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Screening of wheat (*Ttiticum aestivum* L.) cultivars for terminal heat stress tolerance by using biochemical pointer

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ABSTRACT

Among abiotic factors, high temperature is severe constraint limiting crop production. Wheat is a major cereal and staple crop of Pakistan. An experiment was conducted (winter season 2018-19) to expose the effect of warmness induced biochemical alteration on morphological points of Pakistani wheat cultivars. Randomized complete block design (RCBD) with split plot arrangement having three replications was used to experiment. Heat stress treatments H_0 = No heat imposition, H_1 = heat imposition from (Feekes Scale = 10 to 10.5) booting to complete heading were randomized in main plots while varieties in subplots. Galaxy-2013, Gold-2016, Ghazi-2016, Aas-2011, Johar-2016, Pakistan-2013, F-8, Sahar-2006, Jauher-2016 and AARI-2011. Under heat stress, a smaller reduction in chlorophyll fillings and improvement in antioxidant activities were showed by Ghazi-2019, Aas-2011 and Jhor-2016. Among all other cultivars, higher chlorophyll deprivation and depletion in antioxidant activities under heat stress over control was observed. Reasonably lesser grain filling rate, higher duration, number of grains per spike and yield was detected for cultivars Ghazi-2019, Aas-2011, Jhor-2016 and F-8 under heat stress over control. Convincingly, based on biochemical response and morphological indicators genotypes Ghazi-2019, Aas-2011 and Jhor-2016 manifested heat tolerance. Genotype Fareed-2006 and F-8 manifested medium tolerance. Whereas, genotype AARI-2011, Galaxy-2013, Gold- 2016, Jhor-2016, Pakistain-2013, Sher-2006 and Ujala depicted susceptibility to terminal heat stress.

INTRODUCTION

Wheat is the largest growing cereal around the globe. Currently, 218.54 million hectares (mha) of wheat are cultivated globally, with a total world production of 771.71 million tons per year (mts). This accounts for almost 50% of the world grain trade and provides about 20% of the calories consumed per capita (FAO, 2019). China is the top country in wheat production (134,340,630 tons). Pakistan ranked at 8th position in wheat production with a yield of 26,674,000 tons (USDA, 2018). Food security in Pakistan is associated with wheat production and consumption. In Pakistan, the share of wheat in the gross domestic product is 1.6% with a decline of 0.4% relatively to 2017-18 and in value addition of agriculture is 8.9%. It is cultivated on an area of 8.740 million hectares (Govt. of Pakistan, 2019).

The variability between wheat production and yields indicates a large gap between the land areas used and yield output per unit land area. These differences reflect the influence of the environment (temperature, water, soil, geographical location, etc.), the genotypes planted and 'environment × genotype interactions' on plant productivity, and perhaps different governmental policies. Geographical and seasonal variability in wheat

yield is critical economically, as these factors influence the global wheat market, grain supply and price fluctuations (Iizumi *et al*., 2013) Wheat production under changing climate has been a difficult job. According to a special report on global warming, produced by the Intergovernmental Panel on Climate Change (IPCC), global temperatures are expected to continue to increase by a further 1.5°C between 2030 and 2052, if current rates of global warming continue (IPCC, 2018). Concerning crop productivity, yield reductions in bread wheat (*T. aestivum*, AABBDD) are strongly associated with increases in temperatures beyond optimal growth cycle temperatures (17-25°C) and maximum day temperatures (up to 32°C) during grain filling. Temperatures beyond these ranges may elicit stress responses and hence result in yield reductions (Farooq *et al*., 2011). The ability of plants to endure increased temperatures is still relatively poorly understood, especially with respect to how plants prevent early senescence and maintain photosynthesis under elevated temperatures/heat stress (Shibasaki and Rahman, 2013; Dhankher and Foyer, 2018; Khadka *et al*., 2018; Mizoi *et al*., 2018). Therefore, understanding how plants respond to heat stress requires in-depth insights into the aspects of plant metabolism that determine the capacity of crop plants to take up, produce, transport, store and remobilize critical metabolites during vegetative growth, the transition from vegetative to reproductive growth and the maturation stages associated with seed/grain production (White *et al*., 2016; Dhankher and Foyer, 2018).

