

Heat Stress-Induced Cell Death, Changes in Antioxidants, Lipid Peroxidation, and Protease Activity in Wheat Leaves

Amjad Hameed · Madiha Goher · Nayyer Iqbal

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Abstract Six wheat genotypes were evaluated for heat tolerance in terms of seedling growth, antioxidant response and cell death. Based on the heat susceptibility index (HSI), response of the genotypes varied from heat tolerant (Inqilab-91) to heat sensitive (Sitta) along with moderately tolerant (Nesser and Sarsabz) and sensitive (Fareed and FD-83). Heat stress-induced programmed cell death (probably necrosis) in wheat leaves was evident by DNA smear. MDA content increased above twofold in most of genotypes under heat stress, with the lowest increase in the heat-tolerant genotype Nesser. Catalase activity diminished under heat stress in all genotypes. Peroxidase, superoxide dismutase (SOD), protease, and ascorbate peroxidase (APX) activities increased under heat stress. Apparently, heat stress-induced reduction in catalase activity was compensated by a parallel increase in peroxidases to quench H₂O₂. Heat stress-induced decrease (%) in catalase and increase in protease activities showed significant positive correlations, whereas increase (%) in APX activity showed a significant negative correlation with HSI or relative heat tolerance of genotypes. All these correlations signify that catalase, protease and ascorbate peroxidase can be used efficiently as biochemical markers to assess the relative heat stress tolerance of wheat genotypes at the seedling stage. In conclusion, using a multiparametric approach involving morphophysiological and biochemical assays, the sensitivity of wheat genotypes to heat stress could be evaluated to a sufficient level of certainty at the seedling stage.

Keywords Ascorbate peroxidase · Catalase · HSI necrosis · *Triticum aestivum* L.

Introduction

Plants live under the effect of multiple simultaneous stress factors in natural habitats or fields. High temperature can limit productivity of several important crops (Ishag and Mohamed 1996; Rainwater and others 1996). Wheat grows optimally between 17 and 23°C (Porter and Gawith 1999). The lethal maximum temperature for wheat is 47°C for unhardened and 48°C for hardened cultivars (Drozdov and others 1984). Elevated temperature as heat stress inhibits wheat growth (Slafer and Rawson 1995; Ishag and Mohamed 1996) and adversely affects productivity in many parts of world.

Field screening for abiotic stresses like heat stress is difficult due to uncertain environmental changes (for example, rainfall, temperature fluctuation). Several methods based on growth (Hameed and others 2010), physiological traits (Dash and Mohanty 2001; Yildiz and Terzi 2008), and biochemical (Iqbal and others 2010; Hameed and others 2011) traits as selection criteria have been suggested to screen the germplasm for abiotic stresses. Assays like membrane thermostability and ion leakage (Saadalla and others 1990; Shanahan and others 1990) have been used as probes in wheat for heat stress tolerance studies. Moreover, leaf growth, pigmentation, membrane lipid stability, photosynthesis rates and chlorophyll *a* fluorescence characteristics of primary leaves have been tested for use as indices in screening wheat cultivars at an early developmental growth stage for seedling heat-stress tolerance (Dash and Mohanty 2001). Establishment of a reliable

A. Hameed (✉) · M. Goher · N. Iqbal
Nuclear Institute for Agriculture and Biology (NIAB),
P.O. Box 128, Jhang Road, Faisalabad, Pakistan
e-mail: amjad46pk@yahoo.com

and rapid assay system is still required for screening wheat germplasm resistant to elevated temperature.

Environmental stresses lead to the generation of reactive oxygen species (ROS). Heat stress can induce oxidative stress along with tissue dehydration. Generation and reactions of ROS, that is, singlet oxygen, superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\bullet OH$), are common events during cellular injury by high temperature (Liu and Huang 2000) and drought (Farooq and others 2009). Autocatalytic peroxidation of membrane lipids and pigments by ROS leads to loss of membrane semipermeability (Xu and others 2006). Plants have evolved both enzymatic and nonenzymatic mechanisms to scavenge the rapidly evolving ROS. Enzymes, including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), and glutathione reductase (GR) (Zhang and others 1995; Lee and Lee 2000), and nonenzymatic antioxidants such as tocopherols, ascorbic acid (AsA), and glutathione (GSH) (Wingsle and Hallgren 1993; Kocsy and others 1996; Noctor and Foyer 1998; Noctor and others 1998) work in concert to detoxify ROS. The removal of O_2^- by superoxide dismutase (SOD) generates H_2O_2 , which is removed by ascorbate peroxidase and catalase. However, both O_2^- and H_2O_2 are not as toxic as the OH^- , which is formed by the combination of O_2^- and H_2O_2 . The hydroxyl radical OH^- can damage chlorophyll, protein, DNA, lipids, and other important macromolecules, thus fatally affecting plant metabolism and limiting growth and yield (Sairam and Tyagi 2004).

