

Evaluation of twenty genotypes of wheat (*Triticum aestivum* L.) grown under heat stress during germination stage

Yasir MAJEED^{1,2a}, Shaista FIAZ^{2b}, Wan TENG^{3*}, Adnan RASHEED^{4c},
Syed F.A. GILLANI¹, Zhu XI⁵, Mahmoud F. SELEIMAN⁶,
André A. DIATTA⁷, Harun GITARI⁸

¹Gansu Agricultural University, College of Agronomy, Lanzhou 730070, P.R. China; fabemgillani33@gmail.com

²University of Poonch, Laboratory of Plant Breeding and Molecular Genetic, Rawalakot, Azad Jammu Kashmir, Pakistan
yasirmajeed5453@gmail.com (Y.M.); shaistafiaz019@gmail.com (S.F)

³Baicheng Normal University, College of Mechanical Engineering, Baicheng, Jilin, 137000, China; 415980289@qq.com (W.T)
(*corresponding author)

⁴Hunan Agricultural University, College of Agronomy, Changsha 410128, China; adnanrasheed@hunau.edu.cn (A.R.)

⁵Ministry of Agriculture and Rural Affairs of China/Key Laboratory of Hainan Province for Postharvest Physiology and Technology of Tropical Horticulture Product, South Subtropical Crops Research Institute, Key Laboratory of Tropical Fruit Biology, Chinese Academy of Tropical Agricultural Sciences, Zhanjiang 524000, China; zhuxi@catas.cn (Z.X)

⁶King Saud University, Department of Plant Production, College of Food and Agriculture Sciences, P.O. Box 2460, Riyadh 11451, Saudi Arabia; mseleiman@ksu.edu.sa (M.F.S.)

⁷Université Gaston Berger, Département de Vegetables Productions and Agronomie, UFR des Sciences Agronomiques, de l'Aquaculture et des Technologies Alimentaires (S2ATA), Saint-Louis, BP 234 Saint-Louis, Sénégal; andre-amakobo.diatta@ugb.edu.sn (A.A.D.)

⁸Kenyatta University, Department of Agricultural Science & Technology, Kenya; harun.gitari@ku.ac.ke (H.G.)

^{a,b,c}These authors contributed equally to the work

Abstract

Heat stress is one of the most devastating abiotic stresses which causes significant loss of agricultural crop productivity. Thus, it is critical to examine the wheat's response to the heat stress at seedling stage and adopt an appreciated breeding method to develop heat tolerance and to avoid harmful effects. Therefore, twenty wheat genotypes, including two local landraces, were evaluated in the current study to investigate the genetic diversity for heat tolerance at the seedling stage. Grains of wheat genotypes were placed on filter papers in Petri dishes for germinating at different temperature ranges (i.e., 25 °C as control, 30 °C, 35 °C, and 40 °C). The experiment was laid out in a completely randomized design (CRD) with the factorial arrangement and the number of replications was three. Analysis of variance (ANOVA) for seedling traits and biochemical analysis showed that the genotypes had significant differences for coleoptile length, shoot length (SL), root length (RL), shoot fresh weight (SFW), vigor index (VI), glycine betaine (GB) and proline content. The effect of temperature treatments on different wheat genotypes also exhibited highly significant variation for VI. Principal component analysis (PCA) showed that four factors contributed 82.8% to total variability with the Eigen value greater than 0.7 at 35 °C. Correlation analysis showed that coleoptile length and germination percentage (GP) had a highly significant-positive correlation with SL, VI, and SFW. Results showed that wheat genotypes of 'Maraj', 'Fareed', 'Darabi', 'Zincol-16', 'Barsat', 'NARC-2011', and 'Mundar' showed superior performance when grown under different temperatures. 'NARC-2011', 'Inqalab-91', and 'Galaxy' wheat

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genotypes performed well regarding of H₂O₂ and antioxidant activity. These genotypes had a significant level of variability under heat stress and can be used under high temperatures in future breeding programs for further research purposes.

Keywords: genetic variation; germination; heat stress; seedling traits; wheat genotypes

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important staple food crops worldwide, and is the second most important global cereal food crop after maize (Seleiman *et al.*, 2019; Taha *et al.*, 2021; Jones *et al.*, 2023). The main losses in wheat production are more due to abiotic stresses such as high temperature (Coast *et al.*, 2022), than biotic stresses (Ballesta *et al.*, 2020; Ahmad *et al.*, 2022). The genus *Triticum* contains the majority of wheat species. Durum wheat (*Triticum durum* L.) and bread wheat (*Triticum aestivum* L.) make up more than 80% of the global crop productivity (Ali *et al.*, 2019). It is a vital source of proteins used in diets to fulfil the world's food demands, particularly in underdeveloped countries; it is nutritious type of diet that satisfies 50% of the body's protein requirements (Ebel *et al.*, 2018). Wheat grains contain minerals, fiber, carbohydrates, and other essential nutrients (Seleiman *et al.*, 2010). One-fifth of the available energy calories are provided by wheat crop which is used in the human diet. Wheat crop is frequently grown as a winter crop in temperate and tropical zones of the world (Asseng *et al.*, 2011).

