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HEAT-INDUCED REGULATION OF ANTIOXIDANT DEFENSE SYSTEM AND NUTRIENT ACCUMULATION IN HEXAPLOID BREAD WHEAT (*TRITICUM AESTIVUM* L.)

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Abstract

Ten cultivars (five registered S-24, Inqlab-91, Saher-2006, Fsd-2008, and Lasani, and five candidate cultivars P.B-18, M.P-65, S.H-20, AARI-10, and G.A-20) of spring wheat (*Triticum aestivum* L.) were examined for high temperature stress tolerance. Plants were grown in soil filled pots in the Botanical Garden of the Department of Botany University of Agriculture Faisalabad, Pakistan. Three different temperature regimes (30, 40 and 50°C) were applied at two different growth stages (tillering and boot) for three temperature durations 30, 60 and 90 min in a growth chamber. The leaf and root samples were collected after two weeks of temperature treatment and then analyzed for enzymatic and non-enzymatic antioxidants as well as inorganic nutrients (N, P, K^+, Ca^{2+}). At the end, data obtained were statistically analyzed to distinguish heat tolerant from non-tolerant wheat cultivars. After appraisal of growth, antioxidant defense system and uptake of nutrients it was found that cvs. S-24, Inqlab-91, Saher-2006, Fsd-2008, Lasani and G.A-20 exhibited better thermo-tolerance capabilities than the other wheat cultivars (P.B-18, M.P-65, S.H-20, AARI-10). Among the thermo-tolerant wheat cultivars, G.A-20 and Lasani were superior in maintaining shoot fresh weights and shoot length, high antioxidant activities and better nutrient uptake at both tillering and boot stages. The response of all cultivars to heat stress applied at the tillering stage or boot stage was almost the same.

Introduction

Due to anthropogenic gaseous emissions particularly greenhouse gases such as carbon dioxide, methane, chlorofluoro carbons (CFCs), global air temperatures are gradually rising. Therefore, plants are frequently exposed to high temperature regimes during their life cycle which severely limit plant growth and development; particularly the reproductive phases of plants are extremely sensitive to these high temperature regimes (Thuzar, 2010; Bita *et al.,* 2011). To overcome these supra-optimal temperature regimes and maintain homeostatic balance at cellular level, plants have developed a number of defense mechanisms such as osmo-protection, late embryogenesis, early maturation, production of heat shock proteins and antioxidant defense systems (Xu *et al.,* 2011). In response to abiotic stresses including temperature extremes, there is an enhanced production of ROS within the cells which are free oxygen radicals. They cause immense damage to cell membranes and other cellular organelles ultimately leading to cell death (Miller *et al.,* 2010). To overcome this ROS-induced oxidative burst, plants have developed an excellent mechanism of antioxidants including antioxidant enzymes (Sairam & Tyagi, 2004, Shahbaz *et al*., 2008; Ashraf, 2009; Saleem *et al.,* 2011; Akram *et al.,* 2012). Of antioxidant enzymes, superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) are promising. SOD catalyses the conversion of superoxide radical (O_2^-) to H_2O_2 which is then converted to water and oxygen by catalase and peroxidase (Gill *et al.,* 2012; Gondim *et al.,* 2012).

Along with enzymes, the non-enzymatic antioxidants also play a key role in free radical scavenging process. Among non-enzymatic scavenging system, ascorbic acid (AsA), carotenoids, glutathione (GSH) and tocopherols are main antioxidants which play an important role in the detoxification of free oxygen radicals. Ascorbic acid and glutathione are regarded as the essential ROS scavenging metabolites in plant cells (Ashraf, 2009; Ashraf & Akram, 2009; Akram *et al.,* 2012). Thus, both antioxidant systems (enzymes and non-enzymes) act as a protective shield against free radical-induced oxidative damage. Mostly thermo-tolerance conferred by antioxidants to plants is compensatory because these can buffer the heat-induced hazardous impacts (Bo-Shao *et al.,* 2008). However, there exists a remarkable intra- and inter-specific diversity for temperature stress tolerance, because different plant species and genotypes vary in their capability to overcome heat stress (Ashraf *et al*., 1994; Nahar *et al.,* 2010). These tremendous intra- and inter-specific variations provide us an opportunity to select such plants which are tolerant to temperature extremes and then exploit these plants properly to introduce and improve thermo-tolerance in major crop plants (Semenov, 2009).

Wheat is a winter season crop cultivated both in tropics and subtropics in spite of some unexpected high temperature spells during its growth period. In wheat, anthesis and post-anthesis stages are highly sensitive and high temperature during these stages acts as a major limitation on wheat production, ultimately reducing yield and quality of wheat (Taghizadeh & Sharifi, 2010; Wang *et al.,* 2011). Delayed planting of wheat due to high temperature during the reproductive phase results in yield decline and the same factor is of major concern in Pakistan (Joshi *et al.,* 2007). The optimum temperature for photosynthesis in wheat is 20-22°C and a remarkable decline in photosynthetic rate occurs at 30-32°C (Rehman *et al*., 2009). Thermal stress above 32°C causes a huge

decline in grain weight resulting in reduced yield (Mian *et al*., 2007; Balla *et al.,* 2011; Prasad *et al.,* 2011). Optimal temperature for wheat during anthesis and grain filling stage is 25°C or lower (Pradhan *et al.,* 2012). Unexpected occurrence of warm spells (of 35 to 40ºC) is common during the growing season in many wheat growing regions (Asseng *et al.,* 2011) and for each degree rise in mean ambient temperature during grain filling a 3 to 4 percent reduction has been observed in wheat grain weight (Dias & Lidon, 2009).

Undoubtedly, to accomplish this goal such varieties and cultivars might be developed which exhibit efficient tolerance against environmental extremes such as drought, salinity, temperature, etc. (Semenov, 2009; Ashraf *et al.,* 2011). So, the present study was carried out to examine the potential of newly developed wheat cultivars to tolerate high temperature stress by determining the effect of this stress on activities/levels of antioxidants (both enzymatic and non-enzymatic etc.) and inorganic nutrients in different wheat cultivars. Whether or not these metabolic and mineral attributes could be used as potential indicators for temperature tolerance for discriminating wheat cultivars was also one of the premier objectives of this study.

Materials and Methods

Ten cultivars (five registered cultivars S-24, Inqlab-91, Saher-2006, Fsd-2008, and Lasani, and five candidate cultivars P.B-18, M.P-65, S.H-20, AARI-10 and G.A-20) of spring wheat (*Triticum aestivum* L.) were examined for their temperature stress tolerance. Plants were grown in pots containing sandy-loam soil in the Botanical Garden of the Department of Botany, University of Agriculture, Faisalabad, Pakistan. Analysis of soil used in the experiment was carried-out using the standard soil analysis protocols. Data of the soil characteristics are presented in Table 1.

Table 1. Chemical properties of the soil used in the experiment.

Chemical properties	Value
Calcium carbonate ($CaCO_3$)	2.71%
Organic matter	0.95%
CEC (Cation exchange capacity)	17.4 meq 100 g^{-1}
Available P	8.6 mg kg^{-1}
Total N	0.73%
pH	7.80%
ECe (Electrical conductivity of the soil extract)	2.53 dS m^{-1}
SP (Saturation percentage)	34%
Soluble CO_3^{2-}	Traces
Soluble HCO_3^-	4.93 meq L^{-1}
Soluble Cl^-	8.52 meq L^{-1}
Soluble SO_4^{2-}	1.98 meq L^{-1}
Soluble Na^+	2.45 meq L^{-1}
Soluble $Ca^{2+} + Mg^{2+}$	14.30 meq L^{-1}

The whole experiment was conducted in a factorial arrangement. Four temperature treatments i.e., control (25°C), 30°C, 40°C and 50°C were applied to wheat plants at the tillering or boot stage for three temperature durations 30, 60 and 90 min by maintaining the appropriate temperatures using an electric oven in a controlled room. After 15 days of temperature stress treatments, two plants were uprooted and shoot length and fresh weights of shoots and roots were recorded. The leaf samples were also collected to analyze non-enzymatic antioxidants (AsA, phenolics), antioxidant enzymes (SOD, POD, CAT), product of membrane lipid peroxidation, i.e., malondialdehyde (MDA) and nutrients (leaf and root K^+, Ca^{2+}, P and N).