In this study, various biochemical assays were used to test heat sensitivity of wheat genotypes and to assess the potential of different markers for developing a reliable and rapid screen of cultivars. Efforts were made to find the correlation among these biochemical assays and sensitivity to heat stress and to reveal the morphobiochemical indices that can be useful to screen for heat tolerance in wheat at the seedling stage.

Material and Methods

The experiment was conducted using six wheat (*Triticum aestivum* L.) genotypes (Nesser, Inqilab-91, Sitta, Sarsabz, FD-83 and Fareed). Seeds were germinated in plastic pots in triplicate (30 seedlings per replication). The pots were filled with an equal quantity of soil and irrigated at soil water holding capacity. For application of heat stress, 11-day-old seedlings were exposed to $45 \pm 1^\circ C$ for 20 h in an incubator while growth of control seedlings was continued at $25 \pm 1^\circ C$. Leaf samples from control and heat-stressed seedlings were collected and used for biochemical analysis. Seedling growth response to heat stress was also

analyzed. The fresh weight of seedlings was measured immediately after harvesting to avoid any evaporation. Dry weight was measured after complete drying at $90^\circ C$ when there was no further decrease in weight.

For estimation of enzymes, fresh leaves (0.5 g) were ground in cold extraction buffer specific for different enzymes. Samples were centrifuged at $15,000 \times g$ for 20 min at $4^\circ C$. The supernatant was separated and used for the determination of different enzyme activities.

Superoxide Dismutase (SOD)

For the estimation of SOD activity, leaves were homogenized in a medium composed of 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, and 1 mM dithiothreitol (DTT) as described by Dixit and others (2001). The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) following the method of Giannopolitis and Ries (1977). One unit of SOD activity was defined as the amount of enzyme that caused 50% inhibition of photochemical reduction of NBT.

Peroxidase (POD) and Catalase (CAT)

For the estimation of peroxidase, leaves were homogenized in a medium composed of 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, and 1 mM dithiothreitol (DTT). Activity of peroxidase (POD) was measured using the method of Chance and Maehly (1955) with some modification. For measurement of POD, the activity assay solution (3 ml) contained 50 mM phosphate buffer (pH 7.0), 20 mM guaiacol, 40 mM H_2O_2 , and 0.1 ml enzyme extract. The reaction was initiated by adding the enzyme extract. The increase in absorbance of the reaction solution at 470 nm was recorded after every 20 s. One unit of POD activity was defined as an absorbance change of 0.01 min^{-1} .

For the estimation of catalase, leaves were homogenized in a medium composed of 50 mM potassium phosphate buffer (pH 7.0) and 1 mM dithiothreitol (DTT). Catalase (CAT) was estimated using the method described by Beers and Sizer (1952). For measurement of CAT activity, the assay solution (3 ml) contained 50 mM phosphate buffer (pH 7.0), 59 mM H_2O_2 , and 0.1 ml enzyme extract. The decrease in absorbance of the reaction solution at 240 nm was recorded after every 20 s. An absorbance change of 0.01 min^{-1} was defined as 1 U of CAT activity. Enzyme activities were expressed on a fresh weight basis. Total soluble protein concentration was measured by the dye binding assay as described by Bradford (1976).

Protease Activity

For the estimation of protease, leaves were homogenized in a medium composed of 50 mM potassium phosphate buffer (pH 7.8). Protease activity was determined by the casein digestion assay described by Drapeau (1974). By this method 1 U is the amount of enzyme that releases acid-soluble fragments equivalent to 0.001 A280 per minute at 37°C and pH 7.8. Enzyme activity was expressed on a fresh weight basis.

Malondialdehyde (MDA) Content

The level of lipid peroxidation in the leaf tissue was measured in terms of malondialdehyde (MDA, a product of lipid peroxidation) content determined by the thiobarbituric acid (TBA) reaction using the method of Heath and Packer (1968), with minor modifications as described by Dhindsa and others (1981) and Zhang and Kirham (1994). A 0.25-g leaf sample was homogenized in 5 ml 0.1% TCA. The homogenate was centrifuged at 10,000×g for 5 min. To 1-ml aliquot of the supernatant, 4 ml of 20% TCA containing 0.5% TBA was added. The mixture was heated at 95°C for 30 min and then quickly cooled in an ice bath. After centrifugation at 10,000×g for 10 min, the absorbance of the supernatant at 532 nm was read and the value for the nonspecific absorption at 600 nm was subtracted. The MDA content was calculated by using an extinction coefficient of 155 mM⁻¹ cm⁻¹.

