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STUDY OF PHYSIOLOGICAL AND BIOCHEMICAL INDICATORS OF TEMPERATURE TOLERANCE IN WHEAT (*Triticum aestivum* L.) IN RESPONSE TO SHORT-TERM SPATIOTEMPORAL TEMPERATURE VARIATION

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Wheat production is sensitive to minor temperature changes, and thus the identification of indicators of temperature tolerance is of great importance. In the present study, shoots of 20 d old plants of six wheat (*Triticum aestivum* L.) cultivars, namely, Millat-2011, Galaxy-2013, Fsd-2008, Sahar-2006, Lasani-2008 and Shafaq-2006 were exposed to heat stress (37 °C) for 6, 12, 24 and 48 h in the growth incubator while the temperature of root was maintained at 25 °C. The root and shoot temperature of control plants was set at 25 °C. The results indicated that short-term heat stress (STHS) significantly decreased photosynthetic pigments, leaf relative water contents (RWC) and peroxidase (POD) activity. By contrast, the membrane permeability, hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents were increased. In addition, an increase in the activities of catalase (CAT) and ascorbate peroxidase (APX), and the concentrations of anthocyanins, ascorbic acid and proline was recorded irrespective of cultivars under heat stress. Among the cultivars, Galaxy-2013 followed by Millat-2011 had higher chlorophylls, activities of CAT and APX, along with higher RWC and anthocyanins in the shoot exposed to STHS. Further, Galaxy-2013 and Millat-2011 had relatively higher levels of phenolics, flavonoids, soluble proteins and soluble sugars both in the shoot and root under STHS. In contrast, spatiotemporal STHS severely affected membrane permeability, root and shoot metabolism and antioxidant activities in the Shafaq-2006. Taken together, Galaxy-2013, Millat-2011 and Shafaq-2006 proved to be tolerant, moderately tolerant and sensitive to heat stress, respectively. The physio-biochemical traits in the shoot rather than in the root were linked with tolerance to spatiotemporally applied STHS possibly due to the reason that root was incubated at 25 °C irrespective of shoot temperature.

Keywords: growth, heat tolerance, metabolites, root responses, shoot responses, wheat

INTRODUCTION

Approximately 0.5 °C mean surface temperature increase was observed globally in the 20th century while yearly world temperature is expected to increase up to 4.0 °C till the end of the 21st century (Bita and Gerats, 2013). In Addition to these climatic changes, heat waves occur more frequently and affect crop productivity (Lohani *et al.*, 2020). Consequently, to keep away the negative effects of climate change on crop productivity, the understanding of tissue-specific responses of crop species to high temperature is critical.

Among cereals, wheat (*Triticum aestivum* L.) is one of the great sources of protein to the world population. In the developing countries, demand for wheat is probably to increase many folds by the year 2050. It is particularly sensitive to changes in temperature and is grown in areas where high temperatures usually limit its productivity (Rangan *et al.*, 2020). Heat stress can alter metabolism, chlorophyll contents, membrane stability and the accumulation of primary and secondary metabolites in plants (Iqbal *et al.*, 2015). Further, it accelerates the accumulation of antioxidant molecules and up-regulates the activities of antioxidant enzymes (Hussain *et al.*, 2016). High temperature accelerates developmental phases resulting in smaller organs and ultimately reduces productivity. Hence, rises in temperature during developmental stages cause great economic loss in wheat. Being strictly self-pollinated crop, each degree rise in temperature could alter differences in maturity of stamens and stigma causing great yield losses. Thus, the fluctuations in temperature during its different developmental stages largely affect growth and yield particularly in the arid and semi-arid regions.

Wheat resistance to heat stress could differ under the natural environmental conditions where the roots are at the lower temperature being underground as compared to the shoots. Roots having a lower optimum range of temperature for growth play critical roles in plant survival and are highly sensitive to changing environmental conditions as compared

to other plant parts (Huang *et al.*, 2012). Thus, most of the earlier studies conducted in growth incubators using the same root and shoot temperature to understand the effects of heat stress under controlled conditions are not comparable with actual field conditions. Further, the results of the studies conducted under field conditions are usually not reproducible due to different experimental errors that are beyond control under natural environments. Therefore, it is imperative to study the physio-biochemical responses of shoots to spatiotemporally variant temperature while keeping the root temperature constant.