Determination of enzymatic antioxidants

Enzyme extraction: Enzymatic antioxidants of all wheat cultivars were extracted by grinding 0.5 g of fresh leaf material in 10 ml of 50 mM cooled potassium phosphate buffer (pH 7.8). This homogenized material was centrifuged at 15000 x *g* for 20 min at 4ºC. The pellet was discarded and the supernatant used for the estimation of activities of different enzymatic antioxidants.

Superoxide dismutase (SOD): The activity of SOD was determined by monitoring its potential to cause inhibition in photoreduction of nitroblue tetrazolium chloride (NBT) following the procedure given by Giannopolitis & Ries (1977). The reaction mixture (3 ml) contained 50 mM potassium phosphate buffer (pH 7.8), 75 nM EDTA, 13 mM methionine, 50 µM NBT, riboflavin, 1.3 µM and 50 µl enzyme extract. The tubes with reaction mixture lacking enzyme were used as control. Then these tubes were placed under fluorescent lamp (30 W) for 10 min. The reaction was stopped by turning off the lamp and absorbance read at 560 nm using a spectrophotometer (IRMECO U2020). One unit of the enzyme was taken as the amount of enzyme used to cause 50% inhibition in the photochemical reduction of NBT.

Catalase (CAT) and peroxidase (POD): The activities of catalase (CAT) and peroxidase (POD) were determined according to the procedure given by Chance & Maehly (1955) with some minor modifications. The reaction mixture (3 ml) for CAT contained 5.9 m*M*, H_2O_2, 50 m*M* potassium phosphate buffer (pH 7.8) and 100 µl of crude enzyme extract. To initiate the reaction 0.1 ml of the enzyme extract was added to the mixture. The decrease in absorbance was read at 240 nm after every 20s. One unit of CAT was taken as an absorbance change of 0.01 units per min. The POD reaction mixture (3 ml) contained 40 m*M* H_2O_2, 20 m*M* gauiacol, 50 m*M* potassium phosphate buffer (pH 7.8), and 100 µl of enzyme extract. Then the change in absorbance at 470 nm was monitored after every 20 s. One unit of POD activity was defined as the change of 0.01 absorbance unit per min mg of protein.

Determination of non-enzymatic antioxidants

Ascorbic acid (AsA) contents: The AsA contents in the leaves of different wheat cultivars were quantified following the procedure given by Mukherjee & Choudhuri (1983). Fresh leaf (0.25 g) was ground well in 10 ml of 6% TCA. Then the homogenate was centrifuged at 10,000 *g* for 10 min at 4ºC. Then two ml of 2% dinitrophenyl hydrazine solution (in acidic medium) were added to 4 mL of the supernatant. After this, one drop of thiourea (10% thiourea

prepared in 70% ethanol) was added to the mixture. Then the whole mixture was boiled in a water bath for 20 min. After boiling, the mixture was immediately placed on ice for lowering its temperature to 25ºC. Then 5 mL of 80% sulphuric acid (v/v) were added to the mixture at 0ºC and absorbance read at 530 nm. The AsA contents of the samples were quantified against a standard curve prepared using varying ascorbic acid standards.

Malondialdehyde (MDA): The amount of MDA in leaf tissues was measured following the procedure described by Carmak & Horst (1991) with some minor modifications. One gram of fresh leaf material was homogenized in 3 mL of 0.1% (w/v) TCA (trichloroacetic acid) at 4°C. Then the homogenate was centrifuged for 15 min at 2000 x *g*. To 0.5 mL of the supernatant, 3 mL of 0.5% (v/v) thiobarbaturic acid (prepared in 20% TCA) were added. The mixture was then kept at 95ºC in a water bath with continuous shaking. After 50 min, the samples were removed from the water bath and placed in an ice bath. When the temperature of the samples was 25ºC, they were again centrifuged for 10 min. at 10, 000 x *g*. Finally, the absorbance of the mixture was read at 532 nm. The non-specific absorption taken at 600 nm was subtracted from all the readings. Then the concentrations of TBARS were calculated using the absorption coefficient as follows:

$$\text{MDA level (nmol)} = \Delta \text{ (A 532 nm-A 600 nm)}/1.56 \times 10^5$$

Absorption coefficient for calculating MDA is 156 $mmol^{-1}cm^{-1}$

Leaf phenolics: Total phenolics were determined using Folin-Ciocalteau reagent (Julkenen-Titto, 1985). The leaf samples (50 mg) were homogenized with 80% acetone and centrifuged at 10,000 *g* for 10 min. One-hundred microlitres of the supernatant were diluted with 2 ml of water and 1 ml Folin-Ciocalteau's phenol reagent and shaken vigorously. Then 5 ml of 20% sodium carbonate were added and the volume was made up to 10 ml with distilled water. The contents were mixed thoroughly and the absorbance read at 750 nm using a spectrophotometer (IRMECO U2020). The results were expressed as mg/g of fresh leaf.

Determination of mineral elements (K^+, Ca^{2+}, N and P): The dried ground plant material (0.1 g each) was digested following the procedure proposed by Allen *et al.,* (1986). Then the volume of the digested material was brought to 50 mL using volumetric flasks. This extract was filtered and used for the determination of cations (K^+ and Ca^{2+}) using a flame photometer (Jenway PFP 7). Nitrogen was estimated by micro-Kjeldhal's method (Bremner, 1965). The digested plant material (5 ml) was taken in Kjeldhal's tubes. The tubes were placed in Kjeldhal's ammonium distillation unit and 5 ml of 40% NaOH were added to the tube. Boric acid solution (5 ml) was taken in a conical flask with a few drops of mixed indicator. When the distillate was approximately 40 ml, the distillation stopped. The distillate was cooled for a few minutes and treated it with 0.01 *N* standard H_2SO_4 till the solution turned pink. A blank was run for the complete procedure.

N was estimated from the following formula:

$$N\ (\%) = (V2\text{-}V1) \times N \times 0.014 \times 100/W$$

The V1 and V2 in the above equation represent the volume of standard H_2SO_4 required to titrate the blank solution and sample solution, respectively, whereas N represents the normality of the H_2SO_4 used and W, the weight of sample.

Phosphorous (P) was determined spectrophotometrically following Jackson (1964). The extracted material (2 ml) was dissolved in 2 ml of Barton's reagent and total volume was made to 50 ml. These samples were kept for 30 min before analyzing phosphorous.

Statistical analysis: Analysis of variance of the data for each variable was computed using the MSTAT-C Computer Program (Anon., 1989). The Duncan's New Multiple Range test at 5% level of probability was used to test the differences among mean values (Steel & Torrie, 1986).

Results

There was a significant decreasing effect of temperature stress on shoot and root fresh weights of all 10 wheat cultivars (Table 2; Fig. 1). Heat stress, particularly 50°C, imposed at the tillering or boot stage caused a maximum reduction in all wheat cultivars (Table 2). Of all wheat cultivars, cvs. Lasani, G.A-20 and Saher-2006 were better than the other cultivars at all temperature regimes. Imposition of temperature stress at the tillering stage significantly decreased the shoot length of all wheat cultivars, while that applied at the boot stage did not affect the shoot length.