Total Phenolic Content

A microcolorimetric method, as described by Ainsworth and Gillespie (2007), was used for total phenolics assay, which utilizes Folin-Ciocalteu (F-C) reagent. A standard curve was prepared using different concentrations of gallic acid and a linear regression equation was calculated. Phenolic content (gallic acid equivalents) of samples was determined by using the linear regression equation.

Ascorbate Peroxidase (APX) Activity

For the estimation of APX activity, 0.5-g plant samples were extracted in 2.5 ml of homogenizing medium containing 100 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.1 mM ascorbate, and 2% (v/v) β-mercaptoethanol (Dixit and others 2001). For assay of the enzyme activity, the rate of H₂O₂-dependent oxidation of ascorbic acid was determined in a reaction mixture that contained 50 mM potassium phosphate buffer (pH 7.0), 0.6 mM ascorbic acid, and enzyme extract (Chen and Asada 1989). The reaction was initiated by addition of 10 μl

10% (v/v) H₂O₂ and the oxidation rate of ascorbic acid was estimated by following the decrease in absorbance at 290 nm for 3 min.

Detection of DNA Fragmentation

To isolate DNA, the method described by Hameed and others (2004) was used. To check DNA fragmentation, equal amounts of DNA preparations were subjected to electrophoresis in 1.5% agarose gel at a constant 100 V for 3–4 h. The gel was stained with ethidium bromide and observed under UV transilluminator. Stained gels were photographed using the UVI proplatinum gel documentation system (UVitec, Cambridge, UK).

Statistical Analysis

All experiments were conducted in triplicate, and descriptive statistics were applied to analyze and organize the resulting data. The F-test was applied to find differences in variance among samples. The significance of differences between means (for stressed and control) for different parameters was measured using Student's *t*-test (two-tailed), at the 0.01 significance level, and where applicable at the 0.05 significance level. All the statistical calculations were performed using Microsoft Excel 2002 (Microsoft Corp., Redmond, WA, USA).

Results

Findings regarding defense response under heat stress in terms of seedling growth, induction of cell death and antioxidant/biomolecular response are discussed below.

Assessment of Heat Susceptibility of Genotypes

Heat stress significantly ($P < 0.01$) reduced the seedling fresh weight (FW) in all genotypes (Fig. 1a). Genotypes vary in percent reduction in FW. Heat stress-induced reduction in seedling FW ranged from 21% in Nesser to 37% in Fareed. With the exception of only a slight reduction in Inqilab-91, leaf relative water content (RWC) was reduced as a result of heat stress (Fig. 1b). Genotypes also varied in degree of percent reduction in RWC. Heat stress-induced reduction in RWC varied from 2.9% in Inqilab-91 to 41.7% in Sitta. Making use of this variation in percent reduction in seedling fresh weight and RWC among tested genotypes, these parameters were used to calculate an index for heat susceptibility. Therefore, to access the relative sensitivity of genotypes to heat stress, the heat susceptibility index (HSI) was calculated using the following formula.

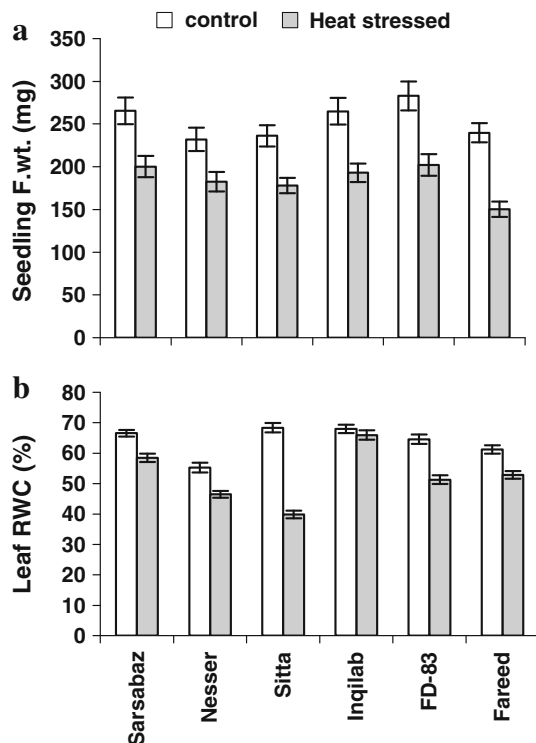


Fig. 1 Effect of heat stress on seedling fresh weight (a) and leaf relative water content (b) of different wheat genotypes

Table 1 Scale for relative heat tolerance of wheat genotypes based on heat susceptibility index (HSI)

Range of heat susceptibility index (%)	Heat stress response
≤10	Heat resistant
11–15	Heat tolerant
16–20	Moderately heat tolerant
21–30	Moderately heat sensitive
31–45	Heat sensitive
≥46	Highly heat sensitive

