Heat Tolerance Evaluation of Wheat (*Triticum aestivum* L.) Genotypes Based on Some Potential Heat Tolerance Indicators

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(Received on 11th December 2012, accepted in revised form 1 march 2013)

Summary: Twenty wheat genotypes including advance lines and cultivated varieties were evaluated for terminal heat stress under glass house conditions in pots using completely randomized design with three replications in 2010-2011. Plants were exposed to 35-40 °C temperature 3 hours daily for five consecutive days. Control plants were kept under normal temperature conditions. The stress tolerance indicators such as Superoxide dismutase (SOD), Peroxidase (POD), Photosynthates stem reserves (PSR), Membrane stability index (MSI) and grain yield revealed significant ($p<0.05$) effect of high temperature on growth and physiological attributes of wheat at anthesis growth stage. Cumulative response of Faisalabad-2008, Lasani-2008, Sussui and AARI-2011 was better in terms of proline accumulation, better antioxidant defense mechanism, PSR, MSI, and grain yield under stress and non- stress conditions. But 4th EBWYT-1, 4th EBWYT-2, 4th EBWYT-3, and NR-397 were found heat sensitive on the basis of used heat tolerance indicators. In addition to the grain yield plant breeders should also include SOD, POD, PSR and MSI as markers in the breeding program for development of heat tolerant wheat cultivars.

Key words: Wheat (*Triticum aestivum* L.) genotypes, Heat stress, Proline, SOD, POD, and Heat stress susceptibility index (HSSI).

Introduction

Wheat (*Triticum aestivum* L.) is a source of protein and rank's second after rice as a source of calories in the diets of consumers in developing countries [1]. Keeping in view agriculture and food security, overall crop yield (wheat, maize and rice) could decrease in South Asia up to 30% by the end of this century (compared with an increase as much as 20% in East and South East Asia) [2].

Climate change-induced temperature increases are probable to reduce wheat production in developing countries (where around 66% of all wheat is produced) by 20-30% [3]. The IPCC [4] also noticed that global climate change (GCC) will have a major impact on crop production.

Heat stress is a particular problem owing to its pronounced spatial and temporal variations leading to declines in plant growth and productivity [5]. According to the estimates a rise in temperature of 1°C in the wheat growing season could reduce the wheat yields by about 3-10% [6].

Wheat is the major staple food crop of Pakistan. In order to meet the demand for food within the country we need to increase the production at least by 4% in accordance with the population growth rate. Wheat yields are sensitive to terminal heat stress due to delayed planting locally because of exposure of the crop to high temperature, from flowering to maturity. At this stage, heat stress causes shortening of the growth cycle forcing premature ripening, reduces yield components, and ultimately resulting in reduced grain yield and quality deterioration [7-9]. Therefore, there is an urgent need to develop wheat cultivars able either to withstand terminal heat stress or mature early and escaping the stress.

Temporary or continuous high temperatures cause an exhibition of physiological and biochemical changes in plants. Understanding heat defense mechanisms is essential to mimic the deleterious effects of heat stress by developing plants with improved heat tolerance. One of the most common responses of crop plants to high temperature stress is increase in proline accumulation [10]. Free proline is involved in osmotic adjustment, protecting pollen and several plant enzymes from heat injury and serves as a source of nitrogen and other metabolites [11, 12]. Accumulation of proline occurs in wheat under heat stress [13].

Heat stress also causes oxidative stress in plants by the generation and the production of reactive oxygen species (ROS) [14]. Plants get rid of

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these harmful ROS products by converting them to less reactive chemicals [15].

High temperature damages most physiological and biochemical processes including loss of membrane integrity, damage to primary photosynthetic processes, phosphorylation, and protein denaturation [16, 17]. Source activity is affected by heat because both leaf area [18, 19] and photosynthesis is reduced [20]. Heat stress damages sink growth potential mainly when stress is imposed during early sink development stages [21]. Membrane thermal stability has been used for screening different wheat genotypes for heat tolerance [22, 23]. Genetic variations in membrane thermostability prevail in various field-grown crops, including wheat [24]. There are many ways of thermo-tolerance from the physiological and agronomic levels, proline accumulating ability, protein denaturing to membrane stability and productivity of wheat during heat stress. Therefore, the current research study was conducted to find out some potential heat tolerance indicators of wheat and their possible use in the breeding program for development of heat tolerant wheat cultivars.

