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Agronomic and physiological response of wheat (*Triticum aestivum* L.) genotypes under terminal heat stress conditions

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Abstract

Heat stress is one of major limiting factor in wheat (*Triticum aestivum* L.) productivity in arid, semiarid, regions of world. Wheat is grown as winter cereal crop in Pakistan. The crop experiences moderate to severe high temperature at most of its physiological stages of growth. A research experiment was carried out to assess the agronomic and physiological response of wheat genotypes under terminal heat stress conditions at Agriculture farm of the University of Haripur in winter, 2012. Six genotypes were sown in pots using Completely Randomized Design (CRD) replicated thrice. Two separate sets of plants were maintained for heat stress and control. Pre-anthesis growth stage (80 days after sowing), one set plants of were subjected to heat stress treatment of 35 to 40°C and 10 to 14h day and night, 50 to 70% relative humidity and illumination of 335 µ mol $m^{-2}s^{-2}$ in glass house. After high temperature treatment for 3h daily for five consecutive days, pots were moved back to normal temperature (average day/night temperature 30 ± 8 and 13 ± 5°C) conditions in open atmosphere. After heat stress treatment, flag leaf from the control and stressed plants were sampled for analysis of proline, chlorophyll *a,* chlorophyll *b* content and membrane thermos-stability. Analysis of variance for agronomic parameters revealed significant ($p \leq 0.05$) differences among wheat genotypes for days to 50% flowering, plant height, number of grains per spike, number of spikelets per spike and seed yield per pot,the proline accumulation could be used as markers in the breeding program for the development of heat tolerant wheat genotypes. Overall PSK-91, LU-26S and SARSABZ showed best performance under imposed heat stress for physiological and yield parameters. There is a dire need to further evaluate the performance these genotypes under field conditions in the areas of heat stressed environment.

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Introduction

High temperature stress is a chief ecological constraint hampering wheat productivity in most parts of the world. The optimal temperature for all physio-chemical processes of wheat from vegetative to reproductive stage is 20°C or lower (Al-Khatib and Paulsen, 1989). The bread wheat, which originated in the Mediterranean region, migrated to the cooler climate of temperate regions of the world. It also started cultivated in warmer regions and occupied the subtropical areas particularly after development of appropriate semi-dwarf varieties. Additional spread of wheat cultivation in subtropical/tropical areas will depend upon ability of this crop to tolerate warmer temperature. The global climate change scenario is likely to change the weather of conventional wheat growing areas and the wheat varieties should have ability to tolerate high temperature. Wheat is the significant staple food crop of Pakistan, where the evaluated per capita utilization is around 124 kg year^{-1}, among most elevated on the planet. Heat stress damages the plant system in a multifaceted way by disrupting cell membranes (Al-Khatib and Paulsen, 1999), enzyme deactivation, anddisruption of metabolic pathways.High temperature variably affects the crop growth especially from pollination to grain filling growth stages. It has been evaluated that an ascent in temperature of only 1°C in wheat amid the growing season lessens wheat yields by around 3–10% (You *et al.,* 2009). So as to take care of the demand for sustenance in Pakistan, an increment in wheat production of no less than 4% is required to stay aware of population growth. Amid this period, heat stressabbreviates the growth cycle and force untimelyaging, reduces the number of grains per spike, number of spikelet's spike^{-1} and at last results in grain yield and quality crumbling (Khan *et al.,* 2007, Wahid *et al.,* 2007 and Din *et al.,* 2010). Along these lines, a pressing need exists to create wheat cultivars which are better ready to with stand heat stressamid later growth stages, or else develop prior to get away from the heat stressordinarily happening later in the growing season.Plants adapt under high temperature conditions by adopting various strategies. A better understanding of ways by which crop plants like wheat can tolerate heat would establish the framework for work to develop plants with enhanced heat stress. A standout amongst the most widely recognized reactions of crop plants to high temperature stress is an increment in proline aggregation (Ahmed and Hassan, 2011). Under high temperature, free proline is included in osmotic conformity to protect dust and plant enzymes from heat harm, furthermore gives a wellspring of nitrogen and different metabolites (Verslues and Sharma, 2010). Amassing of proline has been appeared to happen under heat stress in arabidopsis (Wei-Tao *et al.,* 2011), cotton (Ronde *et al.,* 2001) and wheat (Hasan *et al.,* 2007), and genotypic variety in proline amassing have been accounted for these species. Under high temperature certain heat shock qualities are activated, bringing about the amalgamation of heat shock proteins, though other solvent and insoluble proteins have also been appeared to show changes in abundance under high temperature stress (Simmonds 1995, He *et al.,* 2005).High temperature can adversely affect cellular membranes, can cause injury to essential photosynthetic apparatus, and changethe lipid structure, and cause protein denaturation (Wahid *et al.,* 2007). Membrane thermal stability due to heat stress, normally measured as particle spillage from the cell, has been utilized for screening wheat germplasm for warm resistance (Yildirim *et al.,* 2009). Blum *et al.,* (2001) demonstrated a higher yield in spring wheat lines having more membrane thermos-stability in flag leaves at anthesis. The current research study was undertaken to investigate the physiological premise for heat stress tolerance amid later development stages in wheat, and suggest dependable screening procedures for heattolerancethat can be used in wheat breading projects for Pakistan and elsewhere. Examined genotypes were created for development in rain-fed zones of Pakistan so they could be further utilized asparent material for advancement of heat tolerant wheat cultivars in the residential wheat breading program.The current research study was undertaken to fulfill the following objectives: (ato identify the heat tolerant wheat genotype based on heat tolerant traits; like proline accumulation, leaf

