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Phenotypic and genotypic diversity in some bread wheat genotypes exposed to heat stress

Umar Shahbaz[1], Ummara Waheed[2], Maria Siddique[3], Nadia Riaz[3], Muhammad Waseem Khan[4], Shahab Ud Din[5], Abdur Raziq[6], Fahim Ali Jawad[7], Niaz Muhammad Khan[6], Hayat Ullah[1,6]*

Address:
[1]*School of Biotechnology, Jiangnan University, Wuxi China*
[2]*Institute of Plant Breeding and Biotechnology MNS University of Agriculture Multan*
[3]*Department of Environmental Sciences, COMSATS University Islamabad, Abbottabad Campus*
[4]*Department of Biotechnology, Faculty of Life Sciences and Informatics, Balochistan University of Information Technology Engineering and Management Sciences, Quetta, Pakistan*
[5]*Institute of Biotechnology and Genetic Engineering, The University of Agriculture Peshawar*
[6]*Department of Bioinformatics & Biotechnology, Faculty of Life Science, Government College University Faisalabad, Pakistan*
[7]*Centre of Agricultural Biochemistry and Biotechnology, University of Agriculture, Faisalabad*
Corresponding author:* ***Fahim A. Jawad,* *fahimalijawad10@gmail.com*
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ABSTRACT

Wheat is one of the earliest cultivated plants and second major cereal crop of the world that contains human food essentials. Due to climatic variations, crop is exposed to severe environmental conditions. Rising global temperature is expected to result in severe climate shifts in future. High temperature exerts negative effects on wheat phenology and grain production. The selection of heat-tolerant wheat germplasm is the best stress management strategy. This research study presents the field-based evaluation of 100 wheat genotypes under both normal and heat stress conditions. The data of twelve phenotypic traits were recorded under control and heat stress trials. There were diverse variations observed among genotypes for morphological characters. The high yielding selected wheat lines 68.111-56, CHEN-84, CHEN-74, IWA8611400-65, CHEN-95, CHEN-96, CHEN-86, CHEN-80, 68.111-60 and CHEN-92 were subjected to DNA genotyped with Simple Sequence Repeat marker(SSR) viz; WMC 18, CFA2129, WMC 367, CFD 48, WMC44 and WMC 798. The genetic diversity ranged from 0 to 0.25 with an average value of 0.06. Unweighted pair group of arithmetic means cluster analysis grouped these lines into clusters. Most of the genetic diversity was observed for CHEN-74 and CHEN-96 in cluster. The study has identified genetically diverse, heat tolerant and high yielding wheat genotypes with possible exploitation in development of climate-smart wheat varieties.
Keywords: Abiotic stresses, Crop, DNA fingerprinting, Genotypes, Phenotypic, Wheat

INTRODUCTION

Wheat is one of the major and vital cereal crops grown all around the globe. This crop has significance as a staple food for the rapidly increasing population of the world (Abdelkhalik and Hagras, 2021). Most species of wheat belong to the genus Triticum. More than eighty percent of the world crop is recognized as durum wheat (*Triticum durum*) and bread wheat (*Triticum aestivum*) (Ali *et al.,* 2019). It is an important source of proteins used as diet to cope with the food requirements of the world especially in developing countries; a healthy source of diet fulfilling 50% protein requirements (Ebel *et al.,* 2018). Wheat is also an abundant source of starch, fibers, minerals and other essential nutrients. It provides one-fifth of total energy calories to human food. The crop is usually grown as a winter crop and cultivated in temperate and tropical areas of the world (Asseng *et al.,* 2011).

Heat stress is the main hurdle responsible for a marked decrease in growth, quality and yield of wheat crop (Fleitas *et al.,* 2020). Heat stress affects the wheat during growth-sensitive stages including anthesis, grain filling, and reproductive stages. Morphological parameters i.e., number of spikes, gain weight, grain yield are highly influenced by heat stress (Farooq *et al.,* 2011). A plant having high temperature accelerated growth rate which reduces photosynthesis rate since the life cycle is truncated, while at metabolic level heat stress may also inhibit plant growth directly. It is reported that an increase in temperature from 15-20 °C (day/ night) to 40-15 °C on the third day after anthesis wheat yield was reduced up to 23%. Therefore, it is required to determine wheat genotypes that can grow under adverse environments (Belete *et al.,* 2021; Ramya *et al.,* 2017). Morphological traits are often identified as contributing to enhancing yield potential under heat stress conditions. With environmental change and increasing level of global warming, food security has attained the position of a sensitive and significant issue. High temperatures pose potential threats to wheat production in the world that's why researchers put their attention toward the development of crop genotypes with high tolerance potential (Vinocur and Altman, 2005). The use of molecular markers for the evaluation of genetic diversity of wheat has recently received a great deal of attention from researchers. Wheat genetic diversity was characterized through morphological traits. Simple sequence repeats (SSRs) have become most important genetic markers in a wide range of crop species, including wheat due to higher polymorphism and dispersed throughout genome (Ramya *et al.,* 2017). Incorporating molecular markers and morphological parameters could further facilitate the identification of elite germplasm, new alleles and expedite the breeding efforts in improving heat tolerance. (Sharma *et al.,* 2017).

Through traditional breeding programs efforts have been taken to enhance heat tolerance of wheat plants however only a few studies have been utilized both morphological traits and molecular characterization for wheat genetic diversity.

Heat tolerance wheat genotypes there is limited knowledge available based on morphological traits and molecular characteristics (Al-Doss *et al.,* 2011). The present research study was conducted to evaluate the phenotypic and molecular responses of various wheat genotypes against high-temperature stress. The study is based on phenotypic and genotypic screening of heat stress-tolerant and high yield wheat genotypes based on phenotypic characteristics and molecular level using simple sequence repeats (SSR) markers, and DNA fingerprinting.