MATERIALS AND METHODS

The experiment was performed using genetically diverse six wheat (*Triticum aestivum* L.) cultivars, namely, Millat-2011, Galaxy-2013, Fsd-2008, Sahar-2006, Lasani-2008 and Shafaq-2006 obtained from the Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan. The experiment was laid-out in a completely randomized design with four replicates. The plastic pots (400 mL) were filled with an equal weight of washed and dried sand keeping 1 cm empty at the top, and then fully saturated with half-strength Hoagland's nutrients solution. The whole pots except the top 1 cm were submerged in the water bath put in a growth incubator (Sanyo, Model MLR-351H) set at 25 °C and the root zone temperature was maintained at 25 °C through water circulation around the pots. The seeds of each cultivar were sown in the pots and after germination; plants were allowed to grow up to 20 days in the growth incubator with predetermined root and shoot temperature (25 °C). Later, one set of plants were shifted to another growth incubator where shoots were exposed to heat stress (37 °C) daily for 10 h. The root temperature was kept at 25 °C through continuous water circulation around the pots placed in the water bath. The night temperature was maintained at 25 °C in both the incubators. Further, in both the growth incubators, the other growth conditions such as photosynthetically active radiation (370 µmol m^{-2} s^{-1}),relative humidity (70%, ± 5) and light/dark period (10/14 h) were kept the same. The root and shoot were harvested after 6, 12, 24 and 48 h and the data for various attributes was recorded.

Growth analysis: The plants of each cultivar were harvested after 6 and 48 hours, and the root and shoot were separated with a sharp blade and their lengths were measured in centimeter with a scale. The leaf area of the fully mature top leaf was determined by multiplying the length and width of leaf with 0.75 (correction factor). The root and shoot fresh weights were determined and after drying in an oven at 65 °C for 72 h, root and shoot dry weights determined.

Photosynthetic pigments, leaf RWC and membrane permeability determination: For the pigments determination, the fresh leaf was ground in acetone (80%) and the absorbance of the supernatant was measured with UV-Vis spectrophotometer (U-1800, Hitachi, Japan) at 663, 645 and 480 nm the and chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids were calculated using the formulae as described earlier (Arnon, 1949; Kirk and Allen, 1965). The leaf RWC were determined following the method described earlier (Barrs and Weatherley, 1962). The electrolyte leakage was used to determine the leaf membrane permeability (Lutts *et al.*, 1996).

Oxidative stress indicators (H_2O_2 and MDA): The H_2O_2 concentration was determined as described earlier (Velikova *et al.*, 2000). The H_2O_2 concentration was calculated by using a standard curve made by using different concentrations of analytical grade H_2O_2. For MDA content, the reaction mixture consisted of sample extract in 10% trichloroacetic acid (TCA, 1 mL) and 6% thiobarbituric acid (2 mL). The mixture was boiled for 30 min, and the absorbance of the supernatant was measured at 532, 600 and 450 nm against blank and the MDA contents calculated by using the formula as described earlier (Dhindsa *et al.*, 1981).

Assay of enzymatic antioxidants: The fresh leaf sample was homogenized in potassium-phosphate buffer (50 mM; pH 7.5) using chilled pestle and mortar. The mixture was centrifuged at 15,000 *g* for 15 min. The supernatant was used to measure the activities of enzymatic antioxidants. The CAT activity was measured by the method as described earlier (John *et al.*, 2007). The POD activity was assayed by using guaiacol as substrate (Maehly and Chance, 1954). The activity of APX was determined by following the method of Nakano and Asada (1981). The reaction mixture consisted of 2.7 mL of L-ascorbate (0.5 mM), H_2O_2 (0.2 mL, 0.5 mM) and 0.1 mL enzymes extract.

Total phenolics and flavonoids: Total phenolics were determined following the method of Wolfe *et al.* (2003). The reaction mixture consisted of 1 mL plant extract in 80% methanol, 2.5 mL folin-ciocalteau reagent and 2 mL Na_2CO_3. The total phenolics were calculated by using a standard curve made by using analytical grade tannic acid. The flavonoids were determined by the colorimetric assay (Zhishen *et al.*, 1999). The total flavonoids were calculated from a standard curve made by using analytical grade catechin.

Total soluble proteins, free amino acids and soluble sugars: The estimation of total soluble proteins was carried out as described earlier (Bradford, 1976). The total soluble proteins were calculated from a standard curve made using the bovine serum albumin. Total free amino acids were determined by using fresh tissues (Hamilton and Slyke, 1943). The free amino acids were calculated from a standard curve of analytical grade L-glycine. Total soluble sugars were estimated by using anthrone reagent (Dubois *et al.*, 1956). The total soluble sugars were calculated from the standard curve made using analytical grade glucose.

Determination of anthocyanins, proline and ascorbic acid: The plant material was homogenized in potassium phosphate buffer (5 mL; 50 mM; pH 7.5) and the absorbance of the supernatant was taken at 600 nm and the anthocyanins were

calculated in Units g^{-1} FW (Kubo *et al.*, 1999). The proline concentration was assayed as reported earlier (Bates *et al.*, 1973). The proline concentration was calculated by using the proline standard curve. The plant sample was extracted in TCA and the ascorbic acid (AsA) concentration was determined by using the DTC (2, 4-dinitrophenyl hydrazine, thiourea and copper sulphate) reagent (Mukherjee and Choudhuri, 1983). The concentration of ascorbic acid was calculated from a standard curve of L-ascorbic acid.

Assessment of genetic variation in wheat cultivars: The genetic variation (polymorphism) within six wheat cultivars was assessed by using 40 random amplified polymorphic DNA markers/primers (Fermentas Genelink) (Table S1).