Heat stress has had a major negative impact on the growth, quality, and yield of crops (Mukhtar *et al.*, 2020; Ding *et al.*, 2021). The growth-sensitive periods of anthesis, grain filling, and reproduction are more obvious under heat stress in wheat plants. Indirectly, metabolic plant growth might be vulnerable to heat stress. According to previous studies, wheat productivity declined by 23% on the third day after anthesis when temperature was increased from 15-20 °C to 40-45 °C (Fleitas *et al.*, 2020). In another study, moderate temperature increases can cause significant yield reductions in some temperate wheat cultivars through three effects: reducing sucrose uptake by the endosperm, reducing starch synthesis, and increasing partitioning of carbon into evolved CO₂ (Harris *et al.*, 2023). Consequently, finding wheat genotypes that can grow and adapt to challenging conditions is necessary (Ramya *et al.*, 2017; Seleiman and Abdel-Aal, 2018; Belete *et al.*, 2021). Environmental change and the worsening consequences of global warming have made food security, a sensitive and significant issue. Rising temperatures may endanger the availability of wheat globally, thus scientists have concentrated on developing crop varieties with excellent endurance capability (Vinocur and Altman, 2005).

Due to the physiological nature of wheat as a long-day crop, a rise in temperature over the base temperature (which no growth happens) is necessary for the crop to develop phenologically optimally from seeding to maturity. It takes a certain amount of heat or temperature for a wheat crop to achieve a certain phenophase. The physiological and morphological developments of plants are significantly influenced by temperature, which can affect crop growth, development, and yield. Starting at the early stage of emergence in wheat, heat stress has a negative impact on the wheat crop. Short-term heat stress exposure of wheat seedlings can also result in considerable reductions in root and shoot length, dry mass, chlorophyll content, and membrane stability index, a measure of cell membrane resistance to high temperature (Khan *et al.*, 2020). The plant's germination stage is the stage of growth that is most impacted. Heat stress decreased plant emergence, germination percentage, seedling vigor, aberrant seedlings, and poor shoot and root development in several plant species. The germination of the seed is also inhibited by high temperatures. The rate of seed germination was significantly reduced at high temperatures. High temperature can cause embryo mortality, which slows the development of seedlings (Essemine *et al.*, 2010; Akter and Rafiqul, 2017).

Therefore, twenty wheat genotypes, including two local landraces, were evaluated to different heat stress treatments (i.e., 25 °C as control, 30 °C, 35 °C, and 40 °C) to investigate the genetic diversity for heat tolerance at the seedling stage.

Materials and Methods

The experiment was carried out in a completely randomized design (CRD) with factorial managements and the number of replications was three. Five seeds of each genotype of wheat were sown on filter paper and data was recorded from 3-5 seedlings of each genotype depending upon germination stage and time. Five seeds of each genotype for each replication were spread on fourteen-centimeter petri plates with 2 layers of Whatman filter paper wetted with 10 mL of distilled water. Wheat seeds were allowed to germinate at different temperatures viz., 25 °C, 30 °C, 35 °C and 40 °C, which were used as treatments. The control treatment was 25 °C, because the optimum temperature of wheat germination is 15-25 °C. Twenty genotypes of wheat (two local landraces) were subjected in this research work are given in Table 1.

Table 1. List of wheat genotypes used in the current experiment

Sr. No.	Genotypes	Sr. No.	Genotypes
1.	'Pakistan-13'	11.	'Inqalab-91'
2.	'Zincol-16'	12.	'Barsat'
3.	'Borlaug-16'	13.	'Chakwal-50'
4.	'NARC-2011'	14.	'Darabi'
5.	'NARC-2009'	15.	'Johar'
6.	'Faislabad-2008'	16.	'Punjab-2011'
7.	'Pakhtonkhawa-15'	17.	'Maraj'
8.	'Pirsabak-2008'	18.	'Fareed'
9.	'Ujala'	19.	'Mundar' (local landrace)
10.	'Galaxy'	20.	'Desi wheat' (local landrace)

Seedlings traits

Different seedlings traits were recorded at seedlings stage to check the effect of different heat constraints as given blow.

