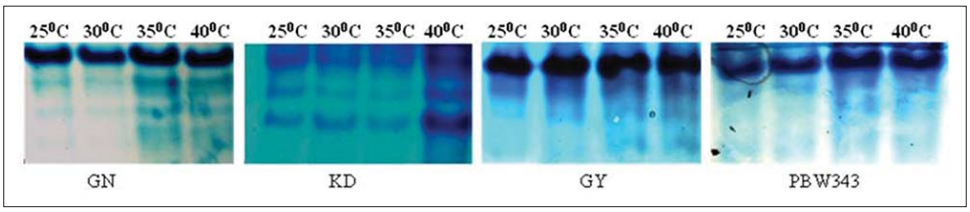

Figure 5: Peroxidase isozyme analysis following heat treatment by polyacrylamide gel electrophoresis

Carotenoid and Chlorophyll

Carotenoid (Figure 6a) enhanced significantly in all four cultivars at 30°C. At 30°C, carotenoid content was 50.01%, 29.50%, 53.48%, and 30.90 % higher than control in GN, KD, GY, PBW343, respectively. In case of GN and KD with further raising of temperature up to 35°C, there was slight increase in carotenoid content, on contrary in other two varieties raising temperature beyond 35°C reduced carotenoid content within leaf tissue.

6 h incubation at 30°C led to an increase in total chlorophyll in all cultivars. Further increase in temperature resulted

in a decline in total chlorophyll content in all cultivars (Figure 6b).

Proline and Total Sugar

Proline content in GN, KD, GY, and PBW343 was 1.87, 2.29, 1.77, and 1.68 times higher, respectively, in relation to control at 35°C (Figure 7a). Raising temperature from 35°C to 40°C resulted in decrease in accumulation of proline in leaf tissues of all four cultivars. There was sharp increase in total soluble sugar within leaf tissue of all four cultivars with gradual increase in temperature suggesting possible role of total soluble sugar in heat-induced oxidative stress regulation. The highest increase was noticed in KD at 40°C (1.34-fold) and lowest noticed in PBW343 (0.974-fold) in relation to control (Figure 7b).

DISCUSSION

High-temperature stress beyond certain limit irreversibly affects plant growth and development (Wahid, 2007). Plants act in response to different abiotic stresses by shifting and changing concentration of metabolite related to different biochemical pathways so that they can acclimatize to unfavorable physiological changes (Kumar *et al.*, 2012). Heat stress produces a significant reduction in fresh weight and RWC in aerial parts of plants (Rodríguez *et al.*, 2015). In this study, FW and RWC gradually reduced with an increase in temperature in all four wheat cultivars and heat susceptibility index was calculated using these two parameters. Based on HSI, PBW343 was identified as more heat susceptible variety, and GN was most tolerant variety. Heat stress generates excessive ROS than optimal level resulting in enhanced lipid peroxidation and H_2O_2 production in leaf tissue (Kipp and Boyle, 2013). H_2O_2 is related to oxidative injury in plants (Ozden *et al.*, 2009) and act as signaling molecule which upregulate genes related to scavenging of free radicals from cell via a number of antioxidant mechanisms including both enzymatic and non-enzymatic (Kumar *et al.*, 2012).

In the present study, lipid peroxidation as indicated by MDA content and H_2O_2 increased significantly in all cultivars, but augmentation was higher in case of heat susceptible lines than in heat tolerant lines. This result was in accordance with previous studies in wheat (Tatar and Gevrek, 2008) and in mung bean (Mansoor and Naqvi, 2013). Increased lipid peroxidation and H_2O_2 level inactivate cell membrane enzymes and proteins,

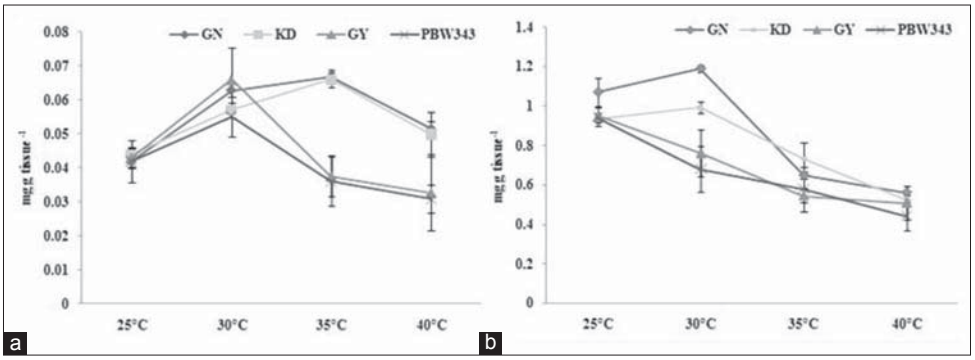

Figure 6: Changes in carotenoid (a) and total chlorophyll, (b) content in four cultivars after heat treatments. Results are expressed as mean±standard deviation. *n*=4. (a) Least significance test difference (LSD) (*P*=0.05) within cultivars=0.02 and within treatments=0.05, (b) LSD (*P*=0.05) within cultivars=0.614 and within treatments=0.915