MATERIAL AND METHODS

Genetic material and planting strategy:
One hundred wheat genotypes were used for a field trial to select best performing genotypes (Table S1). Wheat genotypes were sown in the field of Wheat Research Institute (WRI) at Ayub Agriculture Research Institute Faisalabad. Wheat genotypes were planted in two different plots; 1st plot was planted on their optimum planting period during the first half of November while 2nd plot was sown on 15th of December for heat stress. Planting layout was according to Randomized Complete Block Design in both plots. Inter plant and inter row distance were 8 and 12 inches respectively. Two to four seeds were sown in a single hole. Hoeing was done after germination for proper growth. The length of each row was 5.5 feet. Three plants were selected from each row and tagged properly. This selection was done based on good phenotypic appearance. Morphological data from tagged plants was recorded by following morphological parameters germination percentage, number of tillers per plant, days to heading, days to maturity, number of fertile tillers per plant, Normalized Difference Vegetation Index (NDVI) value, plant height, number of spikes per plant, spike length, number of grains per spike, one thousand grain weight and yield per plant.

Morphological data analysis and molecular analysis:
The documented data were subjected to statistical analysis of variance. The mean and standard error for each character were calculated according to (Steel, 1997). The Analysis of variance was calculated using statistics 8.1. For principal component analysis, the data were subjected to XLSTAT software. High yielding genotypes were selected and subjected to diversity molecular analysis using Simple Sequence Repeats.

Sample collection and DNA extraction:
The seeds of selected genotypes were sown in the lab at 27 °C temperature. The soil texture was 28% silt, 31% sand, 40% clay (a clay loam) and pH 6.5. Leaves were collected from seedlings 12-15 days after sowing. These samples were used for DNA extraction. DNA was extracted from young leaf samples for molecular analysis. For DNA extraction Cetyl Trimethyl Ammonium Bromide (CTAB) (Table S2) method was used according to the protocol described by (Khan et al., 2004) (Table S3).

DNA confirmation test:
Gel electrophoresis with 0.8% agarose was used to determine the presence and quality of DNA and Nanodrop (ND 2000) was used for quantification of DNA. From concentrated DNA solution a working solution was made with the concentration of 30 ng/µl DNA using double-distilled deionized water for polymerase chain reaction (PCR).

Molecular marker simple sequence repeats (SSR):
To investigate the diversity among wheat genotypes, SSR markers were used. Sleeted samples exhibiting different phenotypic characters were subjected to its genotype analysis based on the simple sequence repeats markers in a PCR reaction. (Table S4).

Polymerase chain reaction:
The extracted DNA samples from selected wheat genotypes were subjected to PCR for molecular analysis using SSR markers. Six different SSR markers were used and multiple copies of DNA were formed. Following reagents (Table S5) were used in PCR process with the defined concentration and quantity. PCR profile for SSR markers was designed for amplification of DNA strands (Table S6).

Agarose gel electrophoresis:
The amplified fragments were run using a 3% agarose gel and then visualized under the gel documentation system (BioRad gel documentation system). A dendrogram of 10 selected wheat genotypes based on an unweighted pair group of arithmetic means (UPGMA) analysis was constructed. Furthermore, PopGen 32 was used for the analysis of Nei's genetic relationship with SSR markers.

RESULTS

Phenotypic traits of wheat genotypes
Germination percentage: In control conditions, the germination percentage of wheat genotypes was 33.3-90%. The genotype INDIA-61 and IWA8611400-67 have highest germination rate. In late sowing treatment, germination percentage was 50-70%. The highest value of germination ratio was exhibited by genotype 60 (68.111-60), 86 (CHEN-86) (**Fig. 1** (**A**) and **Table 1**).
Number of tillers: In normal conditions, genotype 61 (INDIA-61) and genotype 4 have a highest number (7.66) of tillers whereas CHEN-97 and CHEN-79 have lowest number of tillers. Under heat treatments, genotype CHEN-86 has highest mean

value and proved to be heat tolerant. While the genotype number 44, 13, 10 proved to be heat sensitive as they exhibit lowest number of tillers (**Fig. 1** (**B**) and **Table 1**)**

Days to heading: Under control condition, genotypes IWA8611400-70 and CHEN-88 started earlier heading (within 90 days from sowing) while the slow response of days to heading was shown by CHEN-74 and CHEN-4. In heat stress condition, the earlier heading response was observed in genotype ARVAND1-10 (with 89 days). However, most delayed heading response under heat stress condition was recorded for genotypes CHEN-88 and CHEN-89 (**Fig. 1** (**C**) and **Table 1**).

Normalized difference vegetation index (NDVI): NDVI values of wheat genotypes under normal field conditions were 0.51 to 0.85. Maximum NDVI values were recorded for genotype CHEN-78 (0.85) and CHEN-92 (0.85) whereas, ARVAND1-8 (0.51) has minimum value. Under the heat stress treatment, genotype GAN-49 and ARVAND1-6 showed maximum NDVI value (0.8) whereas, genotype CHEN-81 and IWA8611400-70 have minimum NDVI values (0.45), (0.46) respectively (**Fig. 1** (**D**) and **Table 1**).

Number of fertile tillers: Under control treatment, number of fertile tillers were 4.66 to 8.66. Wheat genotype 49 and 78 have maximum number of fertile tillers while genotypes 21 and 64 have minimum. Under the high-temperature stress condition, the mean values of fertile tillers were (N=2-7) (**Fig. 1** (**E**) and **Table 1**).

Days to maturity: In normal treatment, wheat genotypes CP18-31 and IWA8611400-71 showed the earliest maturity about 120 days after sowing whereas the lowest response of days to maturity was recorded for genotypes D67.2-39 and CHEN-1 (137 and 136 days). Under high-temperature stress conditions, the highest response of days to maturity was recorded in wheat genotypes 96 and 43. Wheat genotypes 97 and 98 were characterized as heat-sensitive genotypes as they exhibited the most delayed maturity response (**Fig. 1** (**F**) and **Table 1**).