chlorophyll content and membrane thermo-stability test. (Bto recommend the screening criteria based on these heat tolerance traits. And (cto identify the heat tolerant wheat genotypes based on heat tolerant traits.

Materials and methods

Experimental Sites and experimental design;

The present research study was carried out at Agriculture farm University of Haripur, to study the "Agronomic and physiological response of wheat genotypes under terminal heat stress conditions". Six wheat genotypes were obtained from Cereal Crop Research Institute (CCRI) Pirsabak, Nowshera. The pots were supplied with the nitrogen @ 1.5 kg N pot^{-1} according to local standard for high-yielding wheat cultivation, and P_2O_5 and K_2O @ 0.5 and 1.5 kg N pot^{-1}, respectively. The genotypes were sown in pots and replicated thrice using Completely Randomized Design (CRD). Seeds were sown in pots (20 × 22 cm^2) in the glass house. Two separate sets of plants were maintained for heat stress and control. At 90 days after sowing (grain filling stage), one set of plants was transferred to the glass house. The temperature inside the glass house were maintained at 35-40°C for five consecutive days for 3 hours daily. The control set plants were kept in the open atmosphere under normal conditions. After heat stress treatment, plant samples were collected in the ice box for analysis of leaf proline and chlorophyll contents. After sample collection plants were kept in the open atmosphere under normal conditions. Both sets of plants were irrigated as and when required.

Data Observation

The data was recorded on following parameters Days to 50'% flowering, plant height, number of spikelets per spike, number of grain per spike, yield per pot, proline content, chlorophyll *a* and chlorophyll *b* contents.

Data analysis

Data were subjected to analyze difference using Statistix V 8.1. The means were separated by Duncan multiple Range Test at 5% probability level (Steel and Torrie, 1997).

Formula

$$\text{Proline } (\mu g/g) = \frac{\text{Absorbance of sample} \times \text{K value} \times \text{Dilution factor}}{\text{Weight of sample(g)}}$$

$$\text{Chlorophyll } a \text{ (mg/g)} = \frac{(12.7 \times \text{OD at } 663) - (2.69 \times \text{OD at } 645) \times V}{1000 \times \text{Fresh shoot weight (g)}}$$

$$\text{Chlorophyll } b \text{ (mg/g)} = \frac{(22.9 \times \text{OD at } 645) - (4.69 \times \text{OD at } 663) \times V}{1000 \times \text{Fresh shoot weight (g)}}$$

Where,

K = concentration / absorbance

V = Volume of extract (ml)

OD = Optical density

Results and discussion

Analysis of Variance

The data analysis revealed significant differences for all studied parameters such as days to 50% flowering, plant height, number of spikelet's per spike, number of grains per spike and yield per pot.

Anova Table 1. of biochemical parameters days to 50% flowering, plant height, number of spikelets $spike^{-1}$, number of grain $spike^{-1}$, yield pot^{-1}.

Source	Rep.	Varieties	Treatments	Var*Trts.	Error	Total
DF	2	5	1	5	22	35
Days to 50% flowering	0.778	11.028**	240.25	1.517	1.657	340.972
Plant height	2.525	175.538**	152.523	7.649	0.525	1085.06
Number of spiklets $spike^{-1}$	0.03	1.3685**	12.1336	0.4503	0.14	24.3675
Number of grains $spike^{-1}$	1.694	48.894**	616.694	22.228	0.664	990.306
Yield pot^{-1}	0.0118	1.3440**	14.1	0.7091	0.0095	24.5984

The highest reduction in days to 50% flowering was observed in PSK-91 (9.62%) followed by SUNALIKA (7.82%), while least in INQILAB-91 (5.92%) under heat stress conditions as compared to control.Similarly, highest reduction (13.85%) in plant height was observed in (PAK-81) followed by LU-26S (8.70%) under heat stressed conditions, while lowest decrease was found in SUNALIKA (3.70%). The highest reduction in the number of spikelet's spike^{-1} was observed in PAK-81 (7.92%) followed by SARSABZ (7.90%), while the lowest in INQILAB-91 (2.17%).Highest reduction in the number of grains spike^{-1} was found in PSK-91 (27.10%) followed by LU-26S (22.02%) under heat stress conditions respectively.

Means Table 2. of days to 50% flowering, plant height, number of spikelets spike^{-1}, number of grain spike^{-1} and yield pot^{-1}.