For wheat, the production or uptake, transport, storage and remobilization of the critical metabolites required for optimal growth rates and yields are dynamic processes involving feedback and feed-forward sink-source interactions that can be disrupted in plants under heat stress ; Kumar *et al*., 2017; Hütsch *et al*., 2018). Source tissues are the net exporters of the nutrient resources needed for plant growth and development, whereas sink tissues are net importers responsible for the storage and use of these resources (Smith *et al*., 2018). From now, it warrants the precondition of boosting its yield on per unit land area basis and negotiates wheat production under a high-temperature environment . As a temperate climate crop, wheat crop prefers to grow in cool temperature . The temperature optima for terminal spikelet, anthesis, and grain filling for wheat are 12, 23 and 21ºC, respectively . According to an assessment, every 1ºC rise of temperature declines grain yield by 3-17% in Pakistan and India . The higher temperature is foretold to rise further in future and terminal heat stress (>35ºC) deleteriously impacting grain yield in wheat . Exposures of reproductive stages to higher temperature are known as terminal heat stress. Heat stress is of “heat shock” and “chronic heat” types. Heat shock is the abrupt and utmost increment in temperature above 35°C for the duration of 4-5 days. Whereas, chronic heat stress is the occurrence of moderately high temperature (25-30°C) for the relatively long duration.

Harmful effects of warmth stress include photorespiration, pollen infertility, cellular dehydration, rapid phenology, declining availability of assimilates for grain filling, chlorophyll degradation, decreasing number and size of grains and eventually decline in grain yield . The most important adverse effect of heat stress is the generation of excessive reactive oxygen species (ROS) that leads to oxidation of lipids of cellular membranes . Consequently, the plant synthesizes antioxidants to scavenge ROS. Plants also accumulate compatible solutes and osmoprotectants as a defensive mechanism to regain cellular redox balance and homeostasis. Antioxidants and compatible solutes develop heat tolerance and maintain growth.

Various wheat genotypes depict assortment and heterogeneity in response to high temperature . Furthermore, numerous quantitative trait loci exist for a single targeted trait having a complex inheritance pattern . Therefore, the selection of polygenic target traits can be accomplished indirectly employing biochemical markers closely related to heat tolerance . Likewise, diversity among wheat cultivars combined with polyploidy and genes profusion makes it challenging to select a suitable genotype using morphological trait under a high-temperature environment . Hence, the selection of wheat genotypes merely since the response of morphological traits often leads to faulty inferences . Moreover, physiochemical marker-assisted selection of genotypes depicts higher efficacy of selection than mere morphological markers based selection for polygenic traits . Therefore, phonological and biochemical markers assisted screening of wheat cultivars enhances the efficacy of cultivar selection.

Former trials were mainly comprised of heat imposition under controlled environments of the greenhouse.

Studies regarding manipulation of sowing dates are abundantly available to observe adverse effects of high temperature. While relatively a little information is available regarding the imposition of heat stress under field conditions. Moreover, studying biochemical mediated transformations in correlation with morphological traits might prove advantageous for breeding for heat tolerance.

MATERIAL AND METHODS

A research was conducted to detect harmful effects of terminal heat stress on different wheat genotypes. The experiment was conducted at the Agronomic Research Area, Bahauddin Zakariya University, Multan, Pakistan during winter season 2018-19. Randomized complete block design (RCBD) with split plot arrangement having three replications was used to conduct the experiment. Heat stress treatments were randomized in main plots while varieties in subplots.

The seed of various wheat varieties, Galaxy-2013, Gold-1026, Ghazi-2016, Aas-2011, Johar-2016, and Ujala was obtained from Adaptive Research Farm, Bahawalpur, while the seed of Pakistan-2013, F-8, Sahar-2006, Jauher-2016 and AARI-2011, was obtained from the local market. The experiment comprised of two heat stress treatments [H_0= No heat stress (plots without polythene sheet) and H_1= Heat stress (plots covered with polythene sheet from the booting to complete heading of eleven varieties.