There was a significant effect of variety, temperature and growth stages on leaf and root K^+ contents (Table 2). Leaf K^+ increased in cvs. S-24, Inqlab-91, Saher-2006, Fsd-2008, Lasani, and G.A-20, with increase in temperature up to 40°C applied at the tillering stage, but it declined at 50°C. In the remaining cultivars (P.B-18, M.P-65, S.H-20, and AARI-10) leaf K^+ increased up to 30°C and then declined at 40 and 50°C. The temperature stress imposed at the boot stage caused a significant increase in leaf K^+ in cvs. Inqlab-91, Saher-2006, Fsd-2008 and Lasani. However, in contrast, in cvs. S-24, P.B-18, M.P-65, S.H-20, AARI-10 and G.A-20 leaf K^+ contents increased only up to 30°C and then declined in the subsequent regimes of temperature stress. The temperature stress applied at both growth stages caused the maximum increase in leaf K^+ contents in S-24 and the minimum in S.H-20 (Table 2; Fig. 2). Root K^+ contents increased in S-24, Saher-2006, Fsd-2008, and P.B-18 at 30°C and then decreased with a further increase in temperature. The maximum rise in root K^+ was observed in S-24 and the minimum in S.H-20 under varying temperature regimes.

Table 2. Analyses of variance (ANOVA) of the data (mean squares) for growth, accumulation of different inorganic nutrients and activities/levels of antioxidants in heat-stressed and non-stressed plants of ten wheat (*Triticum aestivum* L.) cultivars.

Source of variation	df	Shoot fresh wt.	Root fresh wt.	Shoot length	Leaf K^+	Root K^+
Temperature stress (T)	3	54.21***	1.93***	266.9***	1286.7***	21.01*
Cultivars (Cvs)	9	45.73***	1.06***	24.84***	672.1***	21.98***
Stages (S)	1	48.67***	22.32***	585.1***	27125.6***	58.01**
T x Cvs	27	0.48 ns	0.04 ns	4.32ns	1713.8***	18.34***
T x S	3	8.82***	0.055 ns	162.1***	459.4***	18.97*
Cvs x S	9	1.90 ns	0.17 ns	16.04***	1142.5***	21.12***
T x Cvs x S	27	0.56 ns	0.05 ns	3.12ns	920.9***	16.74***
Error	160	1.00	0.15	3.12	71.4	5.451
		Leaf Ca^{2+}	**Root Ca^{2+}**	**Leaf P**	**Root P**	**Leaf N**
Temperature stress (T)	3	7.17*	59.82***	1101.9***	263.1***	0.130*
Cultivars (Cvs)	9	8.33**	21.05***	509.2***	339.1***	0.101**
Stages (S)	1	204.7***	630.5***	506.2*	4767.6***	2.80***
T x Cvs	27	10.44***	8.963***	381.4***	298.6***	0.034ns
T x S	3	9.54*	26.89***	1483.4***	1451.6***	0.223***
Cvs x S	9	6.25*	18.79***	301.7***	182.9***	0.128***
T x Cvs x S	27	10.66***	13.63***	379.1***	293.2***	0.029ns
Error	160	2.572	2.095	77.56	41.75	0.034
		Root N	**CAT**	**SOD**	**POD**	**AsA**
Temperature stress (T)	3	0.046ns	0.021ns	68.63***	314.1***	151.1***
Cultivars (Cvs)	9	0.087***	0.101ns	104.3***	19.61ns	166.7***
Stages (S)	1	0.221**	0.031ns	123.6***	0.327ns	52.01ns
T x Cvs	27	0.056***	0.048ns	94.67***	158.4***	119.6***
T x S	3	0.201***	0.063ns	25.34**	177.9***	227.02***
Cvs x S	9	0.106***	0.099ns	125.5***	55.99***	193.5***
T x Cvs x S	27	0.069***	0.104ns	104.2***	109.9***	144.6***
Error	160	0.022	0.071	5.03	10.33	26.33
		MDA	**Total proteins**	**Total phenolics**		
Temperature stress (T)	3	232.1***	6849.7***	244497.7***		
Cultivars (Cvs)	9	19.61*	3185.8***	62995.9***		
Stages (S)	1	627.7***	1390791.1***	17581087.0***		
T x Cvs	27	22.07***	3279.4***	30195.8***		
T x S	3	32.02**	10008.7***	238171.6***		
Cvs x S	9	12.18ns	2405.3**	67211.9***		
T x Cvs x S	27	21.00***	2890.4***	30066.1***		
Error	160	8.087	767.3	10308.1		

ns = Non-significant; *, ** and *** = Significant at 0.05, 0.01 and 0.001 levels, respectively

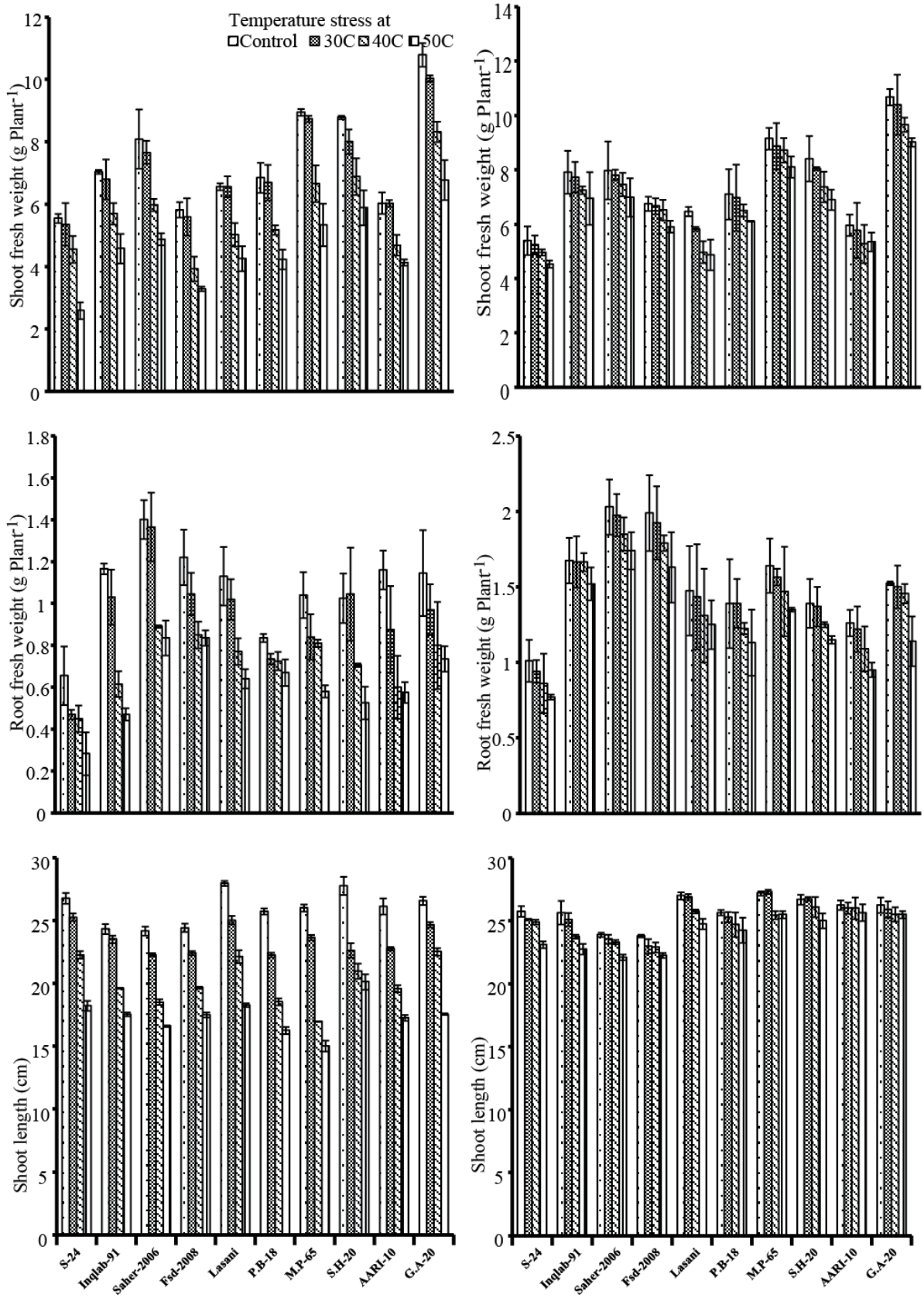

Fig. 1. Shoot and root fresh weights and shoot length of 10 elite cultivars of spring wheat (*Triticum aestivum* L.) subjected to varying levels of temperature stress at the tillering and booting stages (Mean ± S.E.).