Spike length: In control treatment, the average value for spike length ranged from 5.66 cm to 12.3 cm. Wheat genotypes ARVAND1-8 and D67.2-36 were of highest spike lengths 12.3 and 11.4 cm respectively. However, genotype 94 was with lowest spike length of 5.66 cm. Under heat stress, the values for spike length were 6.66 to 11.3 cm. Genotypes 68.111-58 and 68.111-54 were of high spike lengths 11.33 cm and 11 cm respectively. However, genotypes ARVAND1-14 and ARLIN-23 showed the lowest spike length 7 cm and 6.66 cm respectively (**Fig. 1** (**G**) and **Table 1**).

Plant height: Plant height of wheat genotypes was 71.3 to 105.7 cm under control treatment. Genotypes IWA8611400-62 and GAN-49 were of highest height 105.7 cm and 105.3 cm under control conditions. Genotypes ARLIN-20 and D67.2-37 were with lowest height of 82 cm and 73.3 cm. whereas, under heat treatment, genotypes ARLIN-22 and GAN-46 had highest plant height recorded as 99.7 cm and 98.0 cm respectively. Genotypes ARVAND1-18 and ARVAND1-14 showed the lowest plant height 69.7 cm and 71 cm respectively (**Fig. 1** (**H**) and **Table 1**).

Number of grains per spike: The number of grains per spike under control conditions ranged (n=34-54.3). Genotypes D67.2-36 and ARLIN-21 have highest number of grains per spike (n=55.3) and (n=54.3) respectively. Under high-temperature conditions, the number of grains were (n=27-54.5) (**Fig. 1** (**I**) and **Table 1**).

One thousand grain weight: Under control treatment, the average weight of 1000 grains were 48 g, genotypes CHEN-84 and IWA8611400-71 were of highest weight 48 g and 46.7 g respectively. However, under heat stress conditions, average weight of 1000 grain weight was 46 g, genotype CHEN-93 was of highest weight with 35.7 g (**Fig. 1** (**J**) and **Table 1**).

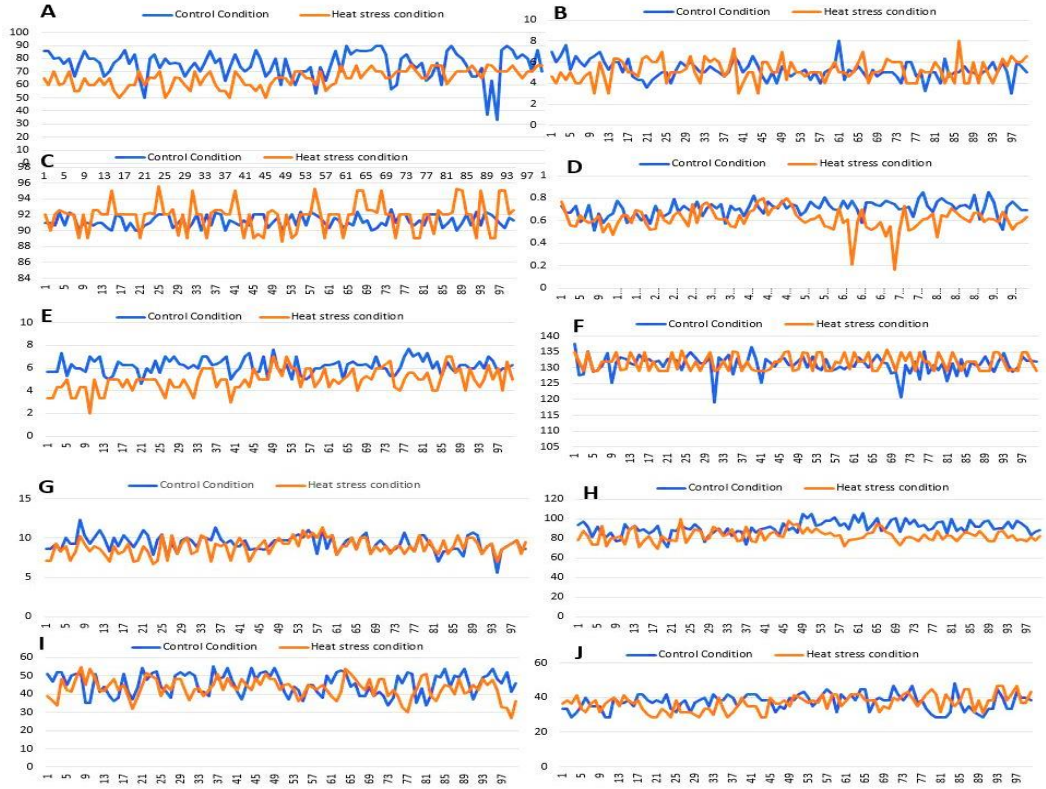

Fig. 1. Comparison of wheat genotypes based on phenotypic traits under control and heat stress conditions.

Table 1. Comparison of wheat genotypes based on phenotypic traits under normal and heat stress condition. Analysis of variance (ANOVA) was based on Degrees of freedom (DF), Sum-of-squares (SS), Mean squares (MS), F ratio (F), and Probability (P).