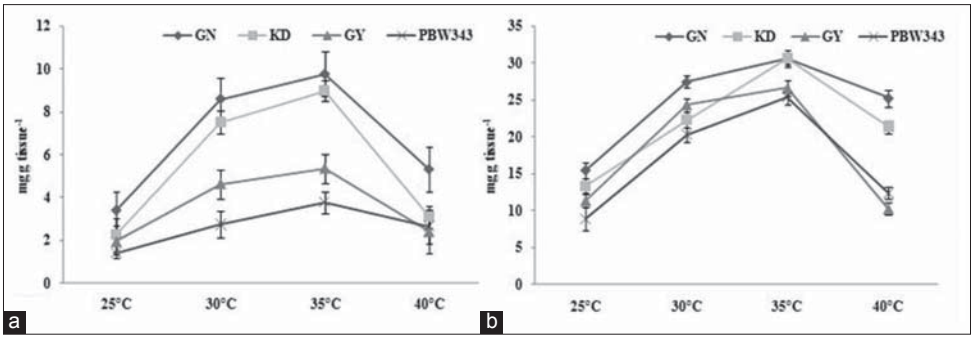

Figure 7: Changes in free proline, (a) and total soluble sugar, (b) contents in four cultivars after heat treatments. Results are expressed as mean±standard deviation. *n*=4. (a) Least significance test difference (LSD) (*P*=0.05) within cultivars=7.33 and within treatments=8.15, (b) LSD (*P*=0.05) within cultivars=16.23 and within treatments=13.60

consequently antioxidative (enzymatic and non-enzymatic) machinery avert cell damage by regulating level of ROS (Ge *et al.*, 2005).

Antioxidative enzymes such as POX, APX, GR, and CAT play important roles in the alleviation of ROS. In the present study, exposure to higher temperature initially upregulated enzyme activity up to 35°C in all cultivars, indicating that higher temperatures could trigger antioxidative enzymes to scavenge H_2O_2 and ROS (Gosavi, 2014). However, further increase in temperature revealed different activities. Peroxidase continued to increase significantly with temperature in all cultivars. Probable role of POX is to defend cells against oxidative damage of H_2O_2 by decomposition through oxidation of phenolic and endiolic cosubstrates (Lin and Kao, 2002). Both APOX and GR are engaged in the ascorbate-glutathione pathway and work in combination for scavenging of the radicals. Maximum GR, APOX, and CAT activity was noticed at 30°C in all cultivars except CAT activity for GN, wherein the activity was found to be highest at 35°C.

Beyond 35°C, all these three enzyme activities (GR, APOX, and CAT) decreased gradually, but downregulation of enzyme activity was minimum in heat tolerant variety and maximum in heat susceptible variety making it prominent that plants cannot endure heat stress beyond certain threshold level. Chakraborty and Pradhan (2010) reported an initial increase of antioxidative enzyme activity in lentil subjected to high temperature up to 45°C. From these finding, it is clear that thermotolerant cultivars better ability to withstand damaging effect of H_2O_2 and lipid peroxidation induced by higher temperature through antioxidative machinery.

Changes in photosynthetic elements and photosynthesis are good indicators of high temperature induced oxidative stress and degree of thermotolerance of the plant. High temperature limits photosynthesis and plant growth (Wahid *et al.*, 2007). In the present investigation, chlorophyll content gradually reduced after an initial increase at 30°C in all cultivars except in relatively tolerant variety GN where it increased 35°C. Since membrane integrity is immediately affected by high temperature, chlorophyll structure damage may be one of the reasons for reduced photosynthesis (Mansoor and Naqvi, 2013). Similar results also found in several crops including maize and rice (Kumar *et al.*, 2012).

Other than enzymes non-enzymatic antioxidant carotenoid and osmoprotectant proline plays crucial role in oxidative stress tolerance. In all the four cultivars, maximum accumulation of proline and carotenoid took place at 35°C. According to Sairam and Tyagi (2004), accumulation of proline and total sugar might contribute to heat tolerance. Increasing concentration of soluble sugar was reported in sugarcane (Wahid 2007). Carotenoids are considered to be the main $^1O_2$ quenchers in chloroplasts. Carotenoids facilitate to defend the plants from oxidative injury by preventing the production of singlet oxygen (Havaux, 2013). Beyond 35°C, carotenoid and proline content continue to decrease. However, the percentage of reduction was higher in heat susceptible cultivars than heat tolerant varieties.

CONCLUSION

Outcomes from our study clearly indicate that high temperature was responsible for the bringing on oxidative stress as shown by reduction in fresh weight and RWC, increase in the MDA content (i.e., lipid peroxidation), H_2O_2 accumulation, degradation of chlorophyll molecule, enhanced antioxidative responses as evident in the differential levels of antioxidative enzyme activities, accumulation of different non-enzymatic antioxidants carotenoid, and osmolytes such as soluble sugars. Out of the four cultivars taken for the study, GN and KD showed a much more distinct antioxidative mechanism after the induction of heat stress, and therefore, they seemed to be cosseted from the harmful damage caused by oxidative stress as a result of heat stress and increased severity of stress. Considering all the above data and comparing with HSI, it was found that among the four cultivars GN was able to withstand the heat stress more effectively than the other three cultivars, whereas PBW343 was more susceptible to heat stress than the other cultivars.

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