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

The scale for relative heat tolerance of wheat genotypes based on the HSI is given in Table 1. The highest value for HSI was observed in Sitta (33.2%) and the lowest HSI value (15%) was observed in Inqilab-91 (Fig. 2). The values of HSI for the other four genotypes varied between these two extremes. Based on HSI, Inqilab-91 was classified as relatively heat tolerant and Sitta was a relatively heat-sensitive genotype. Two genotypes, Nesser and Sarsabz, were classified as moderately heat tolerant, with HSI values of 18.4 and 18.5%, respectively. The remaining two genotypes, Fareed and FD-83 with HSI values of 25.4 and

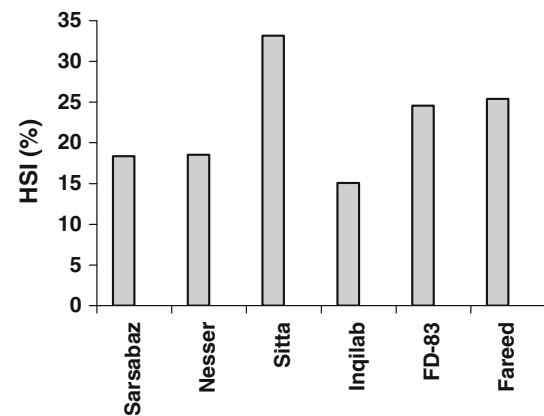


Fig. 2 Variation in heat susceptibility index (HSI) of different wheat genotypes

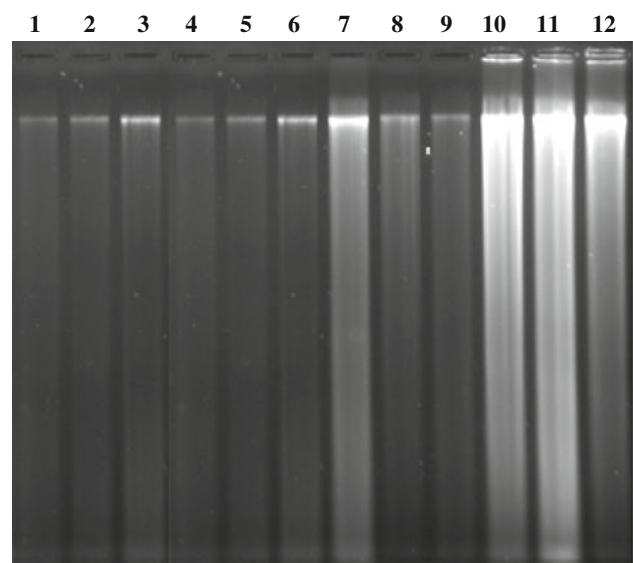


Fig. 3 Electrophoregram of DNA isolated from leaves of wheat seedlings grown under control and heat stress. Lanes 1–6 DNA from control leaves, lanes 7–12 DNA from heat-stressed leaves. 1 and 7 Sarsabz, 2 and 8 Nesser, 3 and 9 Inqilab-91, 4 and 10 Sitta, 5 and 11 FD-83, 6 and 12 Fareed

24.6%, respectively, were classified as moderately heat sensitive.

Cell Death Induced by Heat Stress

Heat stress-induced cell death in wheat leaves was evident by DNA degradation in the form of a smear (Fig. 3). DNA degradation was clear in sensitive and moderately sensitive genotypes Sitta (lane 10), FD-83 (lane 11), and Fareed (lane 12). Degradation was absent in the tolerant line Inqilab-91 (lane 9), whereas in the two sensitive genotypes, Sarsabz and Nesser, it was present in the former (lane 7) and very low in the latter (lane 8). This means that heat

stress-induced DNA degradation varies with relative heat tolerance of genotypes.

Antioxidant and Biomolecular Response

The biochemical response to heat stress was variable among the tested genotypes. Heat stress significantly ($P < 0.01$) enhanced membrane deterioration as reflected by increased MDA content in all genotypes (Fig. 4a). The increase over control was least (70%) in Nesser, and the MDA level was more than twofold higher in most of the genotypes. Total phenolic content was significantly enhanced by heat stress in Sitta and FD-83 (Fig. 4b), with percent increase over control 41.3% in Sitta and 21.2% in FD-83.

Catalase activity was significantly ($P < 0.001$) reduced by heat stress in all genotypes (Fig. 5a); however, the reduction varied among genotypes. The highest reduction in catalase activity was observed in Sitta (89.5 %) and the lowest was in Inqilab-91 (40.3%). A highly significant

positive correlation ($R^2 = 0.835$) was observed in percent reduction in catalase activity and HSI (Fig. 5b). This shows that reduction in catalase activity relates to heat susceptibility of genotypes, that is, catalase activity is reduced to a greater extent in more heat-susceptible genotypes. Therefore, genotypes that can resist loss of catalase activity can perform better under heat stress. This means catalase can be efficiently used as a biochemical marker to assess the relative heat stress tolerance of wheat genotypes at the seedling stage.