Result and Discussion

Proline

Heat stress imposed at anthesis significantly increased proline concentration in leaves of most of the wheat genotypes with respect to their control (Table-1). At anthesis highest increase was in recorded in Faisalabad-2008 (24.2%) and lowest in NR-398 (4.1 %). It was followed by AARI-2011 (24.06 %) and Sussui (17.5 %) respectively. No relative percent increase in leaf proline was observed in 4th EBWYT-4 and 4th EBWYT-5 under heat stress as compared to their respective control.

SOD Activity

Under heat stress, significant ($p<0.05$) increase in SOD activity was noted in most of the tested wheat genotypes as regard to their control (Table-1). The highest significant ($p<0.05$) increase in SOD activity as compared to control was noticed in NR-356 (51.7 %) followed by NR-398 (51.28 %), Faisalabad-2008 (50.3 %) and Pak-07 (47 %) respectively. The overall percent increase in SOD activity in all wheat genotypes ranged from 12.0 to 51.7 (%) as compared to their respective untreated control.

POD Activity

The data depicted significant ($p<0.05$) increase in POD activity in majority of the evaluated wheat genotypes under heat stress at anthesis growth phase as compared to their untreated control (Table.1).The NR-397 ranked on top for increase in POD activity followed by NR-400 (44.1 %), NR-356 (41.3 %) , AARI-2011 (41 %) and NR-398 (40.4 %) respectively. However, the overall quantitative increase in POD activity was highest in Faisalabad-2008 (20.70 Units g^{-1} f.wt).

Photosynthate Stem Reserve Translocation (PSR)

The significant ($p< 0.05$) increase in photosynthate reserve translocation was observed in all the tested genotypes under heat stress treatment as compared to their respective untreated control. The quantitative increase in PSR was highest in Faisalabad-2008 (35.70 %). However, AARI-2011 (49.7 %) was on the top for percent relative increase in PSR as compared to its untreated control. It was followed by NR-399 (46.7 %), Pirsabak-2004 (44.8 %), Faisalabad-2008 (43.1 %) and Pak-07 (41.5 %) respectively (Table-1).

Membrane Stability Index (MSI)

High temperature treatment decreased the MSI in all the tested wheat genotypes. The decrease in MSI of all the tested wheat genotypes ranged from 61.0 to 79.83 %). However, the lowest decrease in MSI was recorded in Faisalabad-2008 (79.83 %) followed by Sussui (76.6 %), Chakwal-50 (74 %) and Lasani-2008 (72.03 %) respectively. The decreasing trend in MSI was almost similar in rest of the tested wheat genotypes under heat stress (Fig. 1).

Grain Yield

The heat treatment significantly ($p<0.05$ %) decreased the grain yield plant^{-1} in all the tested wheat genotypes at anthesis growth stage (Table-1). The relative percent decrease in grain yield of various wheat genotypes ranged from 11.4 to 48.3 % under heat stress at anthesis stage. The relative decrease in grain yield per plant was lowest in Faisalabad-2008 (11.4 %) as compared to its non-treated control. It was followed by Lasani-2008 (18.12 %), GA-2002 (18.4 %), Sussui (18.8 %) and Chakwal-50 (21.6 %). The highest relative percent decrease in grain yield under heat stress was found in NR-398 (48.3 %).

Table-1: Leaf proline contents, SOD activity, POD activity, PSR (%), Grain yield g Plant^{-1} and HSSI of twenty wheat genotypes under control and anthesis stage heat stress conditions.