Sowing was done on 20th of November 2018 with the help of single row hand drill with R × R of 9 inches. Seed rate used during sowing was 100 kg ha^{-1}. Gross plot size used was 3.0 m × 0.90 m having four rows of wheat in each plot with row × row distance of 22.50cm. Fertilizer was applied at the rate of 120:75:60 kg NPK ha^{-1}. 1/3 of nitrogen fertilizer (urea) and all the phosphorus (SSP) and potash fertilizers (SOP) were applied before sowing. Leftover 1/3 nitrogen fertilizer was applied with first irrigation at crown root initiation. And remaining 1/3 nitrogen was applied at the time when crop complete tillering. Irrigations were applied at four critical growth stages viz. crown root initiation, tillering, spike initiation and flowering. The crop was harvested on 10th May 2019. Two hoeings were done in all treatments to control weeds; first after 40 days of sowing and second after 60 days of sowing. Heat stress was imposed at heading stage by covering main plots with polythene sheet while the control plot was left in the ambient environment. Heat stress was imposed from heading to grain filling stage during this, the temperature was recorded in the morning, noon and evening with the help of digital temperature and humidity probe (Digital Multimeter-50302).

Observations recorded

By preparing enzyme extract of leaves samples stored at -80°C in freezer total soluble proteins (TSP) were quantified. Potassium phosphate buffer (pH 4) was used to prepare enzyme extract. Record the absorbance by adding Bradford Reagent using ELISA plate at 595nm (Bradford, 1976).

Superoxide dismutase (SOD) activity was measured as an amount of enzyme that inhibited the photochemical reduction of nitro blue tetrazolium (NBT). Reaction mixture used was 100 µL enzymes extract prepared same as for TSP + 500 µL potassium phosphate buffers (pH 5) + 200 µL methionine + 200 µL triton X + 100 µL NBT + 800 µL distilled water. Placed under ultraviolet light for 15 minutes, added 100 µL riboflavin and noted absorbance at 560 nm with the help of ELISA plate . Peroxidase (POD) activity was recorded as an amount of enzyme required for guaiacol oxidation. Enzyme extract used for TSP was also used to measure POD activity. Reaction mixture comprised of 800 µL potassium phosphate buffer (pH 5) + 100 µL H_2O_2 (40 mM) + 100 µL guaiacol (20 mM). Added 100 µL enzyme extract, 100 µL reaction mixture, and recorded absorbance at 470 nm using ELISA plate . The activity of catalase (CAT) was determined as the amount of H_2O_2 consumed by the enzyme and converted to H_2O and O_2. Same enzyme extract as for TSP determination was used for measurement of CAT activity. Enzyme extract 100 µL was taken in well plate, added 100 µL H_2O_2 (5.9 mM) and recorded absorbance at 240 nm wavelength on ELISA plate .

Grain filling rate (GFR) was recorded by randomly selecting 5 spikes in each treatment. Spikes were harvested at 5 days' interval and oven dried to record dry weight. GFR was determined using the formula by Hunt (1978).

$$GFR = \frac{W2 - W1}{T2 - T1}$$

Where W_1 and W_2 represent the dry weight of spikes at first harvest and second harvest, respectively. To determine grain filling duration (GFD) five plants in each plot were tagged. The number of days from heading to physiological maturity was taken as grain filling

duration (Hunt, 1978). The number of grains per spike was determined by taking the average of 10 spikes harvested randomly from each treatment. For grain yield, each plot was harvested and threshed and converted to tons per hectare.

Statistical Analysis (if any):

The data were analyzed statistically ($p \leq 0.05$) using the Fisher's analysis of variance technique (Steel *et al*., 1997) and treatments' means were compared by using Tukey's Honestly Significant Difference (Tukey's HSD) test at 5% probability level. Moreover, the strength of association among recorded attributes under heat and no heat was determined using correlation analysis .

RESULTS & DISCUSSIONS

Heat stress had an overall deleterious effect at reproductive stages of wheat. However, cultivars specific response was evident on different growth, yield, and biochemical attributes. The varied response of genotypes under heat and control resulted in significant heat × genotypes effect for various parameters.

Table 1. Analysis of variance for the effect of heat stress on growth and yield parameters of wheat varieties.