Germination index (GI)

The sum of seed germinated on day t^{th} divided by the number of days up to germination is called the germination index.

$$\text{Germination index} = \Sigma (Gt/Tt)$$

Gt denoted the number of seeds germinated on day t^{th} and Tt showed the number of days up to germination.

Germination percentage (%)

The number of seeds germinated was counted daily from seven to fourteen days and data was computed. When both plumule and radicle have emerged to 2.5 mm then the seed was considered germinated. The formula for germination percentage was:

$$\text{Germination percentage} = (n/N) \times 100$$

Where N= total number of seeds in each pod, n= number of seeds germinated.

Shoot and root length (cm)

They were measured in cm by selecting three random seedlings from every petri dish and their mean was recorded.

Fresh root and shoot weight (g)

Fresh root and shoot weight were measured with digital electrical balance.

Total fresh weight (g)

Total fresh weight was recorded by taking every shoot and root separately, with digital electrical balance.

Root and shoot dry weight (g)

Root and shoot dry weight were taken by taking roots of seedlings and dried in oven at 60 °C for 24 hours.

Vigor index

Vigor index was calculated by using the following formula: VI = Root length + Shoot length × Seed germination %.

Coleoptile length (cm)

The coleoptile length of seedlings was measured by using a meter rod.

Biochemical analysis

Proline content

It was measured according to method Waterhouse (2002). Two grams of dry sample was grinded with 3% sulfo-salicylic acid and kept for 72 h at room temperature for proline release. Then mixture was centrifuged for 20 minutes at 3000 rpm. The upper layer after centrifuge was taken to treat with Nin hydrin and acetic acid. After this, samples were kept in a water bath for boiling for one hour. At last absorbance of samples was noted at 520 nm on a spectrophotometer.

Determination of glycine betaine content

It was done by using the method of Grieve and Gratan (1983). Dried ground leaves were taken (0.5 g). Extracted these samples with water at room temperature for 24 h and then filtered. One mL of the extract was mixed with 1 milliliter of 2.0 normal hydrochloric acid (HCL) in test tubes. Then approximately 0.5 mL of this mixture was taken. After this 0.2 mL of potassium tri-iodide solution was added. The ice-cooled distilled water of 2 mL was mixed and then 20 mL of 1-2 dichloro-ethane (cooled at 10 °C) was added. By using a spectrophotometer, the optical density of each sample was measured at 365 nm.

Antioxidant activity (%)

Antioxidant activity was determined by using method of Yu *et al.* (2002). Samples were extracted with ethanol. Add 0.25 mm solution of (DPPH) radical to the sample solution in ethanol (1 mL) at 100 concentrations (g/mL). Control and blank were also taken. Absorbance of the all samples with control and blank were recorded at 517 nm. The antioxidant activity was expressed as a percentage by using the following equation:

$$\text{Radical scavenging activity} = \frac{\text{Absorbance of control} - \text{absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Hydrogen peroxide (H₂O₂) content

Plant material (100 mg FW) was homogenized with 0.5 mL of trichloroacetic acid (TCA) in ice bath (Chen *et al.*, 2009). The homogenate was centrifuged at 12,000 rpm for 15 min. 100 mM potassium phosphate buffer and One mM potassium iodide was added to each supernatant. Absorbance was measured at 390 nm. H₂O₂ was quantified based on a standard curve.

Statistical analysis

Data was analysed via MSTAT-C software. Means will be compared by LSD (Bricker, 1991). Simple correlation coefficients between the traits were done (Snedecor, 1956). For principal component analysis, numerical taxonomic techniques were utilized (Rohlf and Sokal, 1981), with the help of computer software past (Hammer *et al.*, 2001), and SPSS 20 (www.spss.com).

Results

Every genotype must have the ideal temperature range to grow and develop. Temperature stress, which has an impact on plant performance, is created when the temperature exceeds the critical limit. Wheat's rate of germination was significantly slowed down at 40 °C, which also induced cell death and decreased the rate of seedling establishment.

Analysis of variance (ANNOVA) for various seedling traits of wheat genotypes with artificial induce heat stress

Table 2, showed that analysis of variance for coleoptile length, germination percentage, shoot length, root length, total fresh weight, shoot dry weight, root fresh and dry weight, vigor index and germination index showed significant differences for treatments and genotypes as well as for the interaction between treatment and genotypes, whereas germination percentage for interaction between treatment and genotypes, total fresh weight for genotypes as well as for the interaction between treatment and genotypes, shoot fresh weight for treatments and genotypes as well as for the interaction between treatment and genotypes and root fresh weight for the interaction between treatment and genotypes showed non-significant results are shown in Table 2.