Table S1. The sequences of 40 random amplified polymorphic DNA markers/primers (Fermentas Genelink) along with details of polymorphic bands in different wheat cultivars.

Primer	Sequence	Total bands	Polymorphic bands
A09	GGGTAACGCC	9	0
M03	GGGGGATGAG	0	0
M06	CTGGGCAACT	4	1
M07	CCGTGACTCA	8	2
M02	ACAACGCCTC	6	1
M08	TCTGTTCCCC	3	0
M09	GTCTTGCGGA	4	2
M10	TCTGGCGCAC	6	6
M11	GTCCACTGTG	2	2
A01	CAGGCCCTTC	0	0
A02	TGCCGAGCTG	8	2
A03	AGTCAGCCAC	7	4
A04	AATCGGGCTG	9	7
A05	AATCGGGCTG	1	1
A07	GAAACGGGTG	9	2
A08	GAAACGGGTG	10	6
A10	GTGATCGCAG	3	3
A11	GTGATCGCAG	8	7
A12	TCGGCGATAG	2	0
A13	CAGCACCCAC	3	1
A14	TCTGTGCTGG	5	2
A16	AGCCAGCGAA	8	2
A18	AGGTGACCGT	8	0
A19	CAAACGTCGG	5	3
A20	GTTGCGATCC	6	6
B01	GTTTCGCTCC	7	3
B03	CATCCCCCTG	8	7
B06	TGCTCTGCCC	9	0
B08	GTCCACACGG	9	0
B10	CTGCTGGGAC	9	0
B12	CCTTGACGCA	12	0
B17	AGGGAACGAG	8	1
B18	CCACAGCAG	9	1
B20	GGACCCTTAC	10	4
N04	GACCGACCCA	9	4
N05	ACTGAACGCC	8	1
N08	ACCTCAGCTC	7	7
J09	TGAGCCTCAC	7	2
J10	AAGCCCGAGG	10	2
J15	TGTAGCAGGG	10	1

The extraction of DNA was carried out by the method as described by (Doyle and Doyle, 1987). The reaction volume of PCR (25 µL) contained distilled water (8.3 µL), 10 × Taq polymerase buffer (Fermentas) (2.5 µL), gelatin (2.5 µL), $MgCl_2$ (Fermentas) (3.0 µL), dNTPs (dATP, dCTP, dGTP) (Fermentas) (4.0 µL), oligonucleotide primer (Gene Link Co. USA) (2.0 µL), Taq polymerase (Fermentas) (0.2 µL) and the template DNA (2.5 µL). The PCR reaction was programmed with the initial temperature of 95 °C for 5 min and 40 cycles (denaturation at 94 °C for 1 min, annealing at 36 °C for 1 min, extension at 72 °C for 2 min) and final extension at 72 °C for 10 min. The PCR products, as well as the DNA ladder (Fermentas), were resolved by electrophoresis and the gels were stained with ethidium bromide, and the amplification data were recorded by taking gel photographs with the gel documentation system (Dolphin–Doc, Wealtec). The PCR data was analyzed and the polymorphic fragments with each primer were noted to identify the polymorphism within cultivars.

Statistical analysis: The data was subjected to analysis of variance technique using CoStat (CoHort, version 6.204). When significant, the Student-Newman-Keuls test at 5% probability level was used to compare treatment means.

RESULTS

The spatiotemporal STHS had a non-significant effect on root and shoot lengths in different wheat cultivars. The STHS significantly reduced the leaf area that exhibited a progressive decrease irrespective of cultivars when compared with control (Table 1).

Table 1. Effect of short-term heat stress to shoot on the leaf area of genetically diverse wheat cultivars.

Cultivars	Control		Heat stress	
	6 h	48 h	6 h	48 h
Millat-2011	14.3±0.145	14.7±0.192	14.1±0.215	12.4±0.368
Galaxy-2013	18.3±1.441	22.0±0.256	16.8±0.899	15.6±0.297
Fsd-2008	9.28±0.179	9.66±0.148	7.13±0.444	8.26±0.532
Sahar-2006	6.68±0.130	11.5±0.201	5.85±0.274	9.07±0.279
Lasani-2008	9.86±0.304	10.2±0.270	9.10±0.425	7.39±0.133
Shfaq-2006	7.66±0.216	10.2±0.126	6.24±0.302	7.35±0.054

$LSD_{0.05}$ = 0.947; (n = 4; Means ± SE).

The maximum and minimum decrease in leaf area was observed in Galaxy-2013 and Shafaq-2006, respectively. Likewise, root and shoot fresh weights reduced significantly ($P < 0.05$) under STHS. Notably, less decrease in fresh weight was observed in Galaxy-2013 followed by Millat-2011, while the decrease in fresh weights was the maximum in Shafaq-2006 (Fig. 1). A gradual decrease in chlorophyll *a*, *b* and total chlorophyll contents was recorded in all cultivars. Afterwards, the chlorophyll contents increased, but remained reduced as compared with control plants. The minimum decrease in chlorophyll contents was observed in Galaxay-

2013 followed by Millat-2011 while the maximum decrease was noted in Shafaq-2006 (Fig. 2). Similarly, the STHS caused a gradual decrease in carotenoids contents under heat stress. The least reduction in carotenoids was found in Galaxy-2013 and Millat-2011 while the maximum reduction was observed in Shafaq-2006 (Fig. 2).