SOV	df	Parameter's Mean Sum of squares				
		GPS	1000-grain weight	GFR	GFD	GY
Blocks	3	478.95	2083.05	0.0041	7.14	22.01
Heat Stress(HS)	1	1109.32**	1889.91*	0.05585**	2209.15**	62.14**
Erorr 1	3	8.34	75.93	0.0041	8.01	0.15
Genotype(G)	10	259.21**	97.13**	0.00524**	182.11**	2.54**
HS ×G	10	9.64*	9.53**	0.00029ns	4.21 ns	0.28**
Erorr 2	60	4.39	3.37	0.0027	4.58	0.11

SOV = Source of variation, DF = Degree of freedom, * = Significant ($p \leq 0.05$), ** = Highly significant ($p \leq 0.01$), NS = Non-significant, GFR = Grain filling rate (g per day), GFD = Grain filling duration (days), GPS = Number of grains per spike, GY = Grain yield (t ha^{-1}).

Heat stress (HS) and varied performance of genotypes (G) significantly affected the grain filling rate (GFR) and grain filling duration (GFD). Whereas, the same trend amongst all cultivars was observed in both main plots resulting in non-significant interaction (HS × G) for GFR and GFD (Table 1). Heat stress accelerated GFR by 31% while diminished GFD by 25%. Concerning GFR, Galaxy-2013 recorded highest GFR (0.14 g per day) and it was statistically similar to F-8, AARI-2011, Pakistan-2013, Sher-2006 and Ujala. Significantly lowest GFR was noted for cultivars Ghazi-2019 and Aas-2011. Regarding GFD, the cultivar Ghazi-2019 recorded the highest value (36.47 days). Ghazi-2011 was statistically comparable to Aas-2011 and Johar. The genotype Sher-2006 recorded lowest GFD (23.61 days) and it was statistically alike to Gold-2016, Pakistan-2013 and Ujala. High-temperature environment caused rapid grain filling rate (GFR) and diminished grain filling duration (GFD). Even though, genotypes Ghazi-2019 and Aas-2011 maintained significantly lower GFR than all other cultivars. Genotypes Ghazi-2019 and Aas-2011 manifested significantly lowest decline in GFD against maximum in cultivars F-8 and AARI-2011.

The weight of 1000 grains was significantly diminished owing to adverse consequences of high temperature, while varied responses of cultivar were also evident. Nonetheless, all genotype revealed evidence of incompatibility between control and heat stress to cause a significant interaction of heat and genotypes (Table 01). The maximum decline in 1000-grain weight under heat stress compared with that in ambient condition was observed in genotype AARI-2011 and Pakistan-2013. The lowest record under heat stress was observed in Sahar-2006.

The varied response of genotypes was obvious under heat and control to manifest significant heat × genotypes effect (Table 1). More heat triggered diminishment in the number of grains per spike over control was observed for genotype Jhor (19%), Sher (18%) and Ujala (20%), whereas, AARI-2011, Gold-2019 and Aas-2011 exhibited smaller decline (<10%) in this regard as shown in Fig 1.