The results of Table 2 indicate that the majority of wheat crop seedling attributes fared well under various heat stress treatments and might be used to select cultivars that are heat resistant. Table 2 displayed the mean square values for each seedling feature that served as the foundation for choosing important and insignificant deviations.

Table 2. Analysis of Variance (ANOVA) for different seedling traits in 20 wheat genotypes at different temperature stresses

SOV	DF	CL	GP	SL	RL	TFW	SFW	RFW	SDW	RDW	VI	GI
T	02	40.47* *	3082.2 *	1420.3 **	3934.4 **	0.15 *	0.005 NS	0.08 *	0.017 *	0.04 *	75667 091.2 **	12.8 *
G	19	1.21 *	614.1 *	15.1 **	18.2 **	0.008 NS	0.004 NS	0.003 *	0.002 *	0.003 *	93276 6.3**	8.6 **
TXG	38	0.19 **	282.2 NS	5.1 *	8.6 *	0.005 NS	0.004 NS	0.002 NS	0.001 *	0.002 *	30848 3.8*	1.8 **
Error	114	0.12	314.2	2.9	6.5	0.005	0.004	0.002	0.001	0.001	30103 1.7	0.6

Where: SOV= source of variation; DF= degree of freedom; T= Treatments; G= Genotypes; TXG= Treatment interaction Genotype; CL= coleoptile length; GP= germination percentage; SL= Shoot length; RL= Root length; TFW= Total fresh weight; SFW= Shoot fresh weight; RFW= Root fresh weight; SDW= Shoot dry weight; RDW= Root dry weight; VI= vigor index; GI= Germination Index; **= $P \leq 0.01$; *= $P \leq 0.05$; NS= non-significant

*Biochemical analysis*Proline content ($\mu\text{mol g}^{-1}$ FW)

The highest values of proline were obtained from 'Darabi' (0.180) followed by 'NARC-2009' (0.149) and 'Ujala' (0.145), whereas the lowest values were recorded by 'Pirsabak-2008' (0.07) followed by 'Galaxy' (0.105) and 'Faisalabad-2008' (0.107) at 25 °C (Figure 1). At 30 °C maximum values were attained by varieties, 'NARC-2009' (0.152), 'Darabi' (0.146) and 'Fareed' (0.142) while minimum values were depicted in cultivars, 'Faisalabad-2008' (0.09) followed by 'Johar' (0.101) and 'Borlaug-16' (0.102). At 35 °C maximum values were obtained by cultivars, 'Darabi' (0.178), 'Ujala' (0.141) and Fareed (0.142) whereas minimum values were calculated for varieties, 'Pirsabak-2008' (0.08), 'Galaxy' (0.105) and 'Inqalab-91' (0.111).

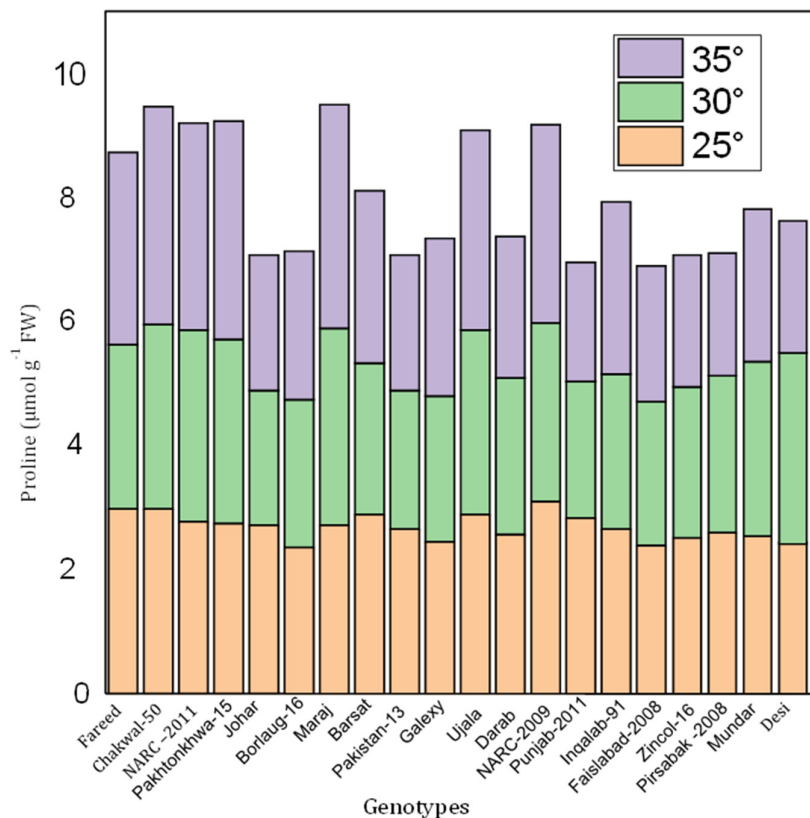


Figure 1. Mean values for proline content in 20 wheat genotypes at different temperature stresses