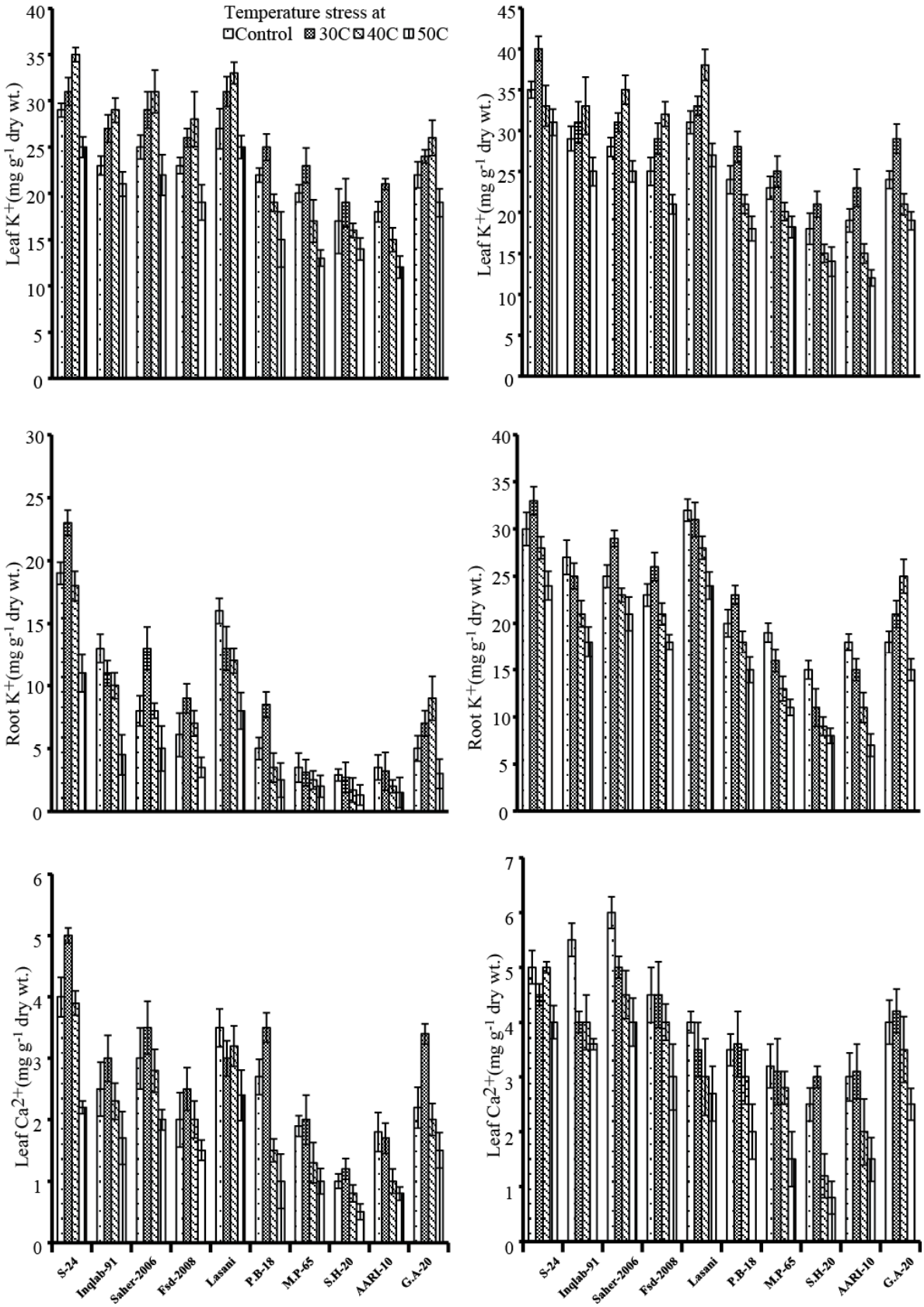

Fig. 2 Leaf and root K^+ and leaf Ca^{2+} concentrations in 10 elite cultivars of spring wheat (*Triticum aestivum* L.) subjected to varying levels of temperature stress at the tillering and booting stages (Mean ± S.E.).

In cvs. S-24, Inqlab-91, Saher-2006, Fsd-2008, P.B-18, M.P-65, S.H-20, and G.A-20, leaf Ca^{2+} increased at 30°C temperature stress level applied at the tillering stage, whereas in Lasani at 40°C, and AARI-10 leaf Ca^{2+} decreased with a rise in temperature. Similarly, heat stress (30°C) applied at the boot stage caused a transient rise in leaf Ca^{2+}, whereas in the other cultivars such as S-24, Inqlab-91, Saher-2006, Fsd-2008, Lasani, and M.P-65 leaf Ca^{2+} decreased with rise in temperature. Like leaf Ca^{2+}, root Ca^{2+} also differed significantly among the cultivars due to heat stress applied at both growth stages. At the tillering stage, root Ca^{2+} like leaf Ca^{2+} increased markedly at 30°C in cvs. S-24, Lasani, M.P-65, S.H-20, AARI-10, and G.A-20, however, in the remaining cultivars no rise in tissue Ca^{2+} was observed at any stress level. The heat-stress induced change in root Ca^{2+} was not consistent in the set of wheat cultivars examined here. (Table 2; Figs. 2, 3).

In the present study, leaf N contents were also influenced significantly in all ten wheat cultivars by the temperature stress imposed at both stages of plant growth and development. Temperature stress imposed at both tillering and boot stages caused increase in leaf N contents with increase in temperature up to 40°C and then it declined at 50°C particularly in cvs. S-24, Inqlab-91, Saher-2006, Fsd-2008 and Lasani (Table 2; Fig. 3). The maximum rise in leaf N was observed in cv. Saher-2006 under temperature stress applied at the tillering stage. Root N contents also exhibited a significant inter-cultivar variation under temperature stress applied at both stages of plant growth and development. Heat stress particularly 30°C imposed at the tillering stage caused a significant increase in root N particularly in cvs. S-24, P.B-18, M.P-65, S.H-20 and AARI-10, and that at 40°C in cvs. Inqlab-91, Saher-2006, Fsd-2008 and G.A-20. The maximum rise in root N was observed in S-24 and the minimum in AARI-10. At the boot stage, root N increased at 30°C temperature stress level in cvs. S-24, Fsd-2008, Lasani, P.B-18, M.P-65, S.H-20 and AARI-10, whereas it increased up to 40°C in cvs. Inqlab-91, Saher-2006 and G.A-20. The maximum rise in root N was observed in S-24 and the minimum in S.H-20 under heat stress applied at the boot stage.