	Heat stress condition					Control condition				
Source	**DF**	**SS**	**MS**	**F**	**P**	**DF**	**SS**	**MS**	**F**	**P**
Germination percentage										
Line	2	39.1	19.6			2	3.52	1.76		
Replication	99	13200.0	133.3	4.44	0.00	99	155.3	1.57	2.09	0.00
Error	198	5944.9	30.0			198	148.3	0.75		
Total	2	39.1	19.6			299				
Grand Mean	65.0					7.75				
CV	8.43					11.2				
Number of tillers										
Line	2	70.9	35.4			2	0.10	0.51		
Replication	99	152.7	1.5	0.77	0.08	99	188.2	1.90	2.11	0.00
Error	198	398.4	2.01			198	178.4	0.90		
Total	299	621.9				299				
Grand Mean	5.33					5.3				
CV	19.6					18.1				
Days to Heading										
Line	2	444.9	222.5			2	0.03	0.01		
Replication	99	937.5	9.47	1.23	0.1	99	144.7	1.46	0.68	0.2
Error	198	1521.7	7.69			198	423	2.14		
Total	299	2904.2				299				
Grand Mean	91.7					91.3				
CV	3.02					1.60				
Normalized Difference Vegetation Index (NDVI)										
Line	2	0.02	0.01			2	33.8	16.9		
Replication	99	1.23	0.01	9.65	0.00	99	1628.9	16.5	1.00	0.49
Error	198	0.26	0.00			198	3264.3	16.49		
Total	299	1.50				299				
Grand Mean	0.63					0.84				
CV	5.67					15.6				
Number of fertile tillers										
Line	2	38.5	19.2			2	3.73	1.85		
Replication	99	88.00	0.89	0.7	0.17	99	126.1	1.27	1.22	0.12
Error	198	251.5	1.27			198	206.9	1.04		
Total	299	378.0				299				
Grand Mean	4.20					6.21				
CV	21.8					16.4				
Days to maturity										
Line	2	518.8	259.4			2	9.51	4.75		
Replication	99	1864.7	18.8	1.57	0.00	99	2233.6	22.6	0.88	0.76
Error	198	2382.6	12.0			198	5091.7	25.7		
Total	299	4766.0				299				
Grand Mean	131.6					131.0				
CV	2.64					3.80				
Spike length										
Line	2	45.1	22.5			2	3.85	1.93		
Replication	99	234.6	2.37	0.88	0.16	99	312.9	3.16	3.67	0.00
Error	198	535.6	2.70			198	170.3	0.86		
Total	299	815.3				299				
Grand Mean	8.18					9.34				
CV	20.1					9.93				
Plant height (cm)										
Line	2	2436.9	1218.4			2	171.2	85.6		
Replication	99	10998.0	111.1	4.90	0.00	99	13391	135.3	2.38	0.00
Error	198	4485.1	22.7			198	11254	56.8		
Total	299	17920.0				299				
Grand Mean	82.6					91.0				
CV	5.76					8.28				
Number of grains per spike										
Line	2	1069.9	534.9			2	6.4	3.22		
Replication	99	9092.7	91.8	6.40	0.000	99	10281.1	103.9	3.51	0.00
Error	198	2840.1	14.3			198	5858.9	29.6		
Total	299	13002.7				299				
Grand Mean	42.9					45.6				
CV	8.81					11.9				

Table 1 continued										
One thousand grain weight										
Line	2	1155.9	577.9			2	9.14	4.57		
Replication	99	6340.0	64.0	5.23	0.000	99	306.6	3.09	0.92	0.66
Error	198	2424.2	12.2			198	664.4	3.35		
Total	299	9920.1				299				
Grand Mean	36.9					30.8				
CV	9.5					12.4				

Principle component analysis of phenotypic traits of wheat genotypes:

The morphological data of the hundred wheat genotypes was observed to correlate the genetic diversity and similarity using XLSTAT software. The main components that exhibited Eigen values higher than one were recognized as highly significant. The first principal component (PC1) showed a high Eigen value with germination percentage and tillers per plant. PC3 was highly related with number of grains per spike, similarly, PC4, PC5, PC6, PC7, PC8, PC10, PC11 and PC12 were highly related to spikes/plant, days to maturity, plant height, NDVI, spike length, yield per plant, 1000 grain weight and number of fertile tillers per plant respectively (**Table 2**). The principal component analysis showed that six principal components out of twelve indicated more than one Eigen value and these showed more variability than the rest of the principal components. The maximum variation (19%) was observed in PC1 with Eigen value 2.39 which then decreased gradually (**Table 2**).

Table 2. Eigen values of twelve principal components (PC).

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12
Germination percentage	0.41	0.33	-0.13	0.08	0.29	-0.14	0.11	-0.13	0.18	-0.63	-0.37	0.01
Tillers per plant	0.48	-0.27	0.18	-0.37	-0.09	-0.17	0.12	-0.03	-0.01	0.08	0.04	-0.68
Days to heading	-0.04	0.11	-0.47	-0.21	-0.45	0.13	0.47	-0.21	0.48	0.13	-0.01	0.05
Days to maturity	-0.05	-0.46	-0.04	0.21	0.54	0.10	0.27	0.18	0.36	0.30	-0.35	-0.01
Fertile tillers	0.52	-0.31	0.08	-0.29	0.00	-0.08	0.06	0.00	-0.08	0.06	0.10	0.71
NDVI* Values	-0.12	0.40	0.16	-0.26	0.21	-0.02	0.62	0.46	-0.28	0.04	0.09	0.02
Plant height cm	0.03	0.25	0.49	-0.23	0.21	0.45	-0.17	-0.09	0.53	-0.01	0.29	0.02
Spike length cm	0.26	-0.09	-0.14	0.08	-0.31	0.58	-0.15	0.62	-0.04	-0.18	-0.16	-0.05
Spikes per plant	0.17	0.01	0.25	0.57	-0.26	-0.39	0.17	0.29	0.35	-0.04	0.35	0.03
Yield per plant	0.34	0.51	0.06	0.15	-0.07	-0.04	-0.19	-0.02	-0.03	0.65	-0.37	0.01
Grains per spike	-0.00	-0.12	0.50	0.29	-0.27	0.33	0.41	-0.39	-0.25	-0.09	-0.28	0.04
100 grain weight	0.32	0.06	-0.36	0.35	0.29	0.33	0.15	-0.24	-0.25	0.12	0.53	-0.11
Eigenvalue	2.40	1.65	1.40	1.21	1.16	1.03	0.82	0.77	0.66	0.43	0.34	0.13
Variability (%)	19.9	13.8	11.7	10.1	9.69	8.59	6.79	6.40	5.49	3.56	2.85	1.11
Cumulative %	19.9	33.7	45.4	55.5	65.2	73.8	80.61	86.9	92.5	96.0	98.9	100.0

* Normalized difference vegetation index.