Wheat Genotypes	Proline contents (µg g^{-1} f. wt)		SOD (U g^{-1} f.wt)		POD (U g^{-1} f. wt)		PSR (%)		Grain Yield Plant^{-1} (g)		HSSI
	Control	Anthesis Stress	Control	Anthesis Stress	Control	Anthesis Stress	Control	Anthesis Stress	Control	Anthesis Stress	
4th EBWYT-1	112.00 ij	128.00 ef	2.10 pq	3.20 j-o	6.80 k-n	9.10 f-i	15.60 i-o	22.60 c-e	15.20 a-d	8.300 jk	1.48
4th EBWYT-2	120.00 gh	129.00 ef	1.80 q	2.70 m-q	5.50 n-p	7.90 h-k	13.80 m-p	24.30 cd	14.90 a-e	9.400 i-k	1.20
4th EBWYT-3	120.00 gh	129.00 ef	1.90 pq	2.50 n-q	6.20 l-p	7.56 i-l	15.40 j-o	19.45 e-h	15.60 a-c	10.42 g-k	1.08
4th EBWYT-4	112.00 ij	112.00 ij	3.30 i-o	3.75 g-n	5.50 n-p	6.85 k-n	14.70 k-o	19.05 e-i	15.20 a-d	12.42 d-h	0.59
4th EBWYT-5	110.00 jk	110.00 jk	3.50 h-n	4.47 f-h	6.80 k-n	7.32 j-m	13.60 m-p	17.57 g-l	14.60 b-e	10.32 g-k	0.96
Raskoh	104.0 k	110.0 jk	2.70 m-q	3.83 g-l	5.40 n-p	6.53 k-o	12.70 n-p	18.10 f-k	15.40 ab	10.22 c-g	1.10
Sussui	139.40 d	169.00 b	6.60 d	9.70 b	12.00 e	16.50 b	17.70 f-l	29.90 b	15.90 ab	12.90 c-g	0.61
Faisalabad-2008	150.30 c	198.50 a	7.90 c	15.90 a	14.80 c	20.70 a	20.30 e-h	35.70 a	17.50 a	15.50 a-c	0.37
Pirsabak-2004	120.00 gh	128.00 ef	3.30 i-o	4.60 fg	5.80 m-p	7.30 j-m	13.40 n-p	24.30 cd	13.50 b-f	10.00 h-k	0.84
Tatara	124.40 fg	131.60 ef	2.40 o-q	3.83 g-l	5.30 n-p	6.50 k-o	12.00 op	18.23 f-k	14.80 a-e	10.30 g-k	0.52
Chakwal-50	134.7 de	153.50 c	6.00 de	8.70 c	9.90 fg	13.73 cd	15.70 i-n	22.23 c-e	15.90 ab	12.45 d-h	0.71
Lasani-2008	130.40 ef	149.150 c	5.50 ef	7.98 c	8.70 f-j	12.90 de	17.00 h-m	21.27 c-f	14.90 a-e	12.20 e-h	0.59
GA-2002	117.00 ghij	124.50 fg	3.50 h-n	4.32 g-i	5.60 n-p	8.00 h-k	13.20 n-p	18.30 f-j	11.40 f-i	9.30 i-k	0.60
NR-400	115.00 hij	120.00 gh	3.90 g-k	4.70 fg	4.80 p	8.60 g-j	12.30 n-p	19.80 e-h	11.00 f-j	7.80 k	0.95
NR-399	117.00 ghij	120.00 gh	4.20 g-j	5.50 ef	6.60 k-n	10.20 fg	10.60 p	19.90 e-h	15.00 a-e	8.90 i-k	1.33
AARI-2011	118.00 ghi	155.40 c	3.80 g-l	5.80 de	5.60 n-p	9.50 f-h	10.50 p	20.90 c-g	14.70 a-e	9.80 h-k	1.09
NR-356	119.00 ghi	127.00 f	2.80 l-q	5.80 de	5.40 n-p	9.20 f-h	12.50 n-p	20.80 d-g	14.30 b-e	9.40 i-k	1.12
NR-398	115.00 hij	120.00 gh	1.90 pq	3.90 g-k	5.30 np	8.90 f-j	13.90 m-p	22.00 c-e	15.30 a-c	7.90 k	1.58
NR-397	110.00 jk	117.00 ghij	2.90 k-p	4.60 fg	4.90 op	10.30 f	14.70 k-o	24.40 c	14.60 b-e	9.20 i-k	1.21
Pak-07	113.00 hij	127.80 ef	2.30 o-q	4.30 g-i	5.70 m-p	9.50 f-h	14.20 l-o	24.30 cd	15.70 a-c	8.50 jk	1.50
LSD	6.397		0.9120		1.402		3.010		2.330		

Means sharing a common letter within a column do not differ significantly at 5 % probability level. SOD; Superoxide dismutase, POD; Peroxidase, PSR; Photosynthate reserve translocation, HSSI; Heat stress susceptibility index.