Data regarding leaf P contents showed that P contents were also influenced significantly due to heat stress imposed at both growth stages, and the leaf P contents varied from variety to variety. For example, heat stress imposed at the tillering stage caused a significant reduction in leaf P in cvs. S-24, Inqlab-91, Saher-2006, Lasani, P.B-18, M.P-65, S.H-20 and G.A-20, whereas heat stress imposed at the boot stage caused reduction in leaf P in almost all cultivars (Table 2; Fig. 4). A significant rise in leaf P contents at 30°C applied at the tillering stage was observed in cvs. Fsd-2008 and AARI-10, whereas the heat stress applied at the boot stage caused an increase in leaf P in only S-24. The maximum rise in shoot P contents was observed in S-24 under heat stress applied at the tillering stage and the minimum in AARI-10 under heat stress imposed at the boot stage. Overall, the maximum rise in leaf P contents was observed in cv. Saher-2006 followed by Lasani and the minimum in M.P-65 under heat stress imposed at both growth stages. Root P contents unlike those of leaf were not influenced by temperature stress but varied significantly among the varieties and at different developmental stages (Table 2; Fig. 4). The maximum rise in root P contents was observed in S-24 and the minimum in S.H-20 under heat stress applied at both tillering and boot stages.

Leaf superoxide dismutase (SOD) activity increased significantly at 30°C in wheat cultivars S-24, Inqlab-91, Saher-2006, Fsd-2008, Lasani and G.A-20, while it decreased with increase in temperature applied at both growth stages in cvs. P.B-18, M.P-65, S.H-20, and AARI-10. The maximum increase in SOD activity was found in S-24, Sahar 2006 and Lasani at 30°C, whereas the maximum decrease in SOD activity due to rise in temperature was recorded in cv. Pb18 and Sh-20 at all higher temperature regimes (Table 2; Fig. 5).

At both tillering and boot stages of growth, peroxidase (POD) activity increased at 30°C and declined at 40 and 50°C in cvs. S-24, Inqlab-91, Saher-2006, Fsd-2008 and G.A-20 compared with that at control while in all other cultivars (Lasani, P.B-18, M.P-65, S.H-20 and AARI-10) it declined at all three temperature stress levels. The POD activity was maximum in S-24 at 30°C followed by Saher-2006, while minimum in SH-20 at both growth stages (Table 2; Fig. 5).

Catalase (CAT) activity increased initially at 30°C in cvs. S-24, Inqlab-91, Saher-2006, Fsd-2008, Lasani, G.A-20, then it began to decline with a further increase in temperature, while in another set of wheat genotypes (P.B-18, M.P-65, S.H-20, and AARI-10) it declined with a rise in temperature (Table 2; Fig. 5). The maximum CAT activity at both tillering and boot stages was observed in cv. S-24 and the minimum in cv. S.H-20.

Leaf ascorbic acid contents in all wheat cultivars were significantly influenced due to high temperature stress when applied at the tillering and boot stages. At both tillering and boot stages, with increase in temperature up to 40°C, leaf AsA contents increased in cvs. S-24, Inqlab-91, Saher-2006, Fsd-2008 and G.A-20, but the contents decreased markedly at 50°C. The maximum increase in leaf AsA contents due to high temperature stress applied at the boot stage was observed in cv. S-24 and the minimum in cv. M.P-65 at 30°C (Table 2; Fig. 6).

Temperature variation significantly influenced phenolic concentration in the leaves of all wheat cultivars at both growth stages. Leaf phenolic contents increased in P.B-18, M.P-65, S.H-20, and AARI-10 but only at 30°C, whereas in Inqlab-91 and Fsd-2008 at 30 and 40°C (Table 2; Fig. 6). The maximum rise in phenolic concentration was observed in S-24 at both growth stages and the minimum in S.H-20 at the tillering stage, and in Fsd-2008 at the boot stage.

The product of membrane lipid peroxidation, malondialdehyde (MDA), was also influenced significantly by high temperature imposed at both growth stages (Table 2; Fig. 6). The MDA contents increased with rise in temperature in all cultivars studied with increase in temperature applied to the wheat plants at both growth stages. MDA contents were relatively higher in P.B-18, M.P-65, S.H-20, AARI-10 and lower in Lasani and S-24 at temperature stress applied at both growth stages.

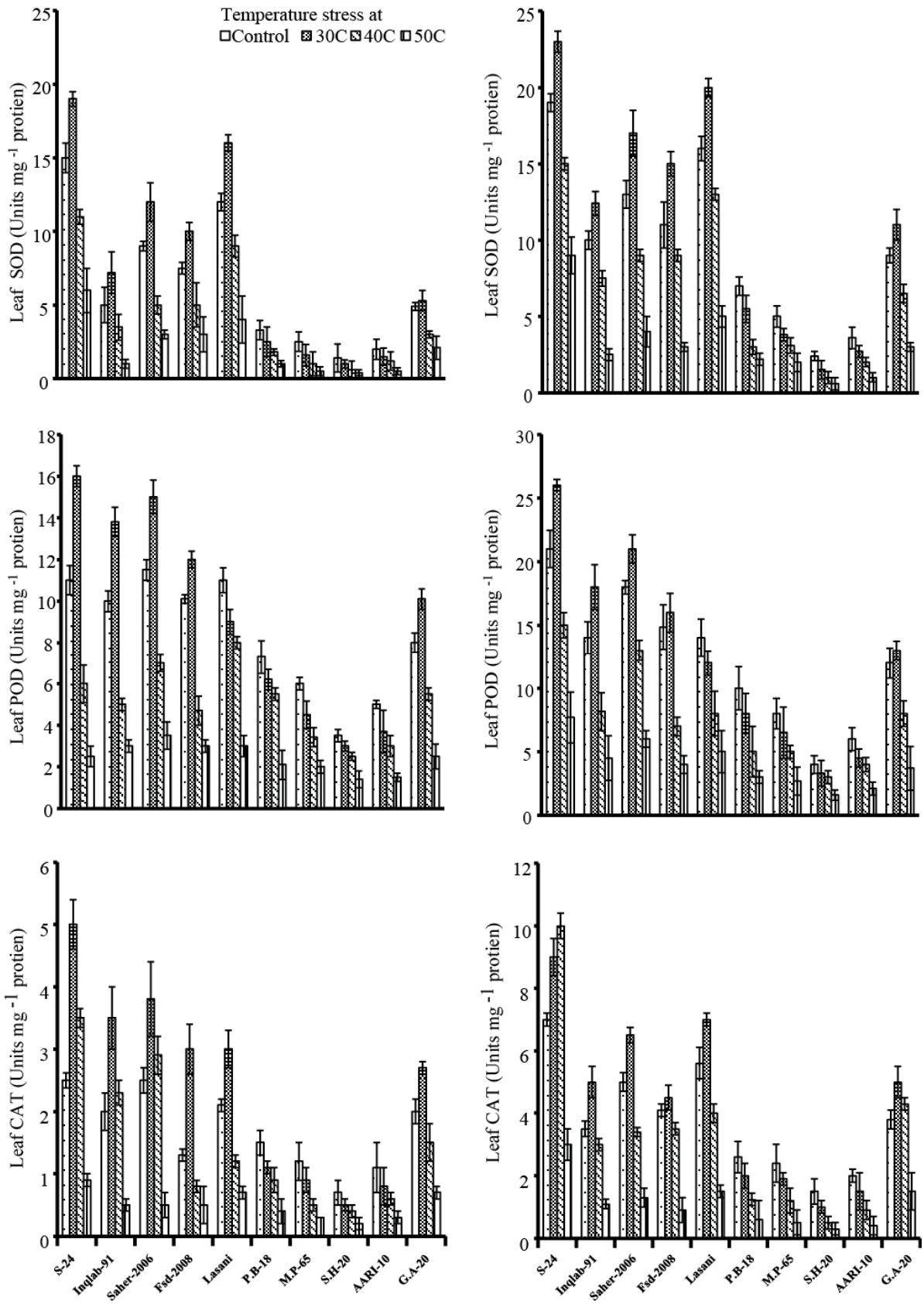

Fig. 3. Root Ca^{2+} and leaf and root N concentrations in 10 elite cultivars of spring wheat (*Triticum aestivum* L.) subjected to varying levels of temperature stress at the tillering and booting stages (Mean ± S.E.).