Scree and score plot:

Scree plot described the graphical representation of total variance related with each principal component. It also indicates the significance of the measuring parameters. The score plot analysis was carried out for 100 bread wheat lines based on 12 parameters (**Fig. 2**(**A**)). The parameters which were away from the central point in the coordinate system considered more significant variables as compared to those which were present near the origin point. The most variable characters in coordinates system were days to maturity, tillers per plant, germination percentage, NDVI and yield/plant (**Fig. 2**(**B**)). The scree plot indicates the percentage of variance by plotting a graph between Eigen value and cumulative variability (%).

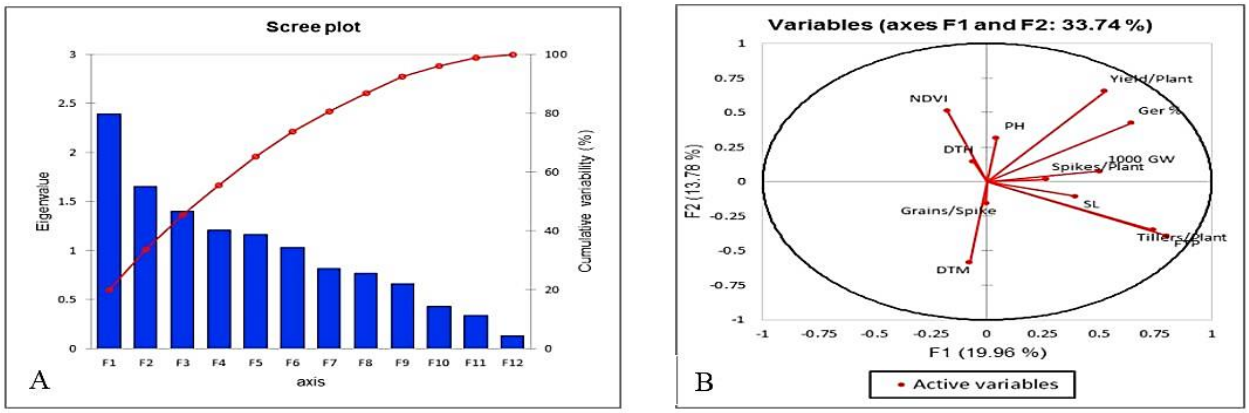

Fig. 2. (**A**) Scree plot between Eigen values and components of principle components for 100 wheat genotypes (**B**) Score plot of two ordination variables on PC1 and PC2 respectively

Bi plot analysis:

Bi plot analysis displayed the relationships between and among the traits of wheat genotypes grown under heat stress. It described the similarities and dissimilarities among the genotypes. Two traits are positively correlated with a high significance value when the angle is less than 90º, traits are negatively correlated when the angle is greater than 90º and traits are independent when the angle is 90º. From the following bi-plot (**Fig. 3**(**A**)) it can be concluded that plant height had a positive correlation with yield per plant, germination percentage and grain weight while it is negatively correlated with days to maturity and tillers per plant. It showed that PC1 and PC2 revealed 33.7% variations. This analysis also explained that traits with longer vectors (away from origin) are more reliable to the treatment combinations and traits with shorter vectors (close to origin) are less responsive to the genotypes. Genotypes 413, 436, 456, 474 and 486 had higher values for PC1 and PC2 and these were recognized as heat tolerant.

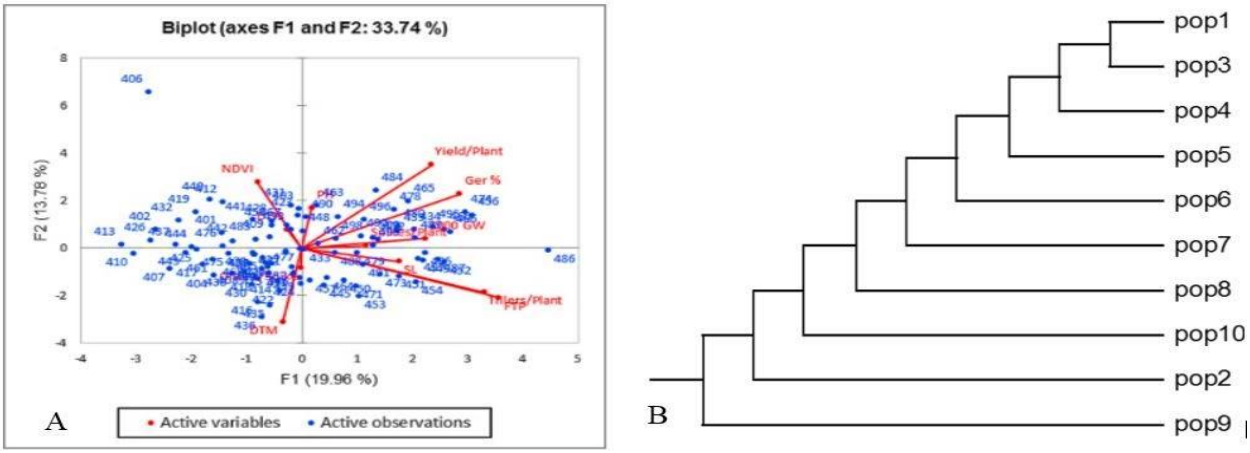

Fig. 3. (**A**) Bi plot representative of variables (**B**) Dendrogram of 10 wheat genotypes based on unweighted pair group of arithmetic means.