Glycine betaine content ($\mu\text{mol g}^{-1}$ FW)

The results for glycine betaine for three different temperatures were shown in Figure 2. At 25 °C maximum values were shown by varieties, 'NARC-2009' (2.99) followed by 'Fareed' (2.97) and 'Barsat' (2.89) whereas minimum values were displayed by varieties, 'Borlaug-16' (2.32) followed by 'Faisalabad-2008' (2.33) and 'Galaxy' (2.40). At 30 °C maximum values were found in varieties, 'Maraj' (3.06), 'NARC-2011' (3.00) and 'Chakwal-50' (2.96) while minimum values were observed in 'Punjab-2011' (2.07) followed by 'Johar' (2.19) and 'Pakistan-13' (2.19). For 35 °C maximum values were obtained by 'Maraj' (3.59) followed by 'Chakwal-50' (3.50) and 'Pakhtonkhwa-15' (3.49). Whereas minimum values were found in varieties 'Punjab-2011' (1.90), 'Pirsabak-2008' (1.92) and 'Fareed' (3.05).

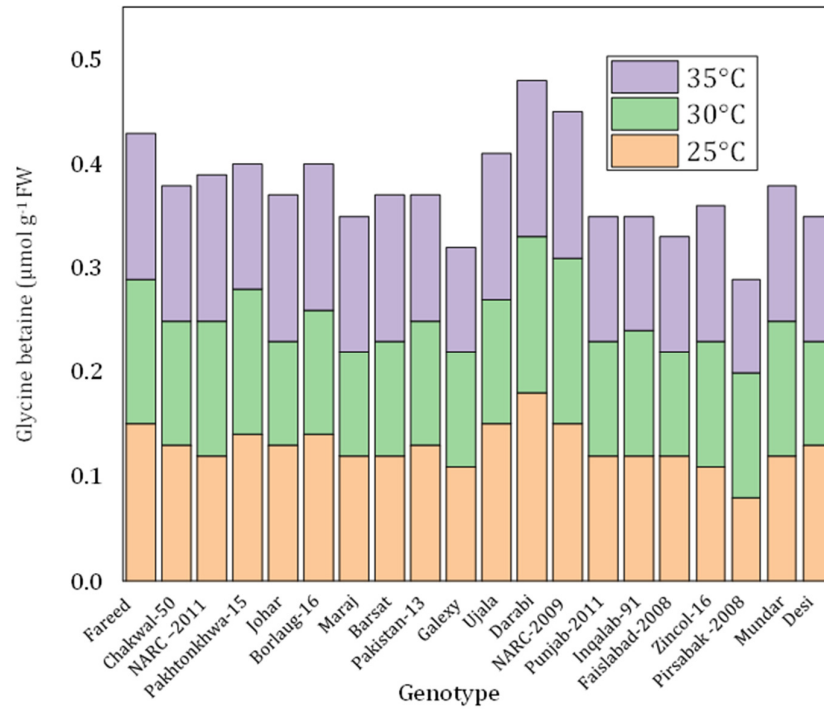


Figure 2. Mean values for Glycine betaine content in 20 wheat genotypes at different temperature stresses

Antioxidant activity (%)

The results for antioxidant activity were shown in Figure 3. At 25 °C maximum values of antioxidant activity were observed in varieties, ‘Galexy’ followed by ‘NARC-2009’ and Fareed and scored values 47.93%, 45.93% and 43.9% respectively while minimum values were recorded in ‘Ujala’, ‘Inqalab’ and ‘Johar’ and values were 11.01%, 11.4% and 11.7% respectively. At temperature 30 °C maximum values were showed by varieties, ‘Fareed’, ‘Borlaug-16’ and ‘Chakwal-50’ and scored values 48.9%, 48.1% and 44.8% respectively. When temperature increased at 35 °C then maximum values were showed by ‘Pakhtonkhwa’, ‘Johar’ and ‘Maraj’ and scored values were 50%, 49.2% and 49% respectively. Minimum values at 35 °C were observed in varieties, ‘NARC-2009’, landrace ‘Mundar’ and ‘Barsat’ and values were 12.4%, 13.8% and 19.3% respectively.