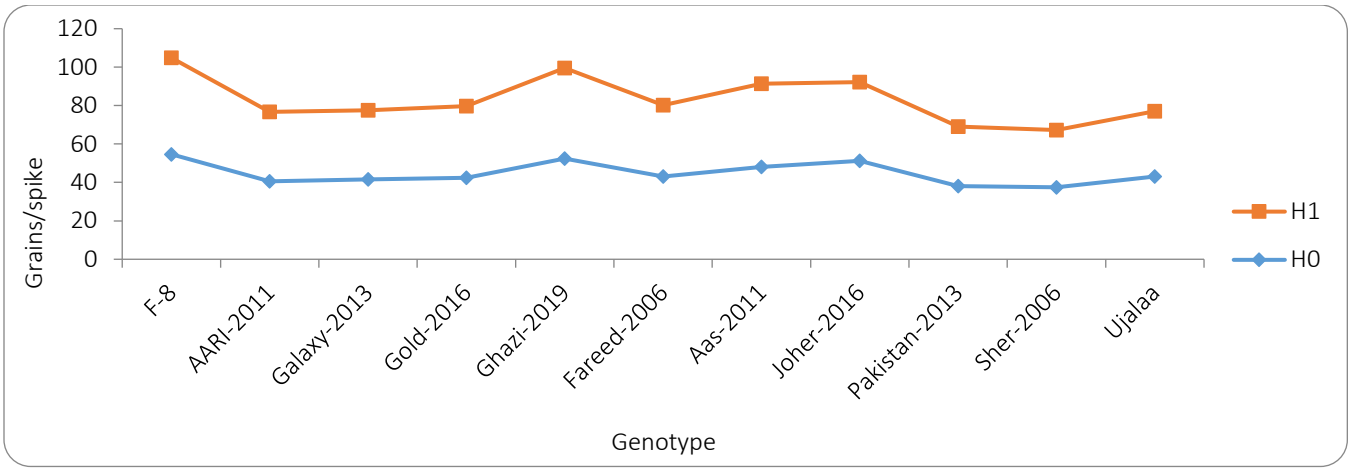

Figure 1. No. of grains/spike of various wheat genotypes under control (H_1) and heat stress (H_0) treatments.

Grain yield (GY) was significantly decreased due to bad implications of heat. The distinct genetic makeup of genotypes was statistically apparent indicating their varied competency to produce yield under different environments. Nevertheless, all cultivars responded differently in control and heat-stressed environment to produce significant interaction of varieties and heat stress. While comparing control plots with heat stress. Maximum decline in GY due to adverse effects of heat stress was observed for cultivar AARI-2011 (46%), Galaxy-2013 and F-8 (44%) shown in figure 2.

Dissimilar performance of different wheat genotypes for antioxidant activities was observed in main plots and resulted in significant interaction of genotypes and heat stress (Table 2). Superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activity of Ghazi-2019 and Aas-2011 was enhanced under heat over ambient conditions. Whereas, all other cultivars recorded a diminishing trend in enzymatic activity under a stressed environment over control.

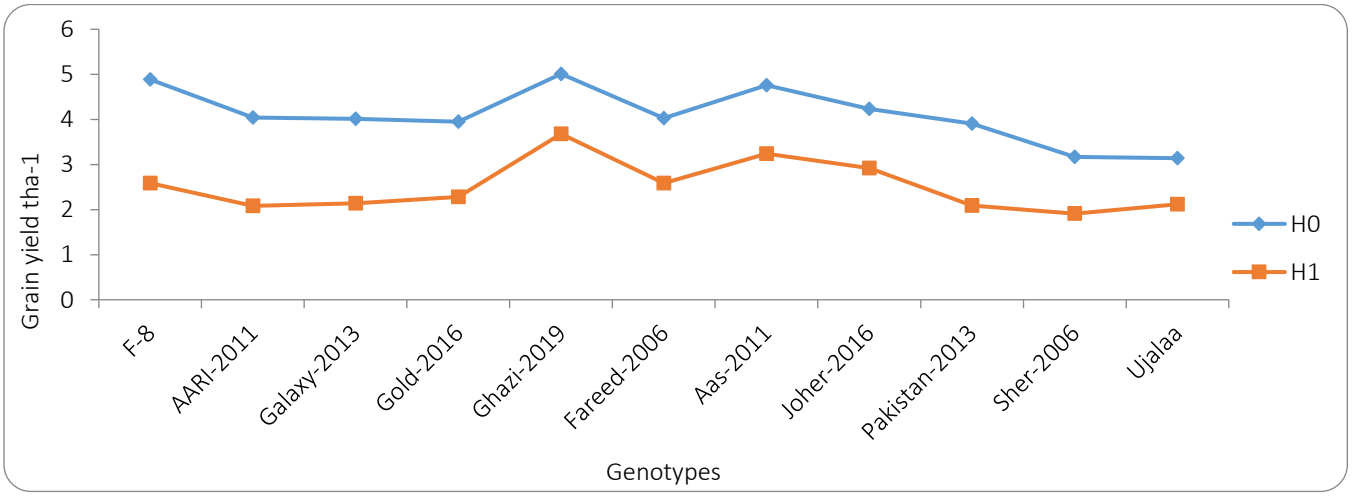

Figure 2. Grain yield of various wheat genotypes under control (H_1) and heat stress (H_0) treatments.

Table 2. Analysis of variance for effect of heat stress on antioxidant activity of various wheat genotypes.