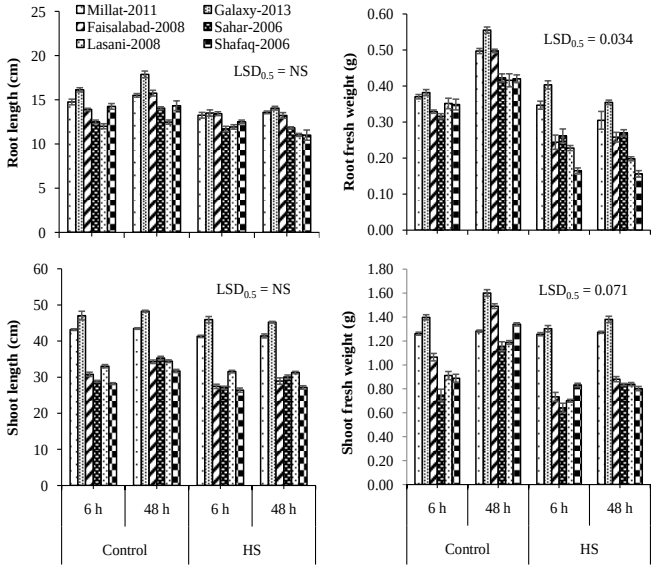

Figure 1. Effect of short-term heat stress on the growth attributes of genetically diverse wheat cultivars (n = 4; Means ± SE); NS, non-significant; HS, heat stress (37 °C)

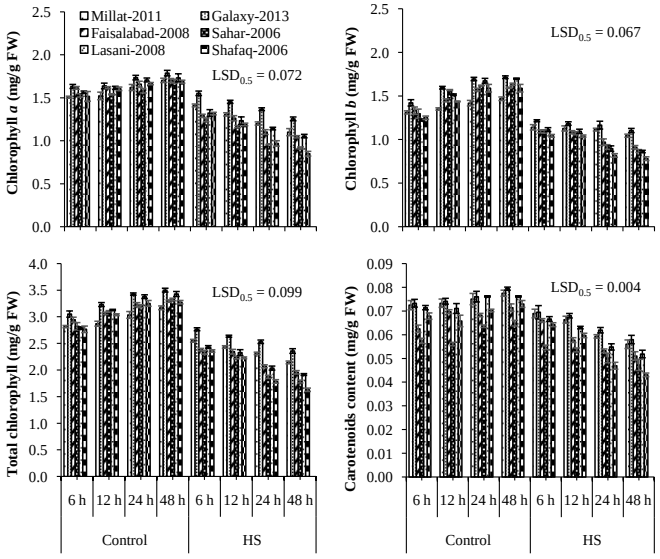

Figure 2. Effect of short-term heat stress on the leaf chlorophylls and carotenoids of genetically diverse wheat cultivars (n = 4; Means ± SE); HS, heat stress (37 °C)

The spatiotemporal STHS significantly ($P < 0.05$) increased membrane permeability in all cultivars. Although a progressive increase in membrane permeability due to increase in temperature duration was noted in all cultivars, the extent of membrane leakage was the minimum in the Galaxy-2013 cultivar. In contrast, the maximum increase in membrane permeability was recorded in Shafaq-2006 after 48 h as compared with control plants. The spatiotemporal STHS significantly ($P < 0.01$) altered leaf RWC. The maximum leaf RWC were recorded in the Galaxy-2013 followed by Millat-2011 while the minimum RWC were recorded in the Shafaq-2006 as compared with control plants (Fig. 3).

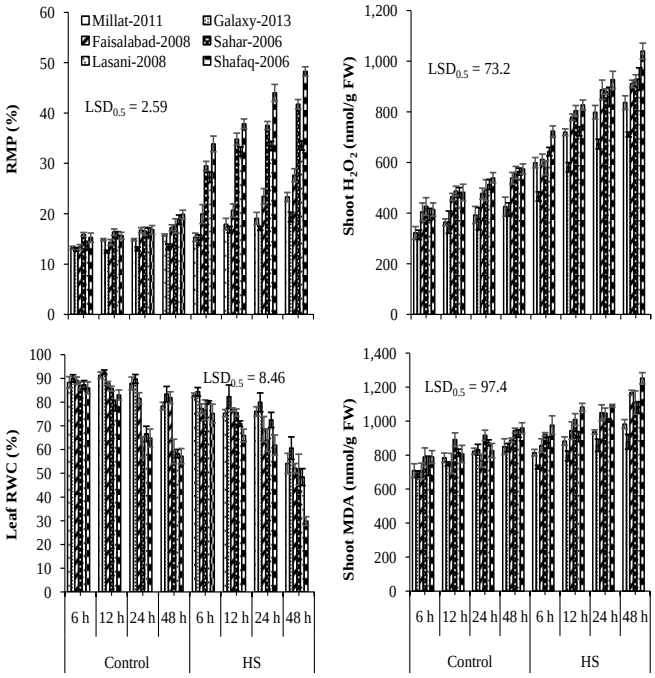

Figure 3. Effect of short-term heat stress on the leaf relative membrane permeability (RMP), relative water contents (RWC), shoot hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents of genetically diverse wheat cultivars (n = 4; Means ± SE); HS, heat stress (37 °C)