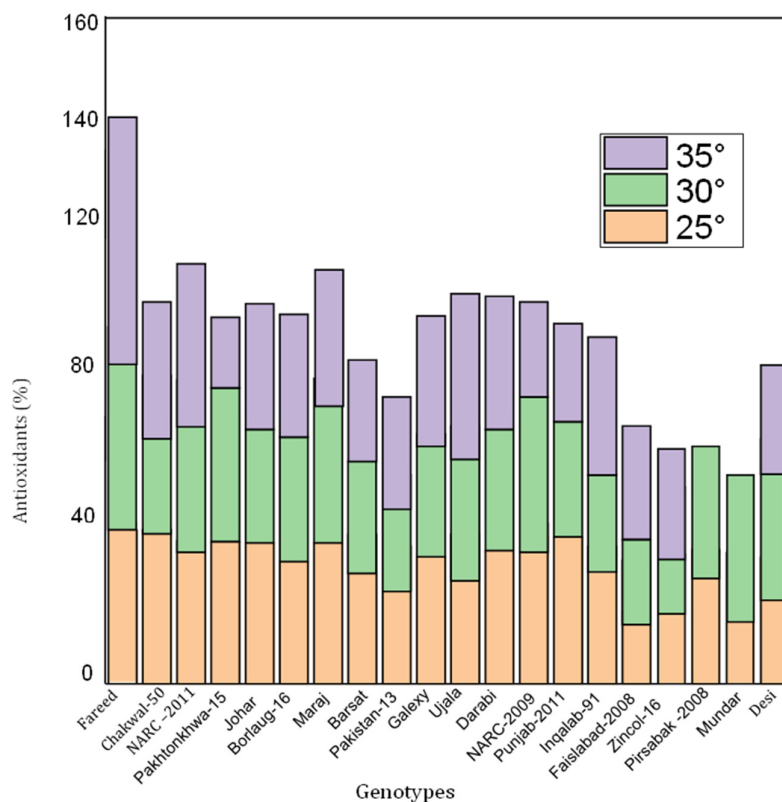


Figure 3. Mean values for Antioxidant activity (%) in 20 wheat genotypes at different temperature stresses

Hydrogen peroxide (H₂O₂)

The results for hydrogen peroxide were shown in Figure 4. At 25 °C maximum values were observed in varieties, ‘Ujala’ followed by ‘Barsat’ and ‘Johar’ and scored values were 0.66, .051 and 0.45 respectively while minimum values were observed by ‘Zincol-16’, ‘Chakwal-50’ and ‘Pirsabak-2008’ and scored values 0.02, 0.06 and 0.07 respectively. At 30 °C varieties showed maximum values were ‘Inqalab-91’ followed by ‘Fareed’ and ‘NARC-2011’ and attained values 0.93, 0.58 and 0.48 respectively whereas minimum values were showed by ‘NARC-2009’, ‘Galaxy’ and ‘Darabi’ and values were 0.02, 0.06 and 0.07 respectively. At 35 °C maximum values attained by varieties, ‘Inqalab-91’, ‘Punjab-2011’ and ‘Pakhtonkhwa-50’ and values were 0.78, 0.60 and 0.58 respectively. Minimum values at 35 °C were showed by varieties, ‘Maraj’, ‘NARC-2011’ and ‘Galaxy’ and values were 0.06, 0.08 and 0.133 respectively.