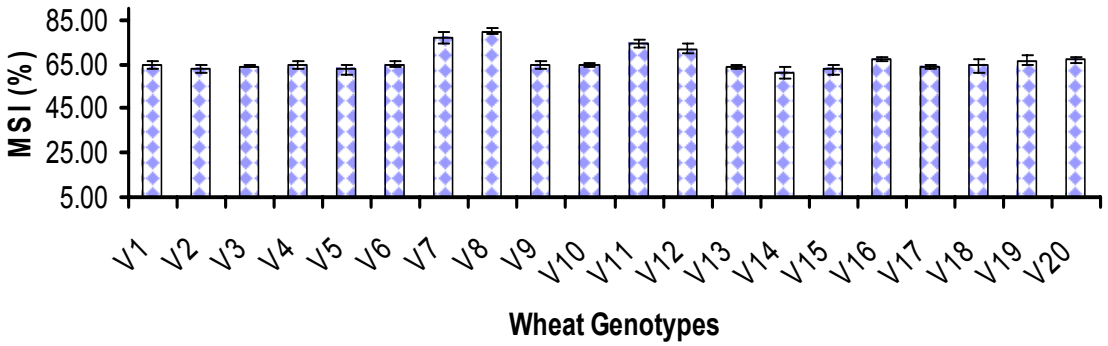

Fig. 1: Effect of heat stress at anthesis growth stage on membrane stability index of wheat genotypes. The vertical bars indicate standard error (SE) of mean (n=3).

Heat stress susceptibility Index (HSSI)

The heat susceptibility values of various wheat genotypes ranged from 0.37 to1.58 (Table-1). The lowest heat susceptibility index was observed in Faisalabad-2008 (0.37) followed by Tatara (0.52), Lasani-2008 (0.59), 4th EBWYT-4 (0.59), GA-2002 (0.60), Sussui (0.61), Chakwal-50 (0.71), Pirsabak-2004 (0.84), NR-400 (0.95) and 4th EBWYT-5 (0.96) respectively. Highest HSI was observed in NR-398 (1.58) followed by Pak-07 (1.50), 4th EBWYT-1 (1.48), NR-399 (1.33), 4th EBWYT-2 (1.20) and NR-397 (1.21) respectively.

In the current research study heat stress imposed at anthesis growth stage resulted changes in the physiological attributes such as leaf proline, SOD activity, POD activity, membrane stability index, photosynthate reserve translocation, yield and its components. Susceptibility of species and cultivars to high temperatures may vary with the stage of plant development, but all vegetative and reproductive stages are affected by heat stress to some extent [24]. Heat stress induced modifications in plants may be implicated from the change in physiological processes or from the varying pattern of development. These responses may vary from one developmental growth stage to another. In the present study, heat stress at anthesis stage significantly increased leaf proline concentration in flag leaves of all the wheat genotypes. Increase in proline accumulation was greater in Faisalabad-2008 (24.2%) and lowest in NR-398 (4.1 %). One of the most common responses of many plant species exposed to various abiotic stresses is the accumulation of compatible organic solutes such as proline. Proline has been suggested to play protective role in plants by acting as a cellular osmotic regulator between cytoplasm and vacuole and by detoxifying ROS, thereby protecting membrane integrity and stabilizing antioxidant enzymes [25]. Under stress conditions, accumulation of proline in plants results

either from increased expression of proline synthetic enzymes or due to repressed activity of proline degradation [25, 26]. In our case increase in proline accumulation was greater in Faisalabad-2008 while lowest in NR-398. Under high temperature genotypic differences in proline accumulation pattern has also been reported in twenty wheat genotypes [27]. The higher leaf proline level can be used as an index to screen wheat genotypes for heat tolerance. The antioxidant defense mechanism plays an important role in the heat stress tolerance of wheat genotypes. Both SOD and peroxidase enzymes protect the cellular systems of plants from cytotoxic effects of the active oxygen species. An increase in SOD and POD activity has been reported in wheat genotypes under pre-anthesis heat stress treatment [28, 29]. It is clear from the data that SOD and POD activities increased in NR-356, NR-398, AARI-2011 and Faisalabad-2008 under heat stress conditions. The increase in the antioxidant enzymes activity demonstrates their ability to scavenge reactive oxygen species and thus imparting tolerance.

The reserves accumulated in vegetative plant parts before anthesis may safeguard grain yield when conditions become adverse to photosynthesis and mineral uptake during grain filling [30]. Data revealed increase in photosynthates stem translocation in AARI- 2011, NR-399, Pirsabak-2004, and Faisalabad-2008 under heat stress at anthesis stage. Increases in stem reserve translocation of these cultivars reflect their ability to transfer assimilates to the grains under adverse environment [31]. The remobilization of assimilates begin from plant senescence, an active process that involves the translocation of stored reserves from stem and shoots to grain [32]. Under high temperature the stored carbohydrates become essential source of transported materials [33]. Our results are in line with those of [34] who observed increase in PSR in heat tolerant wheat genotypes under heat stress conditions. The increase in PSR of aforementioned wheat genotypes could be attributed to their tolerant genetic back.