Traits.	Varieties	LU-26S	PSK-91	PAK-81	SARSABZ	SUNALIKA	INQILAB-91
Days to 50% flowering	Control	77.66	79.33	77.33	80.67	81	78.66
	Heat stress	72	75.66	72.66	74.66	74.66	74.0
Plant height	Control	60.9	55.73	59.2	63.7	68.26	67.63
	Heat stress	55.6	53.13	51	61.03	65.73	64.23
Number of spike spike^{-1}	Control	22.26	21.26	22.33	21.5	21.33	21.63
	Heat stress	21.26	19.86	20.56	19.8	20.7	21.16
Number of grains spike^{-1}	Control	46.33	51.66	47	50.66	41	48.33
	Heat stress	36	37.66	41.33	40.33	36.33	43.66
Yield pot^{-1}	Control	10.36	11.58	10.39	11.44	9.75	10.78
	Heat stress	8.9	9.28	9.78	9.72	9.08	10.02

This indicates that the heat stress imposed at pre-anthesis stage has minimum effect on plant height. The findings also in close resemblance with Maragheh (2013), Dias and Lidon, (2009), (Laghari *et al.*, 2013), Mohammadi *et al.,* (2009) Baloch *et al.,* (2014) Chandra *et al.,* (2014), Zarai *et al.,* (2014),Ubaidullah *et al.,* (2007) and Patil *et al.,* (2010).

Bio-chemical Parameters

Data revealed significant differences ($p \leq 0.05$) among the investigated wheat genotypes for leaf proline accumulation. Chlorophyll *a* and chlorophyll *b* exhibited significant ($p \leq 0.05$) variation in chlorophyll *a* content among different wheat genotypes under pre-anthesis heat stress and control conditions The findings of (Hong *et al.,*2000), (Dhatyani *et al.,* 2013) and Reynolds *et al.*, (1994) are in close resemblance to our results.

ANOVA Table 1. of biochemical parameters proline content, chlorophyll *a* content and chlorophyll *b* content.

Source	DF	Proline content	Chlorophyll *a* contant	Chlorophyll *b* contant
Rep	2	861	0.00030	0.01909
Var	5	172028**	0.00739**	0.34522**
Trts	1	799402	0.56000	0.21314
Var*Trts	5	98642	0.00059	0.02464
Error	22	484	0.00106	0.01570
Total	35	2165127	0.62388	2.44596

Highest leaf proline accumulation was found in SUNALIKA followed by INQILAB-91 under heat stress conditions as compared to their control conditions. However, percent increase in proline accumulation was greater in INQILAB-91(51.9%) followed by SARSABZ (22.50%).Leaf proline level is considered to serve as an effective index to screen wheat genotypesfor relative differences in heat

tolerance. Proline hasbeen suggested to play a protective role in plants acting as a cellular osmotic regulator betweencytoplasm and vacuole, and by its ability to detoxify reactive oxygen species (ROS)and thereby protecting membrane integrity and stabilizing antioxidant enzymes (ASHRAF and FOOLAD, 2007).

Means Table 2. of proline content, chlorophyll *a* and chlorophyll *b* content.

	proline content		chlorophyll *a* content		chlorophyll *b* content	
Varieties	Control	Heat stress	Control	Heat stress	Control	Heat stress
LU-26S	1057.8	1285.7	0.68	0.42	0.50	0.11
PSK-91	834.6	1076.5	0.71	0.45	0.25	0.13
PAK-81	1224.7	1262.5	0.65	0.41	0.72	0.64
SARSABZ	874.3	1128.2	0.71	0.43	0.85	0.76
SUNALIKA	1306.2	1538.1	0.62	0.39	0.69	0.65
INQILAB-91	736.3	1531.0	0.61	0.37	0.65	0.44

In the current research study, enhanced proline accumulation under heat stressed in wheat genotypes might have played role in osmoregulation and scavenging of reactive oxygen species produced under heat stressed conditions and in triggering antioxidant enzymatic system of the tested wheat genotypes similar to the findings of Khan *et al.,* (2015). Leaf proline level is thought as an effective index to screen wheat germplasm under heat stressed conditions.Variations in chlorophyll *a* content are also suggestive of tolerant and susceptible nature of genotypes under heat stress and dehydrated air environmental situations in timely and late planted wheat cultivars, while chlorophyll *b* content highest reduction in chlorophyll *b* was observed in LU-26S (78%) followed by PSK-91 (48%), while the lowest degradation in chlorophyll b was found in SARSABZ.

The current findings are in conformitywith the result of our own previous research study in which heat stress at pre-anthesis and milky growth stages significantly reduced chlorophyll a and chlorophyll b content of wheat genotypes (Khan *et al.,* 2015) and to those of (Ahmed and Hassan, 2011), (Kumar *et al.,* 2012) Reynolds *et al.*, (1994) and (Bhanu, 1997).Similarly, in different tetraploid and hexaploid.

Wheat's chlorophyll content decreases significantly under stress environment (TAS AND TAS 2007) and the same are observed in the current research study.

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