The STHS considerably increased the H_2O_2 concentration in shoots of different wheat cultivars. The Galaxy-2013 and Millat-2011 exhibited the minimum increase in H_2O_2 while the maximum increase was recorded in Shafaq-2006 as compared with respective control plants (Fig. 3). Just like H_2O_2, the maximum increase in MDA concentration was recorded in Shafaq-2006 after 48 h heat stress, whereas, less increase was found in Galaxy-2013 as compared with control plants (Fig. 3). The STHS applied to shoots significantly altered antioxidants activity. There was a significant increase in CAT activity of Galaxy-2013 under STHS while the minimum CAT enzyme activity was recorded in Shafaq-2006

as compared to the control plants (Fig. 4). Generally, the POD activity decreased with time. The same trend in POD activity was noted in plants exposed to STHS. However, the least decrease in POD activity was evident in Galaxy-2013 throughout STHS as compared with other cultivars. Whereas, the minimum POD activity was noted in Shafaq-2006 under STHS (Fig. 4). An increase in APX activity was found irrespective of cultivars under both control and heat-stressed conditions. The maximum increase in APX activity was observed in Galaxy-2013 while the minimum activity was noted in Shafaq-2006 (Fig. 4). The exposure of shoots to STHS considerably increased proline concentration being the maximum after 48 h in Galaxy-2013 while less increase in proline concentration was observed in Shafaq-2006 (Fig. 4).

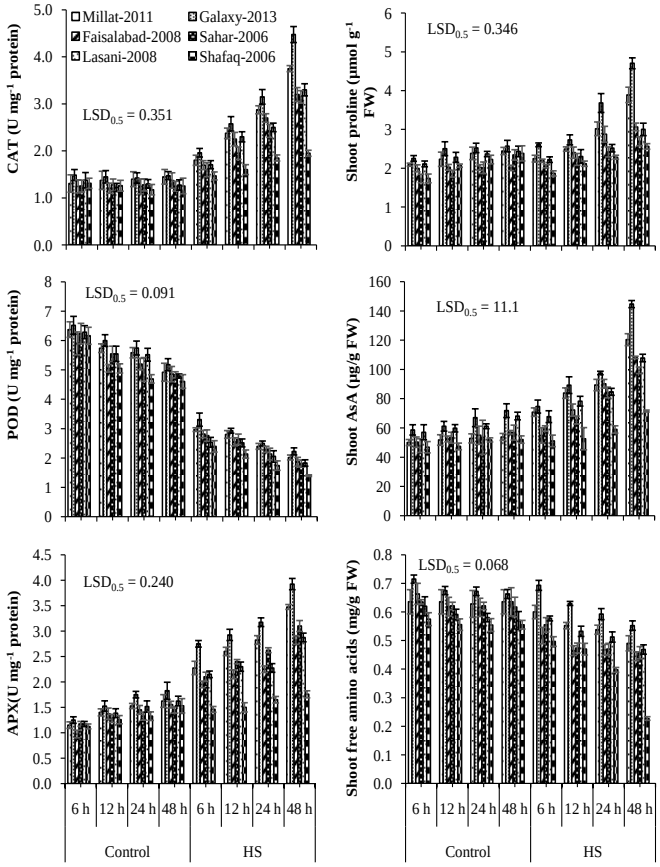

Figure 4. Effect of short-term heat stress on the activity of leaf catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) and concentrations of proline, ascorbic acid (AsA) and amino acids of genetically diverse wheat cultivars (n = 4; Means ± SE); HS, heat stress (37 °C)

The shoot ascorbic acid concentration increased under STHS with more increases after 48 h in Galaxy-2013 followed by Millat-2011, whereas the less increase was found in Shafaq-2006. The total free amino acids decreased in the shoots of wheat under STHS. The maximum reduction in amino acids was recorded in Shafaq-2006 after 48 h heat stress whereas the minimum reduction was recorded in Galaxy-2013 (Fig. 4). The STHS decreased phenolics concentration while it gradually increased in the shoots. The minimum decrease in phenolics concentration was observed in Galaxy-2013 after 6 h heat stress, while the maximum decrease was evident in Shafaq-2006 after 6 h heat stress. Whereas, the maximum increase in phenolics concentration was found in the cultivars Galaxy-2013 and Millat-2011 after 48 h heat stress. The STHS affected the root phenolics concentration differently. For instance, STHS increased phenolics followed by a decrease after 48 h of heat stress. The less decrease in phenolics concentrations was evident in the root of Galaxy-2013, while the maximum decrease was found in Shafaq-2006 (Fig. 5). The STHS significantly increased flavonoids concentration in shoots and rots of wheat cultivars. The maximum increase was evident in Galaxy-2013 while the less increase was found in Shafaq-2006 under STHS as compared to plants grown under control conditions (Fig. 5).