Molecular screening of selected wheat genotypes using SSR primers:

Based on yield data, top twenty high yielding wheat genotypes under high-temperature treatment were assessed to represent the diversity among these by using molecular markers. The seeds of these genotypes were grown in sand and leaves were harvested from seedling stage. These leaves were used for genomic DNA extraction. DNA concentration was measured by Nano drop method. The ten best wheat genotypes with higher DNA concentration values namely; 68.111-56, CHEN-84, CHEN-74, IWA8611400-65, CHEN-95, CHEN-96, CHEN-86, CHEN-80, 68.111-60 and CHEN-92 were evaluated and the dilutions were prepared for amplification of these DNA samples. Six SSR primers WMC 18, CFA2129, WMC 367, CFD 48, WMC44 and WMC 798 were used for molecular screening of ten selected wheat genotypes (**Fig. 4**).

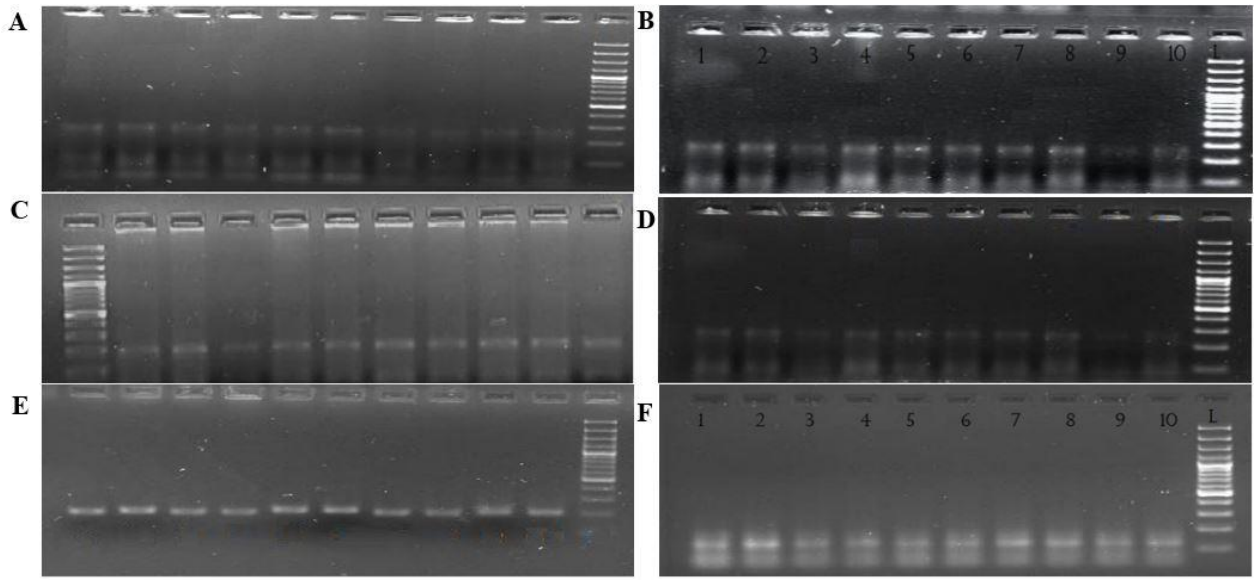

Fig. 3. Amplification of selected wheat genotypes (1-10). (**A**) SSR primer WMC 798. L: Ladder (50bp). (**B**) SSR primer CDF 48. L: Ladder (50bp). (**C**) SSR primer WMC 18. L: Ladder (50bp) (**D**) SSR primer WMC 367. L: Ladder (50bp). (**E**) SSR primer WMC 44. L: Ladder (50bp). (**F**) SSR primer CFA 2129. L: Ladder (50bp).

Population genetic analysis:

SSR primers were used to determine genetic polymorphism at DNA level in ten selected wheat lines. Only the score-able bands were included in the analysis. Every single band was considered as a single locus/allele for all the genetic analyses. The monomorphic alleles were identified by SSR markers. Genetic similarity among wheat genotypes was estimated by Nei's similarity measurement with PopGen software (1.32) (**Table 3**).

Marker diversity:

Power marker software version 3.25 was used to study the marker diversity among wheat lines for 6 SSR loci. The PIC ranged from 0 to 0.22 with a mean of 0.05. The genetic diversity ranged from 0 to 0.25 with a mean of 0.06 suggesting that each of the wheat was stable and that each detected a single genetic locus (**Table 3**). Genetic diversity is usually calculated as the average difference of sequence among any two individuals for specific loci. SSR markers had been effectively used in the identification and differentiation between different wheat cultivars by Zhang et al. (2010).

Table 3. Nei's original measures of genetic identity and genetic distance and summary of marker diversity.

pop ID*	1	2	3	4	5	6	7	8	9	10
1	****	0.92	1.00	1.00	1.00	1.00	1.00	1.00	0.82	1.00
2	0.09	****	0.9129	0.91	0.91	0.91	0.91	0.91	0.89	0.91
3	0.00	0.09	****	1.00	1.00	1.00	1.00	1.00	0.82	1.00
4	0.00	0.09	0.00	****	1.00	1.00	1.00	1.00	0.82	1.00
5	0.00	0.09	0.00	0.00	****	1.00	1.00	1.00	0.82	1.00
6	0.00	0.09	0.00	0.00	0.00	****	1.00	1.00	0.82	1.00
7	0.00	0.09	0.00	0.00	0.00	0.00	****	1.00	0.82	1.00
8	0.00	0.09	0.00	0.00	0.00	0.00	0.00	****	0.82	1.00
9	0.20	0.11	0.20	0.20	0.20	0.20	0.20	0.20	****	0.82
10	0.00	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.20	****

Summary of marker diversity

Markers	Major allele frequency	Allele number	Gene diversity	Heterozygosity	PIC*
WMC798	1.0000	1.0000	0.0000	0.0000	0.0000
CDF48	0.9500	2.0000	0.0950	0.1000	0.0905
WMC18	1.0000	1.0000	0.0000	0.0000	0.0000
WMC367	0.8500	2.0000	0.2550	0.1000	0.2225
WMC44	1.0000	1.0000	0.0000	0.0000	0.0000
CFA2129	1.0000	1.0000	0.0000	0.0000	0.0000
Mean	0.9667	1.3333	0.0583	0.0333	0.0522

*PopGen 32 software identity document; *Polymorphic information content.