SOV	df	Perameter's MSS			
		TSP	SOD	POD	CAT
Blocks	3	0.25	14987.9	54.87	219.87
Heat Stress(HS)	1	1.29*	6994.86**	127.96**	369.62*
Erorr 1	3	0.004	139.93	3.63	22.17
Genotype(G)	10	0.298**	14987.3**	339.83**	1096.87**
HS ×G	10	0.25**	1657.85**	46.37**	139.93**
Erorr 2	60	0.003	34.87	1.09	2.49

SOV = Source of variation; DF = Degree of freedom; * = Significant (p ≤ 0.05); ** = Highly significant (p ≤ 0.01); NS = Non-significant; TSP = Total soluble proteins (mg g-1); SOD = Super oxide dismutase (U per mg protein); POD = Peroxidase (U per mg protein); CAT = Catalase (U per mg protein)

Regarding SOD activity, Ghazi-2019, Aas-2011 and Johar-2016 depicted an increase of 11, 13 and 13%, respectively under heat stress over control. In control Ghazi-2019 and Aas-2011 were statistically alike while in heat stress Ghazi-2019, Aas-2011 and Johar-2016 remained at par. Galaxy-2013 and Gold-2016 depicted maximum decline (36 and 38%, respectively) in SOD activity under heat compared to no heat environment (Fig-3).

The cultivars Ghazi-2016, Aas-2011, and Johar-2016 exhibited 17, 15 and 24% enhancement in POD activity, respectively under heat-induced conditions over no heat imposition. Under high-temperature maximum diminishment (49%) in POD activity was observed for F-8 and Galaxy-2013. Under high-temperature stress, Ghazi-2019, Aas-2011 and Johar-2016 recorded significantly higher POD activity than all other genotypes (Fig-4).

Cultivars Ghazi-2019, Aas-2011 and Johar-2016 depicted an increase of 18, 14 and 17% respectively in. The highest reduction in CAT activity was recorded for genotypes F-8 (48%) and Galaxy-2013 (44%) Fig-5. Ghazi-2019, Aas-2011, and Johar-2016 manifested significantly higher SOD, POD and CAT activity than all other cultivars in heat stressed main plots.

Differential response of wheat genotypes resulted in significant heat × varieties effect for total soluble proteins (TSP). Higher temperature enhanced accumulation of TSP in cultivars Ghazi-2019 (15%) and Aas-2011 (16%) and both cultivars were statistically similar. In control Ghazi-2019 and Aas-2011 also statistically resembled to Johar-2016 for TSP. The highest diminishment in TSP under heat over control was observed for cultivars AARI-2011 (39%) and Gold-2016 (58%) (Fig-6).