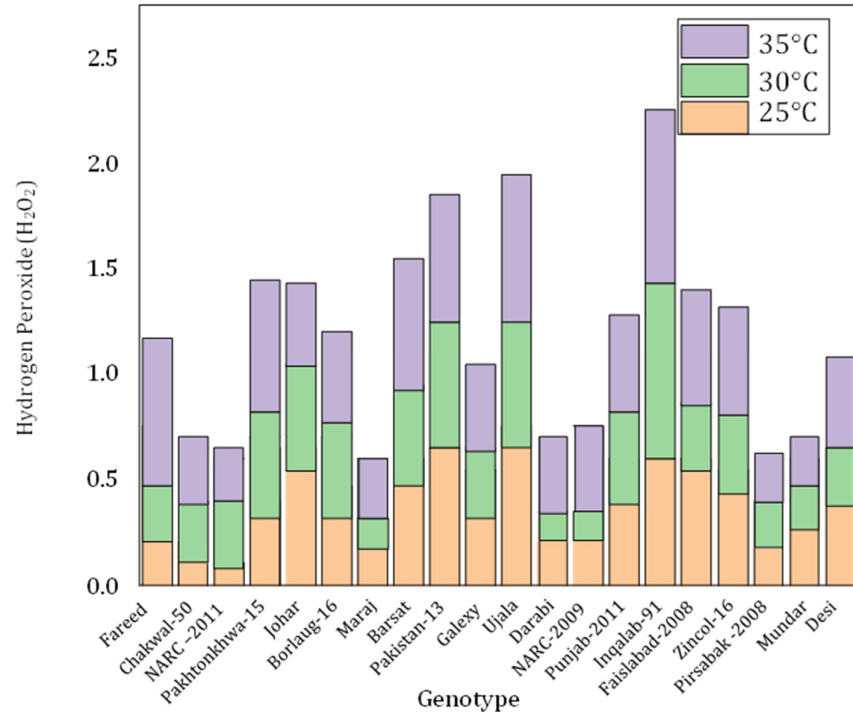


Figure 4. Mean values for hydrogen peroxide (H_2O_2) in 20 wheat genotypes at different temperature stresses

Principle component analysis

Table 3 revealed Principal component analysis (PCA) of 20 wheat genotypes for 11 seedling traits at 35 °C. Out of 11 principal components (PCs), four were significant as Jolliffe cut-off value was 0.7. PCA is a criterion to select significantly important components. Maximum Eigen value was showed by PC1 that is 5.08 whereas PC4 showed minimum Eigen value of 0.80. All the components contributed 82.8% variability among all genotypes. PC1 showed maximum variance of 46% preceded by PC2 (19%) and PC3 (10%) of the entire variance described.

Table 3. Principal component analysis (PCA) for different seedling traits in 20 wheat genotypes at 35 °C

PC	Eigen value	% variance	Cumulative (%)
1(GI)	5.084	46.22	46.22
2(GP)	2.082	18.92	65.15
3(SL)	1.14	10.39	75.55
4(RL)	0.802	7.29	82.84
5(TFW)	0.550	5.00	87.85
6(SFW)	0.434	3.95	91.8
7(RFW)	0.350	3.18	94.98
8(SDW)	0.298	2.71	97.70
9(RDW)	0.203	1.84	99.55
10(VI)	0.040	0.37	99.92
11(CL)	0.008	0.07	99.99

Jolliffe cut-off = 0.7

GI= Germination index; GP= germination percentage; SL= Shoot length; RL= Root length; TFW= Total fresh weight; SFW= Shoot fresh weight; RFW= Root fresh weight; SDW= Shoot dry weight; RDW= Root dry weight; VI= Vigor index; CL= coleoptile length

Scree plot diagram for 11 principal components of 20 wheat genotypes for different seedling traits

Figure 5 showed scree plot of eleven principal components for seedling traits. The scree plot designated that the 4 principal components have contributed towards total variance. But the first 2 PCs contributed maximum towards total variance while later two principal components had a less contribution towards variance. First 2 PCs showed highly significant variance while later 2 showed significant variance. After 4 PCs the line gone about straight indicating less significant differences. Scree plot indicated that the first two components have major contribution towards total variance, outcomes are obtained according to the Jolliffe cut-off is equal to 0.7. However, the Eigen value and contribution of PC5 and other succeeding PCs towards total variance was in small amount, so they were considered as non-significant.