On the other hand, peroxidase, which also detoxifies hydrogen peroxide, was significantly ($P < 0.01$) increased by heat stress in all genotypes (Fig. 6a). The highest stress-induced increase in peroxidase activity was observed in Inqilab-91 (178 %) and the lowest was in Sarsabz (51%). The increase in peroxidase activity induced by heat stress was irrespective of relative heat susceptibility of genotypes. Therefore, no correlation was observed between increase in peroxidase activity and HSI. As heat stress-induced reduction in catalase activity was compensated for

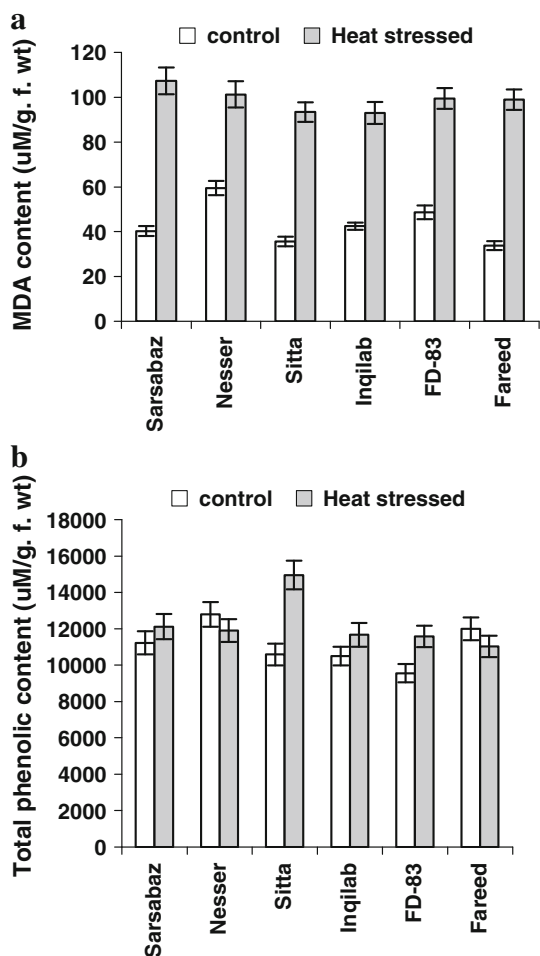


Fig. 4 Effect of heat stress on MDA (a) and total phenolic content (b) in different wheat genotypes

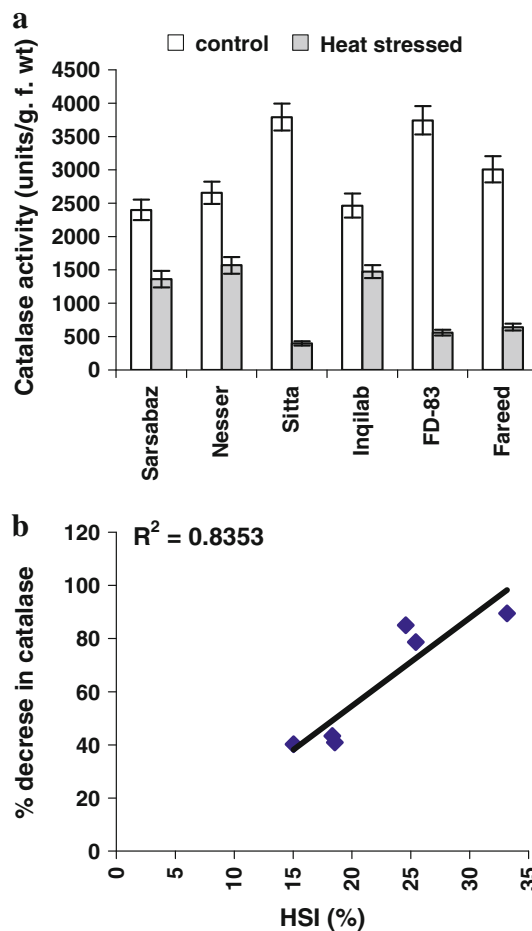


Fig. 5 Effect of heat stress on catalase activity (a) in different wheat genotypes and correlation between % decrease in catalase activity (b) under heat stress and HSI