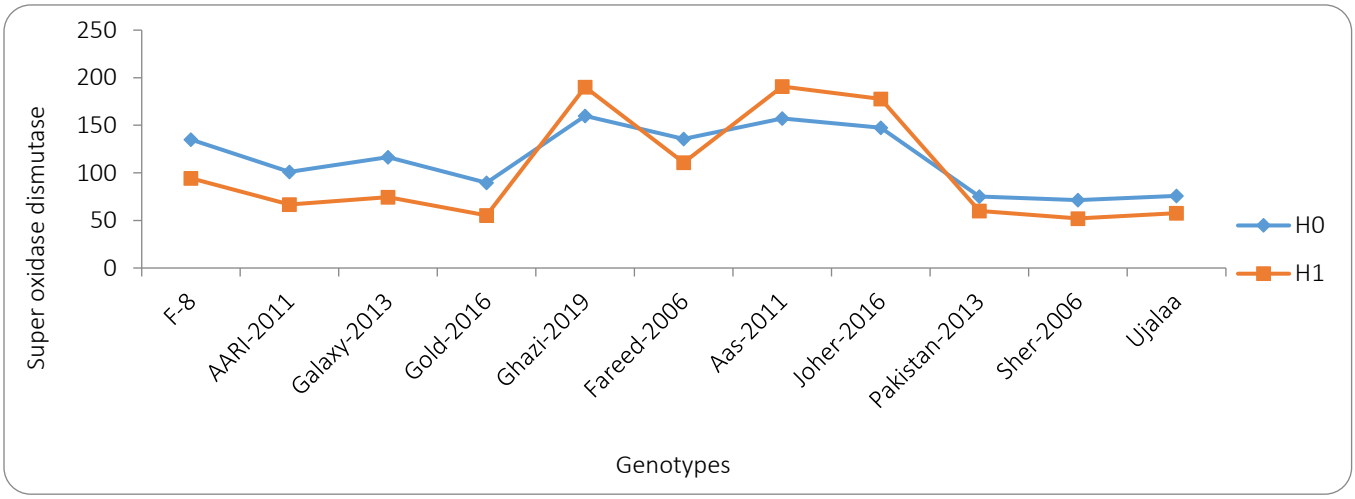

Figure 3. Superoxide dismutase (SOD) of various wheat genotypes under control (H_1) and heat stress (H_0) treatments.

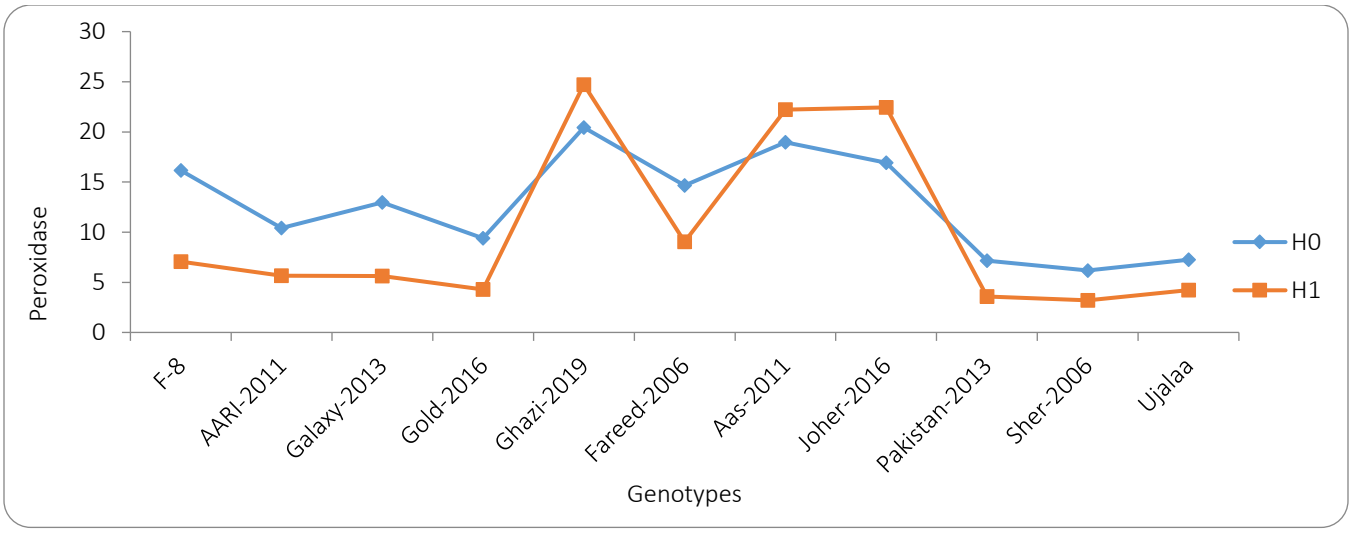

Figure 4. Peroxidase (POD) of various wheat genotypes under control (H_1) and heat stress (H_0) treatments.