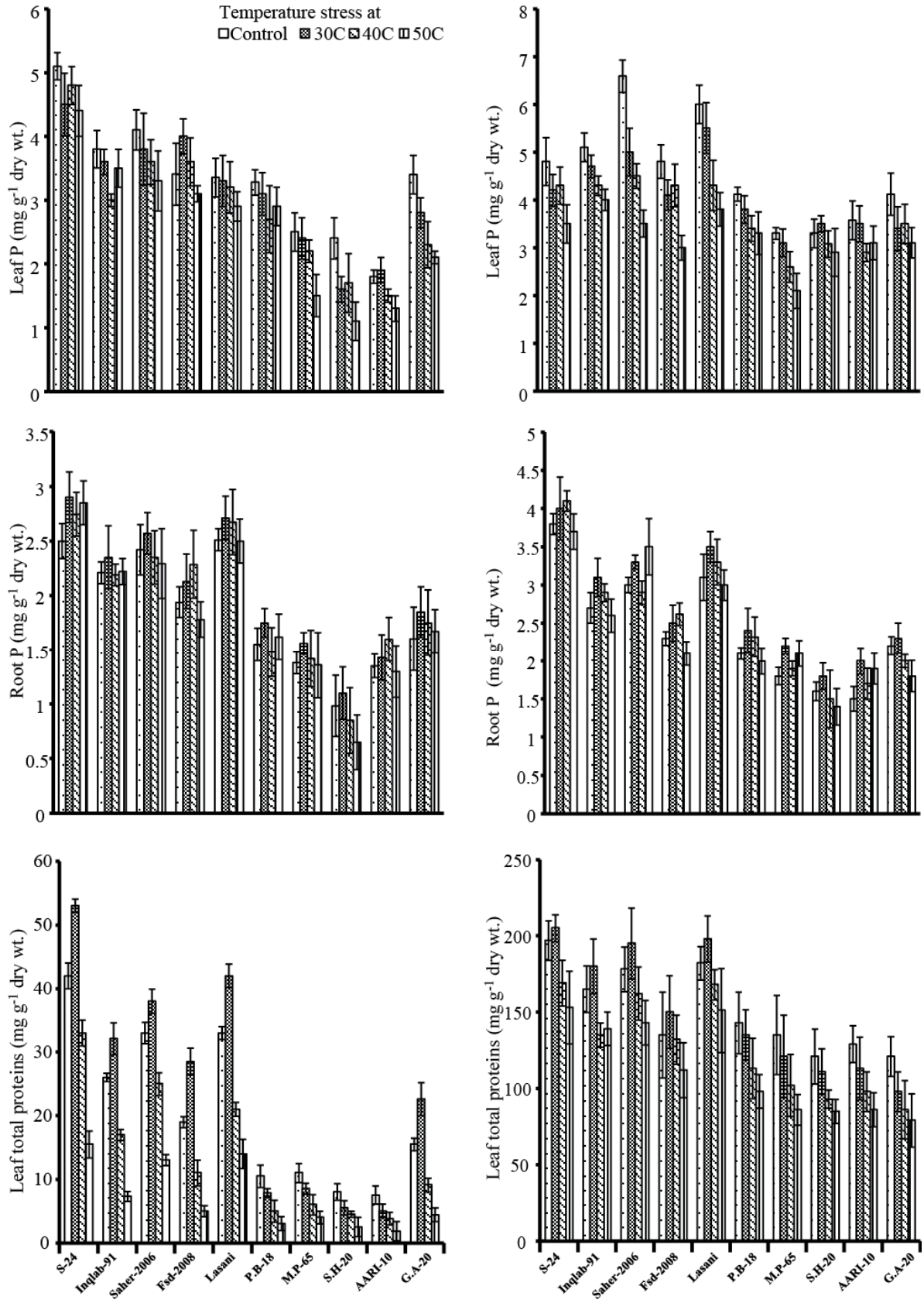

Fig. 4. Leaf and root P concentrations and leaf total soluble proteins in 10 elite cultivars of spring wheat (*Triticum aestivum* L.) subjected to varying levels of temperature stress at the tillering and booting stages (Mean ± S.E.).

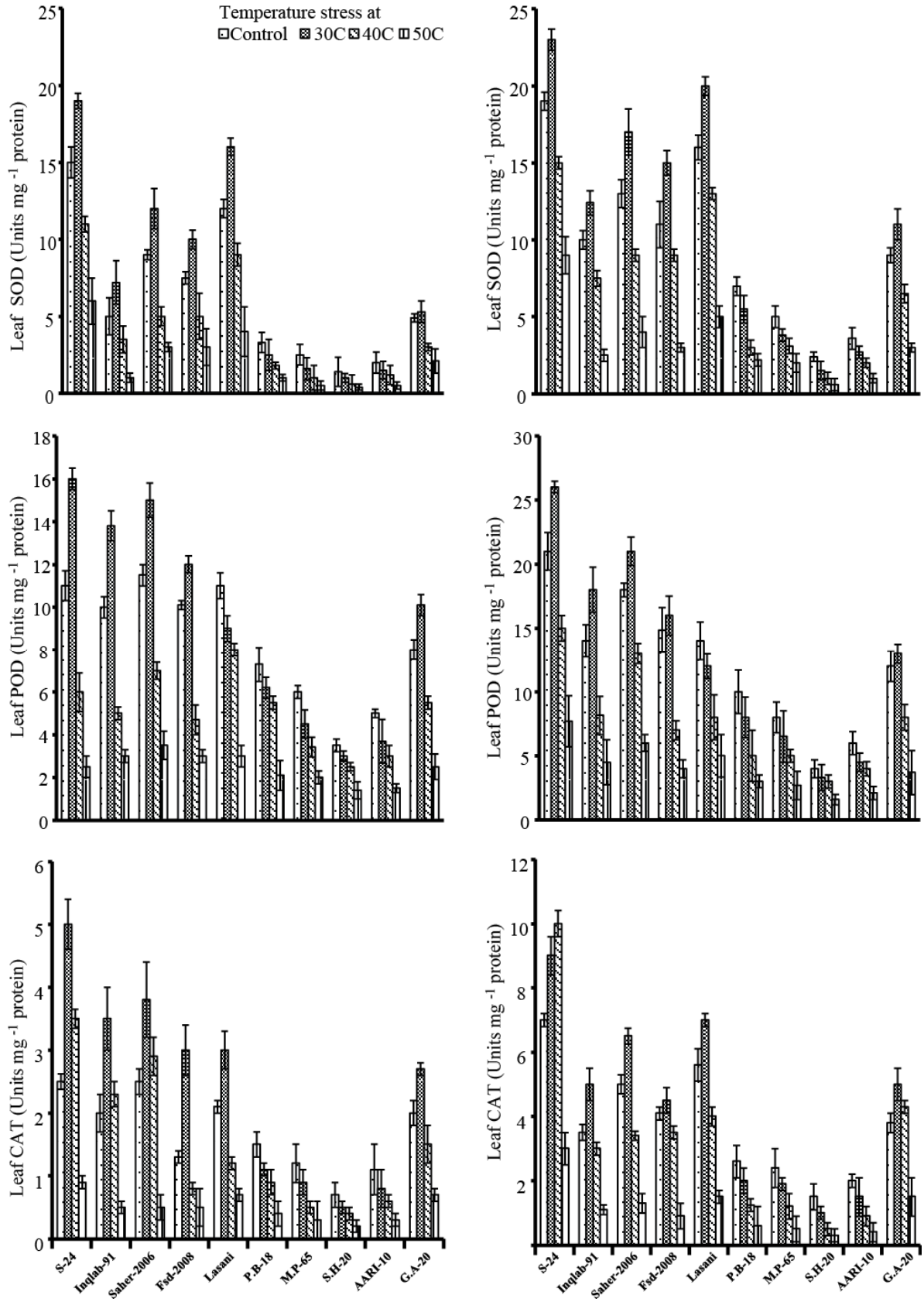

Fig. 5 Activities of leaf superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) in 10 elite cultivars of spring wheat (*Triticum aestivum* L.) subjected to varying levels of temperature stress at the tillering and booting stages (Mean ± S.E.).