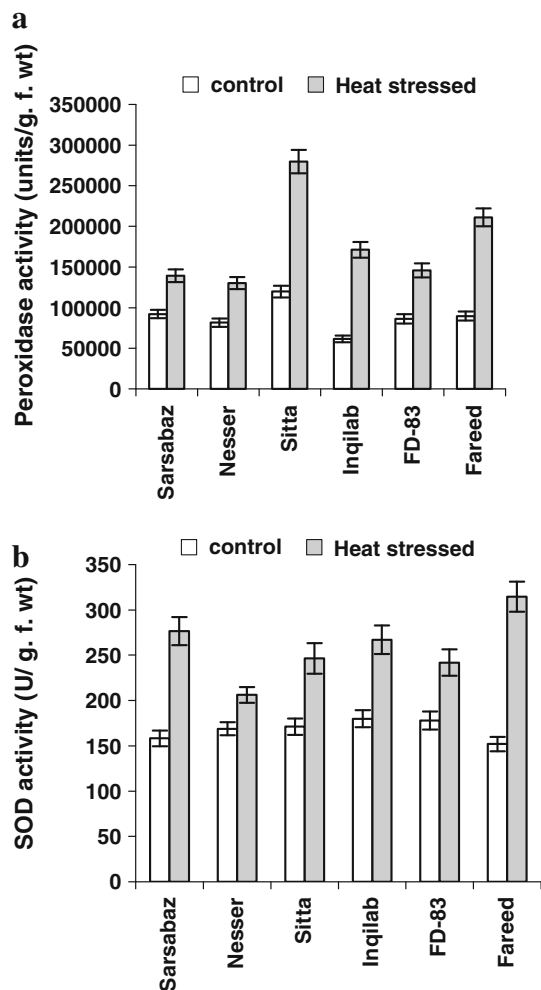


Fig. 6 Effect of heat stress on peroxidase (a) and SOD activity (b) in different wheat genotypes

by a parallel increase in peroxidase activity, it seems that detoxification of hydrogen peroxide under heat stress is mainly by peroxidase.

Superoxide dismutase (SOD) activity also increased significantly in all genotypes (Fig. 6b). The highest increase in superoxide dismutase activity was observed in Fareed where activity was more than twofold higher. Heat stress-induced increase in SOD activity did not relate to relative heat susceptibility of genotypes.

Protease activity was significantly ($P < 0.01$) increased in all genotypes under heat stress (Fig. 7a). The highest increase in protease activity was observed in Sitta and the lowest was in Inqilab-91. A significant positive correlation ($R^2 = 0.818$) was observed in the percent increase in protease activity and HSI (Fig. 7b). This indicates that more proteolytic enzymes are produced in genotypes with higher heat susceptibility. Therefore, heat stress-induced increase in protease activity can be used as a biochemical marker to assess the relative heat stress tolerance of wheat genotypes.

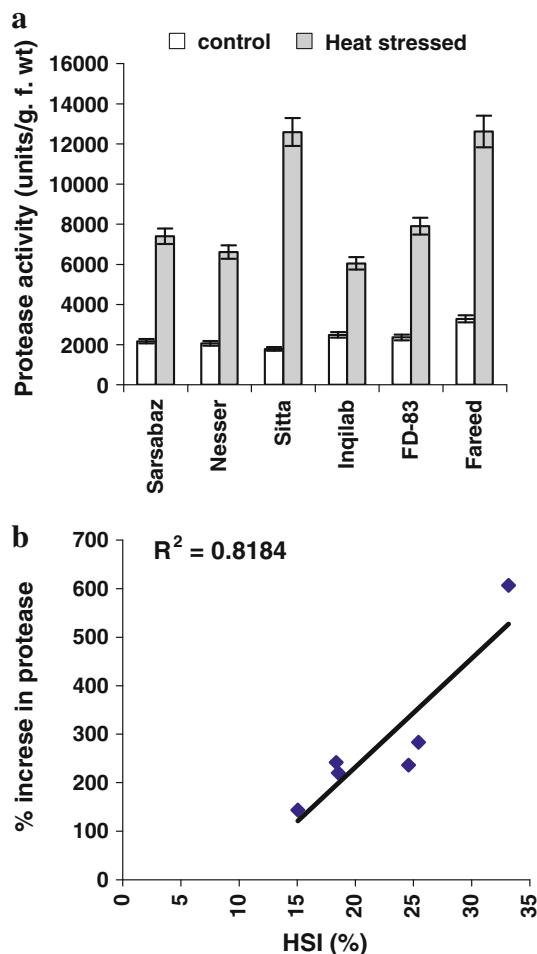


Fig. 7 Effect of heat stress on protease activity (a) in different wheat genotypes and correlation between % increase in protease activity (b) under heat stress and HSI

Heat stress significantly decreased ($P < 0.01$) the total soluble protein content in all genotypes (Fig. 8a). The highest heat stress-induced decrease in total soluble protein was observed in Sitta (78.4%) and the lowest was in Fareed (28.5%). Ascorbate peroxidase activity was increased by heat stress (Fig. 8b). However, the increase was significant ($P < 0.01$) in all genotypes except Sitta. A significant negative correlation ($R^2 = -0.895$) was observed in percent increase in ascorbate peroxidase activity and HSI (Fig. 8c). This indicates that APX activity increases to a lesser extent as heat susceptibility of genotypes increases. This negative correlation suggests that increase in APX activity can be used as a biochemical marker to assess the relative heat stress tolerance of wheat genotypes.