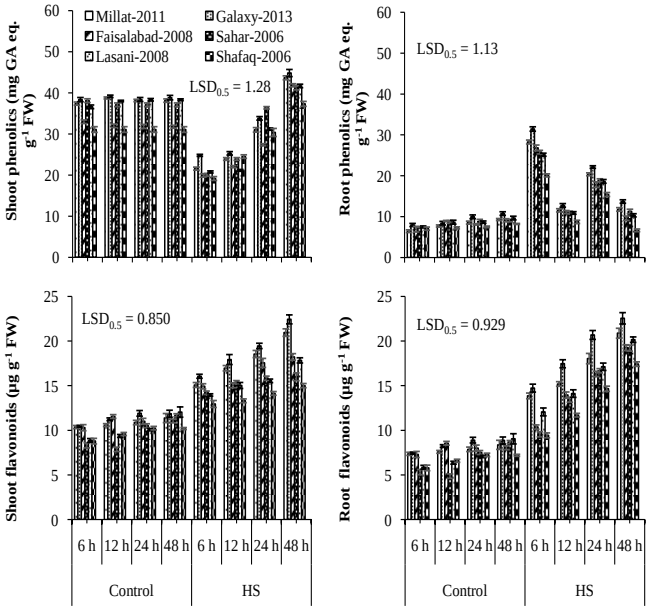

Figure 5. Effect of short-term heat stress on shoot and root phenolics and flavonoids of genetically diverse wheat cultivars (n = 4; Means ± SE); HS, heat stress (37 °C)

The heat stress increased shoot anthocyanins irrespective of cultivars. The maximum increase in anthocyanins was observed in Galaxy-2013 followed by Millat-2011 after 48 h heat stress, while the minimum shoot anthocyanins were recorded in Shafaq-2006 as compared to control (Table 2). The STHS significantly altered the soluble proteins concentration of wheat cultivars (Fig. 6). The shoot total soluble protein concentration gradually increased from 6 h to 48 h in the case of control plants while decreased when

Table 2. Effect of short-term heat stress to shoot on anthocyanins content of genetically diverse wheat cultivars.

Cultivars	Control (25 °C)				Heat stress (37 °C)			
	6 h	12 h	24 h	48 h	6 h	12 h	24 h	48 h
Millat-2011	2.74±0.075	2.64±0.049	2.79±0.050	2.79±0.055	4.10±0.059	4.71±0.125	5.04±0.076	5.37±0.147
Galaxy-2013	3.01±0.052	2.88±0.066	2.85±0.043	2.89±0.058	4.20±0.126	5.12±0.048	5.28±0.060	5.64±0.131
Fsd-2008	2.46±0.053	2.51±0.025	2.51±0.062	2.51±0.032	3.63±0.110	4.25±0.065	4.55±0.158	4.78±0.048
Sahar-2006	1.95±0.050	1.93±0.008	1.93±0.012	1.94±0.009	3.79±0.092	4.51±0.051	4.55±0.139	4.54±0.039
Lasani-2008	2.60±0.070	2.65±0.051	2.65±0.057	2.69±0.035	3.62±0.129	4.34±0.125	4.60±0.072	4.57±0.043
Shfaq-2006	2.20±0.129	2.23±0.128	2.29±0.088	2.23±0.114	3.40±0.089	4.08±0.039	4.34±0.084	3.58±0.128

$LSD_{0.05}$ = 0.235; (n = 4; Means ± SE).

exposed to heat stress. The reverse was true in the case of root where a gradual decrease in case of control while an increase in case of heat-stressed plants was evident. However, less reduction in shoot soluble proteins and the maximum increase in root soluble proteins was found in Galaxy-2013 whereas the reverse was true for Shafaq-2006 as compared with control plants. The total soluble sugars significantly decreased in the shoot and root under STHS. The maximum decrease was found in Shafaq-2006 while the minimum was observed in Galaxy-2013 followed by Millat-2011 as compared with control plants. Further, the reduction in sugar concentration was drastic in the roots of all wheat cultivars exposed to STHS (Fig. 6).