Genetic relationship among wheat genotypes:

From PopGen software, genetic relationship among wheat genotypes was identified by Nei's UPGMA method. The UPGMA-based dendrogram grouped genotypes as shown in (**Fig. 2** (**B**)) exhibiting the genetic diversity among genotypes. Genetic similarity and dissimilarity between genotypes can be determined by the distance between genotypes. The genotypes close to each other have more genetic similarity than other ones. Most genetic difference was observed for genotype 1 (CHEN-74) and genotype 9 (CHEN-96). These two genotypes are at a greater distance from each other. Line 2 (CHEN-65) and 9 (CHEN-96) had most diverse genome from the rest of genotypes.

DISCUSSION

For wheat crop development, temperature is the main factor that affects the growth of plant. The optimum temperature for a wheat crop is 18°C to 24°C under normal conditions. The severity of possible reduction in crop yield is determined by developmental stage at which the plant is exposed to high temperate (Dwivedi *et al.,* 2017). In this study, wheat genotypes (N=100) with twelve parameters/traits were morphologically evaluated, based on control (normal/optimum temperature) and late sowing (heat stress) in field trials. Morphological statistical analysis (Statistix 8.1) showed highly significant results for germination percentage, days to heading, number of spikes per plant, NDVI value, days to maturity, spike length, plant height, number of grains, 1000 grain weight and yield per plant. According to Ihsan *et al.* (2016), morphological diversity between wheat genotypes was higher. However, morphological diversity within replications was low with different wheat genotypes. According to Sareen *et al.* (2020), 2-6 alleles were present per locus. PIC value indicates the discriminating ability of locus and is estimated from number of alleles per locus and their relative frequency.

Germination percentage, wheat genotypes under normal condition were high germination ratio. According to Nahar *et al.* (2010) long term effects of heat stress on developing seed might delay the germination of seed or loss of vigor that reduce the emergence and seedling establishment. Number of tillers, wheat plant ability to produce tillers depends upon genotype, agronomic, spacing, nutritional management and environment (air temperature). Heat stress affects the growth of wheat by causing a reduction in duration of grain filling phase and a number of tillers. Under heat stress conditions, effects on the days to appearance of first node, number of tillers and spikelet's per plant are observed that result in reduction of plant sink capacity (Sharma *et al.,* 2017). Days to heading, days of heading reduction is due to heat stress condition which is caused due to plant life cycle shortening as a result of terminal heat stress. Wheat plant having early maturing genotypes has been an effective strategy to overcome the yield loss from heat-stressed condition (Sehgal *et al.,* 2018). Normalized difference vegetation index (NDVI), temperature (>30°C), during grain filling period confirms terminal heat stress that causes a sharp decline in the NDVI. Although high yielding genotypes generally exhibit a slower decline in NDVI, noted during heat stress conditions. Therefore, under heat stress condition for late sowing region, these genotypes would have been selected for cultivation (Ramya *et al.,* 2015). A number of fertile tillers, during anthesis and grain filling period, terminal heat stress is a major threat, which speed up maturity and significantly reduce grain size.

Heat stress resulted in premature plant senescence and reduce photosynthetic activity. The number of plant fertile tillers under normal condition were approximately eighteen tillers per plant however under the heat-stressed condition the number of plant tillers decreased up to five tillers per plant (Dwivedi *et al.,* 2017). Days to maturity, Wheat genotypes having highly significant ($p<0.76$) for days to maturity under control condition suggested high yield. Environment and genotype interaction had high effects on plant growth. It has been previously reported that under stress condition, days to maturity is reduced that negatively affects yield of plant. Heat stress (28°C to 30°C) may alter plant growth duration by reducing maturity period and seed germination. Plants grown under optimum conditions have comparatively higher biomass than that grown with heat stress conditions (Yin *et al.,* 2009). Spike length, Tall genotypes and late maturation have enough time and capacity to accumulate photosynthetically assimilates that results in high grain yield that explains the moderate to the high correlation of days to heading, days to maturity, plant height, stem length and grain yield (Ramya *et al.,* 2015). Plant height delayed

planting remarkably reduced days of heading, days of maturity and plant height, due to plant life-shortening as a result of heat stress. Under late sown condition, a decrease in plant height might be due to increase in temperature in vegetation stage that stops vegetation development and shortens the size of plant (Poudel *et al.,* 2020). Number of grains per spike, according to previous literature, number of spikes are decreased under heat stress conditions, late emergence of seedlings due to low temperature and early maturity due to heat stress condition during plant reproductive stage lead to reduce number of grains per spikes. Temperature (>20°C) between spike initiation and anthesis speed up spike development however reduce number of grains per spike (Senapati *et al.,* 2019). One thousand grain weight, delay sowing progressively decline grain weight and flour yield, due to injury during grain development caused by heat stress. Heat stress (temperature >30°C) affects photosynthesis that inhibits starch synthesis in the endosperm and reduces the duration of grain filling. It is also observed that heat stress during pre and post anthesis under filed condition reduce the grain weight (Dwivedi *et al.,* 2017).