Discussion

Establishment of a reliable and rapid assay system is required for screening germplasm having tolerance to

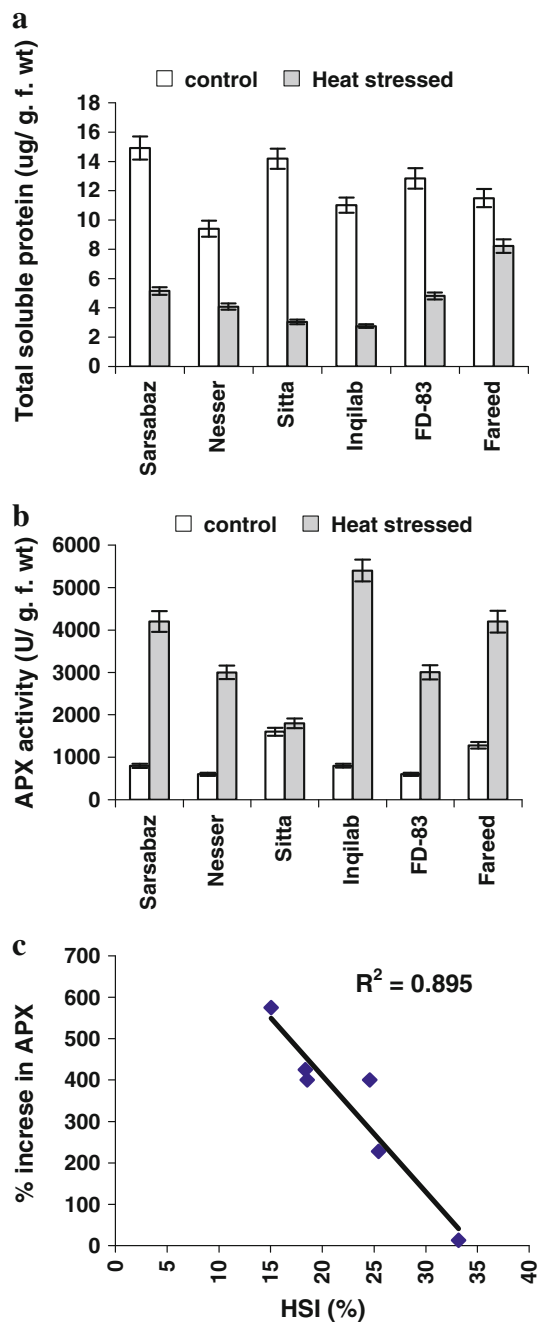


Fig. 8 Effect of heat stress on total soluble protein (a), APX activity (b) in different wheat genotypes, and correlation between % increase in APX activity (c) under heat stress and HSI

elevated temperature. Several bioassays such as electrolyte leakage measurement by electroconductivity (Howarth and others 1997; Ibrahim and Quick 2001), chlorophyll (Burke 1998; Ristic and others 2007), thylakoid lipid stability (Ristic and others 2007), triphenyl tetrazolium chloride (TTC) assay (Fokar and others 1998), and chlorophyll *a* fluorescence (Havaux and others 1988) have been used as probes of heat stress in many crops, including wheat.

Regardless of their reliability and common use, these techniques have some limitations. Electroconductivity and TTC assays have limited applications because of the amount of labor involved in variable field conditions (Ristic and others 2007). Similarly, measurements of chlorophyll *a* fluorescence require use of expensive instrumentation and in some cases necessitates dark adaptation of the leaf tissue, which limits the number of plants that can be screened in a given day. There is a need to develop more efficient, less expensive alternatives for high-throughput screening for heat tolerance. Therefore, one of the objectives of this study was to develop a screening technique for reliable and rapid assessment of heat sensitivity in wheat cultivars.

In the present study, variations in seedling fresh weight and leaf relative water content were observed in wheat genotypes. These parameters were utilized to calculate a heat susceptibility index (HSI) derived from % reduction in these two parameters on exposure to heat stress. This newly derived simple index proved to be a good heat stress-responsive marker for rapid and reliable screening of wheat cultivars at the seedling stage. Based on HSI, Inqilab-91 was classified as relatively heat tolerant and Sitta as relatively heat sensitive. Genotypes Nesser and Sarsabz were classified as moderately heat tolerant and Fareed and FD-83 as moderately heat sensitive. It is important to mention here that results based on HSI showing Inqilab-91 as a relatively heat-tolerant line at the seedling stage are in accordance with the field performance of this genotype as heat-tolerant material. This genotype has been used as a check (heat-tolerant control variety) in field experiments (heat stress trails by late sowing method) (Khan and Hussain 2006) and as a source of heat tolerance for breeding new heat-tolerant wheat lines (Aziz-ur-Rehman and others 2009). Actually, Inqilab-91 has a long growing period (142 days) and remains photosynthetically active longer during the rising temperatures of March and April in Punjab, Pakistan. Thus, HSI also showed relevance to the field performance of the wheat cultivars under heat stress and therefore can be used as a reliable tool for screening heat tolerance in wheat.