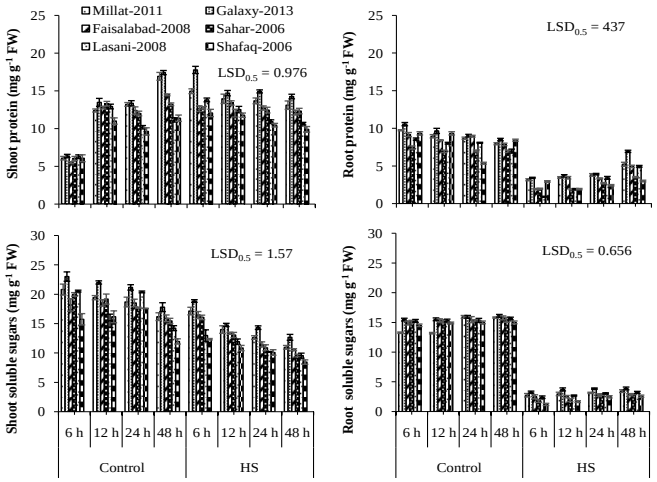

Figure 6. Effect of short-term heat stress on shoot and root soluble proteins and soluble sugars of genetically diverse wheat cultivars (n = 4; Means ± SE); HS, heat stress (37 °C)

The genetic difference among six wheat cultivars was evaluated with RAPD analysis. A total of 40 primers were used for this purpose. Of the 40 primers, 34 have shown polymorphism in six wheat cultivars. The percent polymorphism was calculated in each cultivar (Table 3). Briefly, 42.5% polymosphism was present in Millat-2011, 37.5% in Galaxy-2013, 35% in Fsd-2008,35% in Sahar-2006, 37.5% in Lasani-2008 and 32.5% in Shafaq-2006.

Table 3. The details of respective polymorphic primers and percent polymorphism in six wheat cultivars.

Wheat cultivars	Total primers	Polymorphic primers	Polymorphism (%)
Millat-2011	40	17	42.5
Galaxy-2013	40	15	37.5
Fsd-2008	40	14	35.0
Sahar-2006	40	14	35.0
Lasani-2008	40	15	37.5
Shafaq-2006	40	13	32.5

DISCUSSION

High temperature is one of the major environmental stresses that severely affect plant growth and productivity. Consequently, plants have developed several adaptations to cope with the temperature changes. Within the same species, different cultivars could exhibit different mechanisms to cope with environmental changes provided the same growth conditions (Mishra *et al.*, 2017). In the present study, heat stress was applied on shoots of six genetically diverse wheat cultivars, whereas root temperature was maintained at 25 °C. The high shoot temperature decreased leaf area, and root and shoot fresh mass, however, the minimum decrease was noted in the Galaxy-2013 and Millat-2011 cultivars. Further, the effect of STHS was more evident on root fresh mass rather than shoot fresh mass and leaf area in the cultivars. The decrease in the root fresh mass could be due to heat-mediated over evapo-transpiration in the aerial tissues. The heat stress-mediated decrease in root activity has already reported in foxtail millet (Aidoo *et al.*, 2016). Although heat stress affects several physiological processes, photosynthesis is adversely affected due to the loss of photosynthetic pigments (Aidoo *et al.*, 2016; Hussain *et al.*, 2016). The STHS caused a gradual reduction in chlorophylls and carotenoids contents as compared with control irrespective of cultivars. However, a more decrease in the pigments was noted in the Shafaq-2006 cultivar. The results are in agreement with the earlier finding of Iqbal *et al.* (2015) who found high temperature-mediated inhibition in chlorophyll contents of wheat. The accumulation of carotenoids could be helpful to protect different cellular structures from ROS damage leading to membrane stability under stressful environments. The cultivars Galaxy-2013 and Millat-2011 accumulated more carotenoids whereas the least

concentration was noted in Shafaq-2006. The exposure of maize to low temperature suppressed while high temperature promoted carotenoids accumulation in maize (Xiang *et al.*, 2019). The difference could be because the corn seedlings were grown in trays and were shifted to the chamber set at high temperature where both root and shoot were exposed to the same temperature.

The highest membrane permeability was observed in Shafaq-2006, while the least membrane permeability was recorded in Galaxy-2013. In consistent with membrane permeability, Galaxy-2013, displayed the least decrease in leaf water concentration. The maintenance of higher RWC in Galaxy-2013 could have helped to better tolerate stress by adaptation in photosynthesis and other metabolic processes involved in plant development. Taken together both membrane permeability and RWC, it is expected that distinct responses of cultivars might be a collective effect of their adaptability and survival by readjustment of metabolism under high-temperature stress. Further, heat stress increased H_2O_2 and MDA concentration in all cultivars particularly in the Shafaq-2006. The oxidants such as H_2O_2 and MDA have been suggested as markers for heat stress tolerance (Hussain *et al.*, 2016). Thus, the cultivars that accumulated more oxidants had higher membrane permeability and showed less tolerance to high temperature.

The heat-tolerant plants tend to protect themselves from the damaging effects of ROS with the synthesis of enzymatic antioxidants. Antioxidants maintain the equilibrium between the production and detoxification of ROS. Both CAT and APX enzymes play an important role in heat stress. CAT reacts with H_2O_2 to decompose it, and its enhanced activity is related to stress tolerance (Hussain *et al.*, 2016). The higher CAT and APX activity was observed in plants of Galaxy-2013 under heat stress, while Shafaq-2006 exhibited comparatively low activities. Such variations in CAT activity could be either due to an increased level of enzyme degradation or failure of enzyme synthesis. Further, increases in CAT and APX activities are also reported under heat stress (Sgobba *et al.*, 2015). The enhanced CAT and APX activities in the present study enabled plants to reduce ROS under STHS. However, heat stress decreased POD activity in the present study. This could be due to differential root zone and shoot temperatures that altered POD activity. Further, different varieties of the same species could show differential response to heat stress in terms of POD activity. For instance, high rhizosphere temperature increased POD activity in the leaves and decreased in the stems and roots of super rice, while contrasting results were found in the non-super rice (Li *et al.*, 2019). An increase in POD activity was reported in wheat (Almeselmani *et al.*, 2006) under high-temperature stress.