Increased solute leakage is an indication of decreased cell membrane thermo-stability, has long been used as an indirect measure of heat-stress tolerance in diverse plant species, including wheat [8, 35]. In present investigation, high temperature decreased the MSI in all the tested wheat genotypes but least decrease was noted in Faisalabad-2008 followed by Sussui, chakwal-50 and Lasani-2008 respectively.

Decrease in MSI or heat sensitivity reflects the extent of lipid peroxidation caused by active oxygen species [36]. Increase in membrane stability of these genotypes might be due to their heat tolerant genetic back. [25, 37] suggested membrane thermal stability as a selection criterion for heat stress tolerance. Similarly [38] suggested that membrane thermo-stability test, can be used to determine the heat tolerance of wheat varieties under heat stress conditions. Their results suggested that genetic variation among genotypes for membrane stability can be utilized in wheat breeding in heat-stressed environment.

Heat treatment significantly decreased the grain yield per plant in all the tested wheat genotypes at anthesis growth stage. The least percent decrease in grain yield per plant was found in Faisalabad-2008, GA-2002, Sussui and Chakwal-50 while highest in NR-398. Grain yield reduction and genotypic variation above optimum temperature has already been reported in wheat and other crops species [39-41]. Our results are in consonant with those of [42] who found variation in grain yield plant^{-1} of various wheat genotypes under high temperature stress. In wheat, at reproductive growth stage, during the onset of meosis in the male generative tissues till the completion of anthesis, grain setting is reduced by temperature rise above optimum [43].The high grain yield attained by Faisalabad-2008 may be due to its mobilization efficiency of reserves from leaves, stem or other plant parts towards sink [44, 45]. Similarly low grain yield attained by NR-398 may be due to either an incomplete remobilization of reserves or a reduction in the quantity of reserves available for remobilization [46].

The lowest HSSI exhibited by Faisalabad-2008, Tatara, Lasani-2008, 4th EBWYT-4, GA-2002, Sussui, Chakwal-50 demonstrate their tolerant genetic back. Similarly highest HSSI shown by NR-398 closely followed by Pak-07, 4th EBWYT-1, NR-399, 4th EBWYT-2 and NR-397 might be due to their sensitive nature.

Experimental

Twenty wheat genotypes, viz., 4th EBWYT-1, 4th EBWYT-2, 4th EBWYT-3, 4th EBWYT-4, 4th EBWYT-5, Raskoh, Sussui, Faisalabad-2008, Pirsabak-2004, Tatara, Chakwal-50, Lasani-2008,GA-2002, NR-400 (advance line), NR-399 (advance line), AARI-2011, NR-398 (advance line), NR-397(advance line) and Pak-07 obtained from Wheat Program, Crop Sciences Institute, National Agricultural Research Centre (NARC) were used in the study. The wheat plants were grown in pots (30×40 cm size) containing 10 kg sandy loam soil in

a glass house under natural daylight and 25/20°C day night temperature at NARC, Islamabad (latitude 33. 38 °N, longitude 73. 00 °E) during the winter/spring with average day/night temperature 30 ±8ºC and 13 ±5ºC respectively. A recommended dose of NPK @ 120-100-60 kg ha^{-1} was applied as urea, di ammonium phosphate and potassium sulphate. Before planting soil organic matter in the soil used for the experiment was 2.45 %, extractable P and K was 37.5.0 mg kg^{-1} and 191.5 mg kg^{-1} respectively. The pots were arranged in factorial, randomized, complete block design. Plants were subjected to heat shocks of 35-40°C for 3 hours for five consecutive days immediately at anthesis (80 days after sowing). At anthesis when the first anther extrusion occurred, the pots of three replications, each containing 3 plants were moved to a glasshouse for heat shocks where temperature was maintained at 35-40ºC and 14/10 h day/night, 50/70 % relative humidity and illumination of 335 µmol m^{-2} S^{-2}. After the heat shock treatments, flag leaf was sampled for analyzing proline, SOD, POD, MSI and PSR. The pots were moved back to the natural conditions in open atmosphere after sampling. The control potted plants were kept in another tunnel with similar environmental conditions except temperature. Control potted plants temperature was maintained closer to the atmospheric temperature (23 ºC). Control and heat stress plants were regularly irrigated as and when required. The grain yield data was recorded at physiological maturity.