In this study, using XLSTAT software the principal component analysis of 100 wheat lines showed population structure with respect to twelve studied traits. The results indicated that plant height had positive correlation with yield, germination percentage and grain weight while it was negatively correlated with days to maturity and number of tillers. The PC1 and PC2 revealed 33.7% variations. Similarly, Kaur *et al.* (2016) described those variable characters in coordinates system were days to maturity, spike length, yield per plant, plant height and grains per spikes. A positive correlation is present among these traits and they are negatively correlated with other remaining parameters. According to Sareen *et al.* (2020), markers with PIC value <0.25 are slightly informative, those with value between 0.25 and 0.5 are informative, however,>0.5 value is more informative markers. PIC values of the markers have a vital role in diversity studies to study different genotypes.

DNA fingerprinting was used for molecular study, top 10 high yielding wheat cultivars were selected for molecular screening using six SSR primers to screen out variability for genetic diversity analysis. The genetic diversity ranged from 0 to 0.25 with a mean of 0.06, which shows that each of the wheat lines was stable. Similarly, Haile *et al.* (2013) reported higher polymorphism among wheat genotypes using SSR markers. The primer WMC 44 showed monomorphic alleles in all genotypes of bread wheat (Haque *et al.,* 2014). PopGen analysis and Nei's genetic relationship for Six SSR markers showed the diversity among wheat genotypes Etminan *et al.* (2016). In this study, the mean PIC value of ten selected genotypes was 0.05. The highest PIC value 0.22 was observed by SSR marker WMC 367 and followed by the CDF 48 with 0.09 PIC value. These findings were also supported by Mason *et al.* (2010).

CONCLUSION

In conclusion, ten promising wheat genotypes 68.111-56, CHEN-84, CHEN-74, IWA8611400-65, CHEN-95, CHEN-96, CHEN-86, CHEN-80, 68.111-60 and CHEN-92 have high potential to tolerate heat stress and better yield. These genotypes will be valuable in breeding programs aiming to develop promising heat-tolerant wheat varieties.

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التنوع المظهري والوراثي في بعض الطرز الوراثية لقمح الخبز المعرضة للإجهاد الحراري

عمر شهباز [1] ، أمراء وحيد [2] ، ماريا صديق [3] ، نادية رياض [3] ، محمد وسيم خان [4] ، شهاب الدين [5] ، عبد الرازق [6] ، فهيم علي جواد [7] * ، نياز محمد خان [6] ، حياة الله [1،6]

[1] كلية التكنولوجيا الحيوية ، جامعة جيانغنان ، ووشى الصين
[2] معهد تربية النباتات والتكنولوجيا الحيوية MNS جامعة الزراعة مولتان
[3] قسم العلوم البيئية ، جامعة كومساتس إسلام أباد ، أبوت آباد الحرم الجامعي
[4] قسم التكنولوجيا الحيوية ، كلية علوم الحياة والمعلوماتية ، جامعة بلوشستان لهندسة تكنولوجيا المعلومات وعلوم الإدارة ، كويتا ، باكستان
[5]معهد التكنولوجيا الحيوية والهندسة الوراثية ، جامعة الزراعة بيشاور
[6] قسم المعلوماتية الحيوية والتكنولوجيا الحيوية ، كلية علوم الحياة ، الكلية الحكومية ، جامعة فيصل أباد ، باكستان
[7] مركز الكيمياء الحيوية والتكنولوجيا الحيوية الزراعية ، جامعة الزراعة ، فيصل آباد
* بريد المؤلف المراسل: fahimalijawad10@gmail.com

الملخص

القمح هو أحد أقدم النباتات المزروعة وثاني محصول حبوب رئيسي في العالم يحتوي على أساسيات غذائية بشرية. بسبب التغيرات المناخية ، يتعرض المحصول لظروف بيئية قاسية. من المتوقع أن يؤدي ارتفاع درجات الحرارة العالمية إلى تحول مناخي حاد في المستقبل. تؤثر درجات الحرارة المرتفعة سلباً على فينولوجيا القمح وإنتاج الحبوب. يعتبر اختيار الأصول الوراثية للقمح الذي يتحمل الحرارة أفضل استراتيجية لإدارة الإجهاد. تقدم هذه الدراسة البحثية التقييم الميداني لـ 100 نمط وراثي للقمح تحت كل من الظروف العادية وظروف الإجهاد الحراري. تم تسجيل بيانات اثني عشر صفة مظهرية تحت السيطرة وتجارب الإجهاد الحراري. ولوحظت اختلافات متنوعة بين الأنماط الجينية للصفات المورفولوجية. تم التفريق بين جينوتيب القمح المختارة عالية الإنتاجية 56-68.111 و CHEN-84 و CHEN-74 و IWA8611400-65 و CHEN-95 و CHEN-96 و CHEN-86 و CHEN-80 و 60-68.111 و CHEN-92 بواسطة التنميط الجيني للحمض النووي. باستخدام تقنية تكرار التسلسل البسيط (SSR) أي ؛ WMC 18، CFA2129، WMC 367، CFD 48، WMC44 and WMC 798. تراوح التنوع الجيني من 0 إلى 0.25 بمتوسط قيمة 0.06. أن تحليل الكتلة قام بتجميع هذه ال جينوتيب في مجموعات. ولوحظ معظم التنوع الجيني لـ CHEN-74 و CHEN-96 في هذة المجموعة. حددت الدراسة طرزًا وراثية متنوعة وراثيًا ومتحملة للحرارة وعالية الغلة للقمح مع إمكانية استغلالها في تطوير أصناف قمح ذكية مناخيًا.

الكلمات المفتاحية: الاجهاد اللاحيوى ، المحاصيل ، بصمة الحمض النووي ، الأنماط الجينية ، النمط الظاهري ، القمح