Different metabolites such as phenolics, flavonoids, lignins and anthocyanins play critical role in plants tolerance to abiotic stresses. Initially, the total phenolic concentration decreased in the shoot followed by gradual increase with the passage of time under heat stress. The reverse was true in the root when under heat stress. The STHS increased flavonoids contents both in the root and shoot of all cultivars. The maximum flavonoids concentration was recorded in the Galaxy-2013 cultivar. Under the STHS, anthocyanins increased in the shoots of all cultivars especially in Galaxy-2013 followed by Millat-2011. The phenolics, flavonoids and anthocyanins could protect plants from oxidants and are part of the acclimation mechanism against heat stress (Bartwal *et al.*, 2013). Further, the soluble protein concentration of shoots increased under STHS indicating the newly synthesized stress proteins. The accumulation of a variety of proteins including heat shock proteins and other stress-related proteins is an important general response of plants to heat stress. The root soluble proteins decreased and then gradually increased under STHS. The maximum soluble proteins were observed in the root of Galaxy-2013 while the minimum in the Shafaq-2006 after 48 h of heat stress. The root was maintained at a constant temperature while the shoot temperature was raised. In this context, root initially didn't face heat stress so had less soluble proteins while later signals from shoot might have triggered the synthesis and accumulation of proteins particularly in the tolerant cultivars. The increased stability and less degradation of proteins are linked with thermotolerance of root (Huang *et al.*, 2012).

The accumulation of different soluble and low molecular weight compatible osmolytes that don not interact with normal metabolic processes are usually considered useful under stressful environments including heat stress (Hussain *et al.*, 2016; Iqbal *et al.*, 2015). Sugars are involved in signaling process, can scavenge ROS and their accumulation could induce heat stress tolerance (Sugio *et al.*, 2009). The STHS decreased total soluble sugars in the shoot and root of wheat cultivars. However, Galaxy-2013 had relatively more sugars and the minimum were noted in Shafaq-2006 when under heat stress. The proline and ascorbic acid concentrations increased considerably after 48 h of heat stress in Galaxy-2013. Whereas, total free amino acids decreased due to heat stress and the minimum concentration was noted in Shafaq-2006. Consistent with the present results, heat shock mediated rise in proline and decline in free amino acids concentration is earlier reported in maize seedlings (Hussain *et al.*, 2016). The proline is involved in stress signaling and can scavenge ROS and modulate cellular functions to generate cross adaptability in stressful environments (Kaur and Asthir, 2015). Similarly, ascorbic acid not only scavenges ROS, but also can modulate various growth and developmental processes (Akram *et al.*, 2017). Thus, the tissue accumulation of sugars, proline and ascorbic acid in the present study suggested their role as non-enzymatic antioxidants, and the modulator of heat stress-driven responses in genetically diverse wheat cultivars.

Of the six wheat cultivars studied, some cultivars exhibited better adaptability than the others did. Therefore, we assessed

genetic variation among cultivars using the random-amplified polymorphic DNA (RAPD) molecular marker. Being cost-effective and easy to perform, RAPD can be used effectively to assess genetic variations among cultivars (Kapteyn *et al.*, 2002). The maximum polymorphism (42.5%) was observed in the Millat-2011 followed by the Galaxy-2013 (37.5%). However, the minimum polymorphism (32.5%) was noted in the Shafaq-2006. The results showed that wheat cultivars used in the present study were genetically diverse, and thus showed different physio-biochemical responses to STHS. Taken together physio-biochemical responses of wheat cultivars to heat stress, and the genetic diversity among cultivars, the Shafaq-2006 was attributed a heat susceptible cultivar compared with other cultivars. In contrast, the Galaxy-2006 and Millat-2011 that exhibited relatively greater polymorphism were more tolerant to heat stress. Thus, the cultivars, namely, Galaxy-2006, Millat-2011 and Shafaq-2006 were ranked as the tolerant, moderately tolerant and sensitive cultivar, respectively to STHS.

***Conclusions*:** The more polymorphic cultivars (Millat-2011 and Galaxy-2013) showed higher tolerance to STHS than the less polymorphic cultivar (Shafaq-2006) that was evident from their higher growth and metabolic stability under heat stress. The abilities of wheat plants to maintain higher leaf chlorophylls and RWC coupled with the higher shoot enzymatic and non-enzymatic antioxidants, and the membrane stability were attributed as physio-biochemical markers for tolerance to STHS.

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