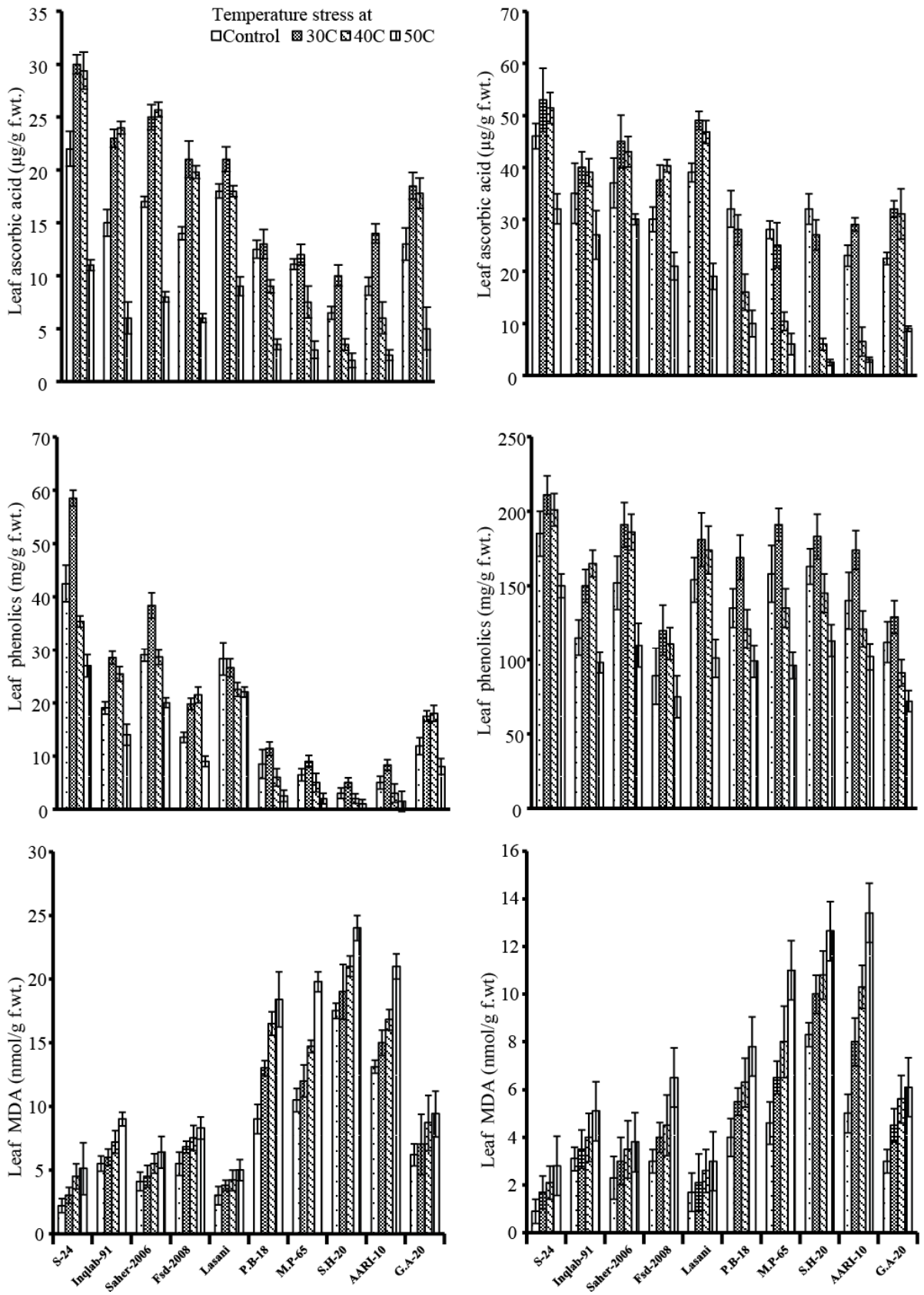

Fig. 6. Leaf ascorbic acid (AsA), total phenolics and malondialdehyde (MDA) contents in 10 elite cultivars of spring wheat (*Triticum aestivum* L.) subjected to varying levels of temperature stress at the tillering and booting stages (Mean ± S.E.).

Discussion

In the present study, a significant decreasing effect of temperature stress (30, 40 and 50°C) applied at the tillering or boot stage was observed on shoot and root fresh weights and shoot length of all 10 wheat cultivars. The most effective temperature in this regard was found to be 50°C. Among all wheat cultivars, cvs. Lasani, G.A-20 and Saher-2006 were better at all temperature regimes. As there is a narrow temperature range for proper growth and development of staple cereal crops including wheat, so any temperature beyond this particular range can disturb fertilization, grain production and ultimately reduce average crop yield (Wang *et al*., 2011). In order to survive under high temperature regimes plants have developed a variety of mechanisms that enable them to tolerate heat stress conditions (Xu *et al.,* 2011). Antioxidant defense system is one of the defense mechanisms which plays an important role in the detoxification of ROS produced under abiotic stress conditions including heat stress (Sairam & Tyagi, 2004; Mustafi *et al.,* 2009**).**

Superoxide dismutase (SOD) is one of the major antioxidant enzymes which acts as a first line of defense against free oxygen radicals (Ashraf, 2009; Akram *et al.,* 2012). In the present study, leaf SOD increased transiently at 30°C in cvs. S-24, Inqlab-91, Saher-2006, Fsd-2008, Lasani and G.A-20 while it decreased in cvs. P.B-18, M.P-65, S.H-20, and AARI-10 with a further increase in temperature imposed at both growth stages. An increase in SOD activity as observed in S-24, Inqlab-91, Saher-2006, Fsd-2008, Lasani and G.A-20 at 30°C has also been reported in some cultivars of creeping bentgrass (Huang *et al.,* 2001) and Kentucky bluegrass (He *et al.,* 2005). The observed decline in SOD activity in cvs. P.B-18, M.P-65, S.H-20, and AARI-10 with increasing temperature may have been due to increased rate of enzyme degradation or low synthesis of this enzyme within the cell at high temperatures. The high temperature induced decrease in SOD activity was found to be due to reduced transcript levels of genes encoding SOD in tobacco plants which were exposed to heat stress (Rizhsky *et al.,* 2002). The observed decline in SOD activity in the wheat cultivars is parallel to the findings of Gur *et al*., (2010), who while working with cotton (*Gossypium hirsutum*) reported that SOD activity in the leaves of heat (38 and 45°C) treated plants was lower than that in the control plants.