Proline was determined in the leaf using the method of [47]. Fresh plant tissue was extracted with 3% aqueous 5-sulfosalicylic acid and the filtrate was reacted with ninhydrin solution at 100°C for 1 hour. The reaction mixture was extracted with toluene and the absorbance of the chromophore contain toluene was read at 520 nm.

Determination of Enzyme Assays

The SOD activity (SOD, EC 1.12.1.1) was estimated by measuring its ability to inhibit the photochemical reaction of nitro-blue tetrazolium (NBT) in the presence of riboflavin in light following [48]. One unit of enzyme activity was determined as the amount of the enzyme needed for the inhibition of 50 % NBT reduction rate by observing absorbance at 560 nm with spectrophotometer. Activity of Peroxidase (POD, EC 1.11.1.7) was assayed using the method of [49]. Both the enzyme activities were expressed on leaf fresh weight basis. The Leaf MSI was determined according to [50]. Leaf strips (0.2 g) of uniform size were taken in test tube containing 10mL doubled distilled water in two sets. Test tubes in one set were kept at 40 ºC in a water bath for 30 min and electrical conductivity of the water containing the sample was measured (C_1) using conductivity bridge. Test tubes of the other set were incubated at 100ºC in the boiling water for 15 min and their electrical conductivity was measured as above (C_2). MSI was calculated using formula as below:

$$MSI = [1-(C_1/C_2)] \times 100$$

Photosynthate stem reserve translocation (PSR) was determined following [51].

$$\text{PSR translocation (\%)} = \frac{S1-S2}{G1-G2} \times 100$$

where, S1 = Stem dry weight (g) at anthesis, S2 = Stem dry weight (g) at maturity, G1 = Grain dry weight (g) at anthesis, G2 = Grain dry weight (g) at maturity

The heat stress susceptibility index (HSSI) was used as a measure of heat tolerance in the form of minimization of the reduction in grain yield caused by high temperature versus normal temperature conditions. HSSI was calculated following the formula given by [52].

HSSI = (1-yh/yp)/H.

where: yh = mean yield under heat stress, yp = mean yield under control or potential yield, H = heat stress intensity = 1-(mean yh of all genotypes/mean yp of all genotypes). Minimum, maximum temperature (ºC) and rainfall (mm) data during the experimental period 2010-2011 is presented in the Fig. 2.

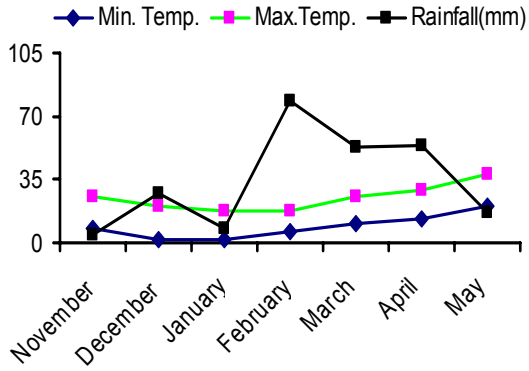

Fig. 2: Minimum, maximum temperature (°C) and rainfall (mm) data during 2010-2011.

Statistical Analysis

The analysis of variance of the data for each attribute was carried using Minitab version 13.1. The mean values were compared with the Duncan's Multiple Range Test at 5 % level of probability [53].

Conclusions

Heat tolerance is a complex phenomenon. There is dire need to identify some potential heat tolerance indicators for screening genotypes that can perform well under both stress as well as non-stress conditions. The stress tolerance indicators particularly antioxidant defensive mechanism, PSR, MSI and better yielding ability under heat stress environment could be used to screen and identify heat tolerant genotypes. Using these indicators wheat genotypes Faisalabad-2008, Lasani-2008, Sussui, GA-2002, AARI-2011 performed good under terminal heat stress. But 4th EBWYT-1, 4th EBWYT-2, 4th EBWYT-3, NR-397 were found heat sensitive while rest of the genotypes were found moderately heat tolerant. In addition to the yield, plant breeders should also use these indicators as selection markers in the breeding program for development of heat tolerant wheat varieties.

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