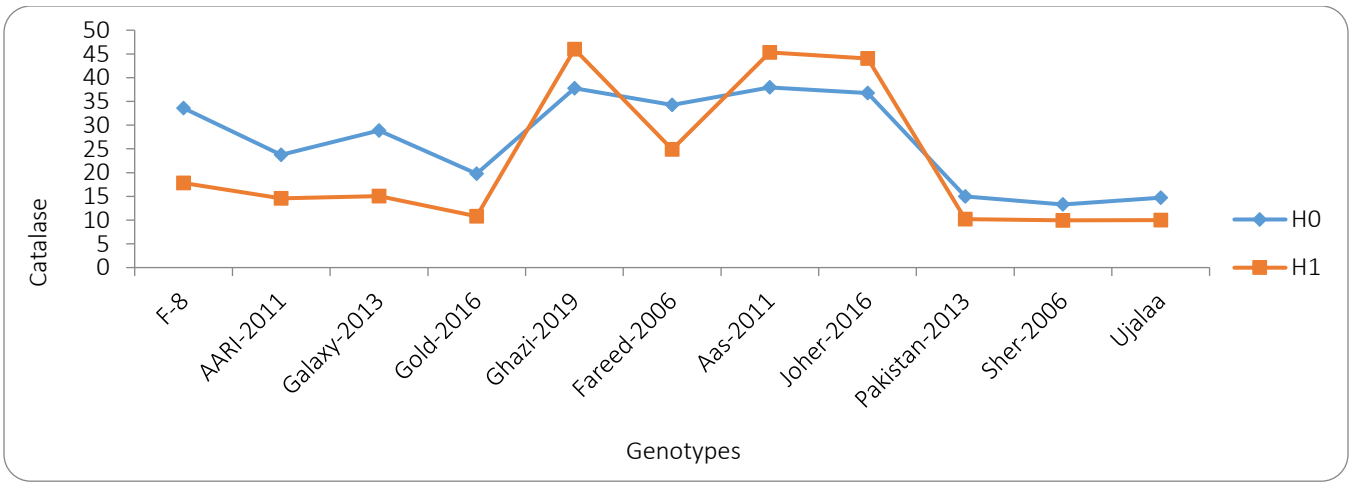

Figure 5. Catalase (CAT) of various wheat genotypes under control (H_1) and heat stress (H_0) treatments.

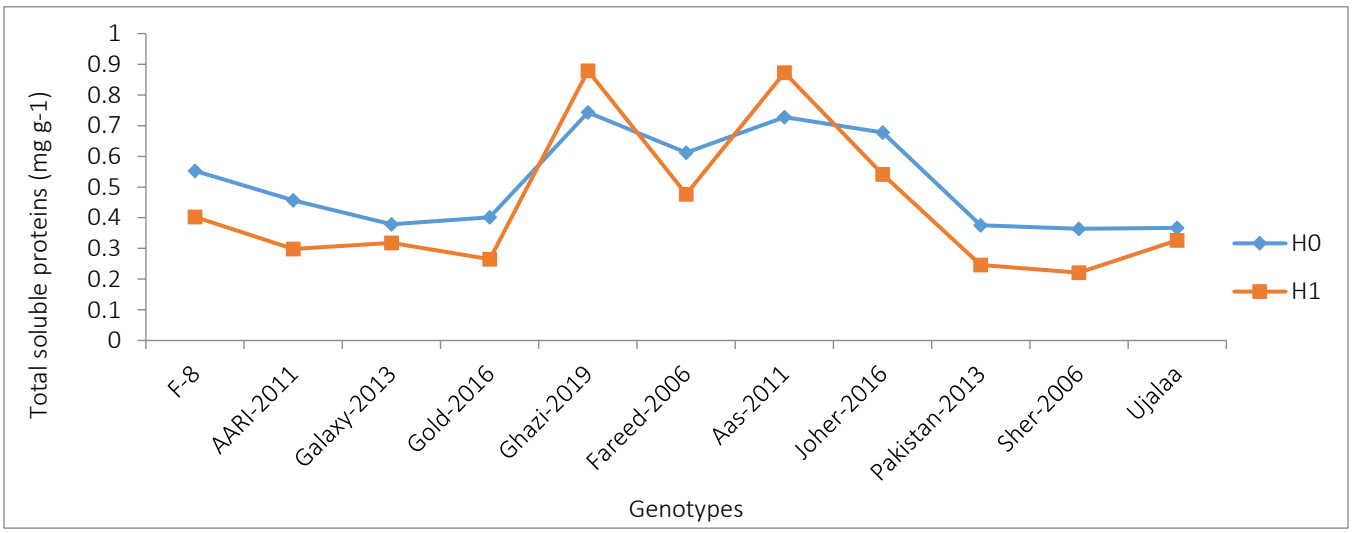

Figure 6. Total soluble proteins (TSP) of various wheat genotypes under control (H_1) and heat stress (H_0) treatments.

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