In the present study, analysis of cell death in leaves of wheat genotypes in response to severe heat stress revealed that it was evident by a DNA smear instead of a clear DNA ladder. Cell death induced by heat stress seems to be through senescence or necrosis. Previously reported cell death by senescence in plants did not appear to involve accumulation of distinct oligonucleosomal DNA fragments. In some previous studies, nuclear DNA fragmentation was also detected during leaf senescence of *Ornithogalum virens*, tobacco (Simeonova and others 2000; Rosa and others 2007), and rice (Lee and Chen 2002), but no DNA laddering was observed.

The second possibility of cell death induced by heat stress is through necrosis, which is accidental cell death often caused by harsh environmental stresses, and DNA smear and rupture of nuclear, organelle, and plasma membranes are often observed (Danon and others 2000). It has been reported that wheat leaves heated at 80°C for 10 min underwent necrosis, as no DNA laddering but DNA smearing was observed, while heat stress at 42°C for 15–120 min induced cell death that was evident by DNA ladder and TUNEL assay (Fan and Xing 2004). Moreover, Swidzinski and others (2002) also reported that *Arabidopsis thaliana* cells treated at 80°C for 10 min underwent necrotic cell death. In view of these previous reports, it seems that the prolonged heat stress at 45°C for 20 h applied in the present study may have resulted in necrosis. A significant increase in protease activity upon heat stress in the present study also strengthens the possibility of necrosis since DNA laddering is the product of chromatin digestion catalyzed by nucleases but without protease involvement (that is, no histone digestion) (Fan and Xing 2004), whereas concurrent nuclease and protease activity normally causes necrosis and a DNA smear on agarose gels (Wyllie and others 1980).

An increase in membrane lipid peroxidation due to abiotic stresses, including heat shock, is a common response (Liu and Huang 2000; Jiang and Huang 2001). Similarly, in the present study, MDA content also was increased by heat stress and the increase was more than twofold in most of the genotypes, with the lowest increase in the relatively heat-tolerant genotype Nesser. Catalase activity was significantly reduced by heat stress in all genotypes. Therefore, genotypes that can resist loss of catalase activity can perform better under heat stress. On the other hand, peroxidase and SOD were significantly increased by heat stress in all genotypes; however, no correlation was observed with the heat susceptibility index. Ascorbate peroxidase activity also increased under heat stress. As heat stress-induced reduction in catalase activity was compensated for by a parallel increase in peroxidases, it seems that detoxification of hydrogen peroxide under heat stress is mainly by peroxidases. Previously, decreased catalase activity with a simultaneous increase in peroxidase activity under heat stress has been reported in leaves and roots of creeping bentgrass (Liu and Huang 2000).

Interestingly, heat stress-induced percent decrease in catalase activity and percent increase in protease activity show a significant positive correlation with HSI or relative degree of heat tolerance of genotypes, whereas percent increase in ascorbate peroxidase shows a significant negative correlation. This means that reduction in catalase activity relates to heat susceptibility of genotypes, that is, catalase activity decreases to a greater extent in more heat-susceptible genotypes. Moreover, increased protease

activity indicates that more proteolytic enzymes are produced in genotypes with higher heat susceptibility. Further, APX activity increases to a lesser extent as heat susceptibility of genotypes increases. All these correlations signify that catalase, protease and ascorbate peroxidase can be used efficiently as biochemical markers to assess the relative heat stress tolerance of wheat genotypes at the seedling stage.

Previously, some other relatively expensive parameters have been correlated with heat tolerance of genotypes. For example, correlation between heat stability of thylakoid membranes and loss of chlorophyll has also been reported in winter wheat under heat stress and was proposed to be useful for high-throughput screening for heat tolerance in wheat (Ristic and others 2007). Moreover, cell membrane thermostability was positively and significantly correlated with yields of spring wheat cultivars in hot environments (Fokar and others 1998).

In conclusion, a heat susceptibility index based on the decrease in seedling fresh weight and leaf relative water content can be used efficiently for assessing heat tolerance in wheat breeding. Heat stress induced the necrosis that was evident by DNA smear formation. Compromised antioxidant activities and enhanced protein, DNA and lipid degradation were common phenomena playing central roles in the heat stress-induced cell death process. Enzymes like catalase, peroxidases, superoxide dismutase and protease have association with heat tolerance in wheat and thus can be used as efficient biochemical markers for germplasm screening.

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