Mild temperature (30°C) applied at both tillering and boot stages of growth, increased POD activity, but higher temperatures (40 and 50°C) decreased POD activity particularly in cvs. S-24, Inqlab-91, Saher-2006, Fsd-2008 and G.A-20 compared to the controls. These findings are in line with the work of Saleh (2007) on mungbean in which leaf POD contents decreased at 40, 45 and 50°C. Chakraborty & Pradhan (2012) reported that in six varieties of lentil (*Lens culinaris* Medik.) POD activities decreased in response to high temperatures ranging from 30-50°C. The maximum POD activity observed in cv. S-24 indicates its better defensive mechanism against oxidative stress. Earlier Al-Meselmani *et al*., (2009) reported that heat tolerant genotypes of wheat possess efficient activities of antioxidant enzymes than non-tolerant genotypes.

Catalase (CAT) is another free oxygen radical scavenging enzyme which takes part in controlling signal transduction pathways involving H_2O_2 (Bechtel & Bauer, 2009). In the present study, it was found that in genotypes S-24, Inqlab-91, Saher-2006, Fsd-2008, Lasani, and G.A-20) catalase activity increased initially at 30°C, then it began to decline with a further increase in temperature, while in the other genotypes P.B-18, M.P-65, S.H-20, and AARI-10 it declined with a rise in ambient temperature. The observed decline in CAT activity has also been reported in wheat and some other plants species. For example, Dash & Mohanty (2002) showed that in wheat (*Triticum aestivum* L.) seedlings CAT activities increased at 30°C but thereafter it declined at 40°C. Saleh *et al*., (2007) also reported a decline in CAT activity in mungbean in response to heat stress (40, 45 and 50°C).

Among non-enzymatic scavenging system, ascorbic acid (AsA), carotenoids, glutathione (GSH), tocopherols and phenolics are main antioxidants which are involved in counteracting free oxygen radicals. Leaf AsA contents in all wheat cultivars were significantly influenced due to high temperature stress applied at the tillering and boot stages. Leaf AsA contents increased in cvs. S-24, Inqlab-91, Saher-2006, Fsd-2008 and G.A-20 at 40°C and then decreased at 50°C. Shen *et al*., (2009) reported that in two cool season turfgrass species, AsA contents declined significantly at 38°C and a further decrease was observed at higher temperatures (42 and 46°C).

Phenolics also act as antioxidants in response to multiple stress conditions including high temperature stress. Temperature stress imposed at different growth stages significantly influenced phenolic concentration in the leaves of all wheat cultivars. Temperature stress particularly 30°C imposed at both tillering and boot stages increased leaf phenolic contents in cvs. P.B-18, M.P-65, S.H-20, and AARI-10, whereas such increase in phenolics was observed in cvs. Inqlab-91 and Fsd-2008, when the temperature increased to 40 and 50°C. Some previous reports also show that in some plants (strawberry, spearmint) leaf phenolics increased at 30°C (Wang & Zheng, 2001; Fletcher *et al.,* 2005). In cvs. S-24, Lasani, G.A-20 and Saher-2006, quite a different trend was observed when heat stress applied at two different growth stages. For example, in cvs. S-24 and Saher-2006, leaf phenolics increased at 30°C applied at the tillering stage and up to 40°C applied at the boot stage. These differences in phenolic contents at different growth stages among different cultivars (S-24, Lasani, G.A-20 and Saher-2006) might have been due to differences in their thermo-sensitivity at different phenological stages. This is analogous to what has been observed earlier in some other wheat cultivars while examining their degree of salt tolerance at different growth stages (Ashraf & Khanum, 1997).

The product of membrane lipid peroxidation, malondialdehyde (MDA), was also influenced significantly by high temperature imposed at different growth stages. Heat stress imposed at both tillering and boot stages increased MDA contents in all cultivars examined. MDA contents were relatively higher in P.B-18, M.P-65, S.H-20, and AARI-10 and lower in Lasani and S-24. MDA is believed to be an indicator of plant cellular injury induced by oxidative stress (Liu *et al.,* 2012). In the present study, MDA levels were comparatively higher in the cultivars in

which antioxidant enzymes (SOD, POD, CAT) and antioxidant compounds (AsA & phenolics) exhibited comparatively low activities. These findings coincide with the findings of Liu *et al*., (2012) who reported that in two cool season turfgrass species in response to heat stress (38, 42, 46°C) antioxidant activities were lower and MDA levels higher in thermo-sensitive species than those in thermotolerant ones.

Potassium (K^+), is necessary for photosynthesis, movement of photosynthates into sink organs, maintenance of turgor pressure, and activation of some key metabolic enzymes. It also influences uptake different elements in plants subjected to stress conditions (Cakmak, 2005). In cvs. S-24, Inqlab-91, Saher-2006, Fsd-2008, Lasani, and G.A-20, leaf K^+ contents increased with increase in temperature up to 40°C imposed at the tillering stage and then declined at 50°C. In the remaining cultivars P.B-18, M.P-65, S.H-20, and AARI-10 leaf K^+ increased up to 30°C and then declined at 40 and 50°C. Ashraf & Hafeez (2004) reported a significant increase in K^+ uptake in pearl millet and maize under high temperature stress conditions. Increase in K^+ contents in the wheat cultivars up to 40°C is in agreement with a previous report in which increase in shoot K^+ contents has been observed in wheat up to 40°C temperature stress (Dias *et al.,* 2009). Cakmak (2005) reported that a higher and better nutritional status of K^+ in a plant can mitigate free radical-induced oxidative stress by inactivating NAD(P)H oxidases and sustaining electron transport chain in chloroplasts.

Despite a variety of roles played by nitrogen (N), it can facilitate the proper utilization and uptake of K, P and other elements in plants. In the present study, N contents were also influenced significantly in all wheat cultivars due to heat stress applied at both stages of plant growth and development. A maximal rise in leaf K^+ in cv. Saher-2006 was observed at temperature stress imposed at the tillering stage and in cv. S-24 when heat stress applied at the boot stage. Pedersen *et al*., (2004) reported that absorption of N in different species of *Porphyra* increased with a rise in external temperature. Root N contents like those of leaf also exhibited a significant inter-cultivar variation under heat stress imposed at two different growth stages. Falah *et al*., (2010) reported that root nutrient uptake increases in response to temperature but prolonged temperature exposure reduces root capabilities to uptake nutrients.

Leaf P contents were influenced significantly under heat stress imposed at two different growth stages. However, the pattern of leaf P accumulation differed from cultivar to cultivar under varying temperature regimes. For example, heat stress applied at the tillering stage decreased leaf P in cvs. S-24, Inqlab-91, Saher-2006, Lasani, P.B-18, M.P-65, S.H-20 and G.A-20, whereas that applied at the boot stage caused reduction in leaf P in almost all cultivars. A similar decline in leaf P contents with increase in temperature has previously been reported in tomato (Klock *et al.,* 1997), cool season Kentucky bluegrass (*Poa pratensis* L.) (Shen *et al.,* 2009) and a genotype of wheat (Dias *et al.,* 2009).

The pattern of Ca^{2+} accumulation in the leaves and roots of the heat stressed plants of 10 wheat cultivars was inconsistent. Generally, both leaf and root Ca^{2+} increased at the mild temperature (30°C) in most of the cultivars, whereas at the higher temperature regimes (40 and 50°C) a decline in tissue Ca^{2+} was observed. A transient increases in cytosolic calcium levels under heat stress have been observed in many plants. For example, Gong *et al*., (1998) reported a transient increase in cytosolic calcium in response to heat stress in tobacco, Liu *et al*., (2003) in wheat, Liu *et al*., (2006) in suspension-cultured *Arabidopsis* cells, and Saidi *et al*., (2009) in moss plants.

In conclusion, wheat cvs. S-24, Inqlab-91, Saher-2006, Fsd-2008, Lasani and G.A-20 exhibited better thermo-tolerance capacity than the other wheat cultivars P.B-18, M.P-65, S.H-20, and AARI-10, which was found to be associated with their better antioxidant defense mechanism and optimal nutrient uptake under varying high temperature regimes.

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