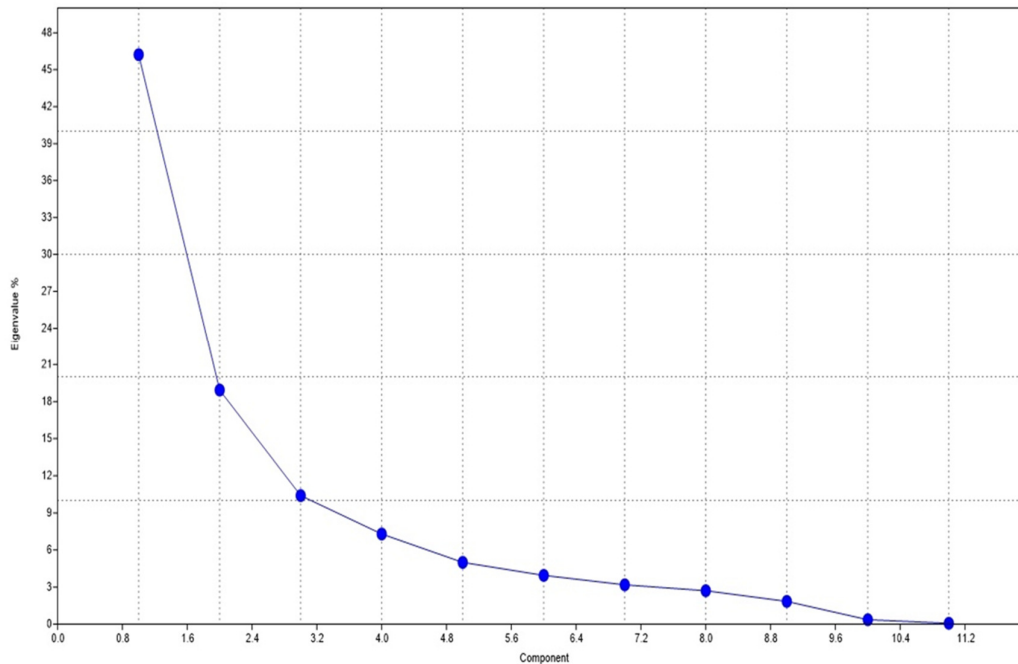


Figure 5. Scree plot for eleven principal components in 20 wheat genotypes for different seedling traits

Biplot diagram

Maximum variance was stimulated by first 4 PCs used to construct a scatter biplot diagram for seedling traits of 20 wheat genotypes shown in Figure 6. Some traits such as root dry weight and germination % showed more variation as they were present far away from the point of origin. Whereas traits like shoot dry weight, coleoptile length and shoot length were detected on same point indicated that these parameters were closely associated with each other. Traits like total fresh weight, root length and shoot fresh weight were located close to the origin, so these traits were less variable. Scatter biplot illustrated 8 wheat genotypes 'Darabi', 'Borlaug-16', 'Faisalabad-2008', 'Barsat', 'NARC-2009', 'Inqalab-91', 'Johar' and 'NARC-2011' showed more variations from other wheat genotypes.

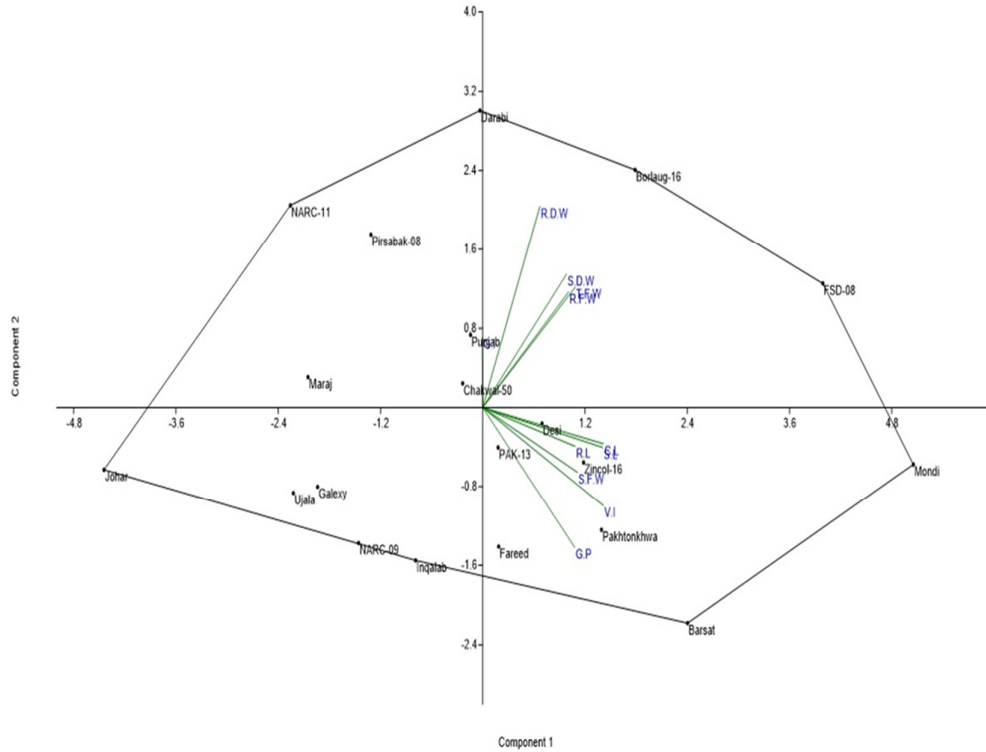


Figure 6. Biplot diagram of 20 wheat genotypes for different seedlings traits

Loading factor for 20 wheat Genotypes at 35°C

Loading for factor 1

Figure 7 showed loading for factor one. Maximum positive load was noted for vigor index (0.879) and coleoptile length (0.92) for factor 1, representing a robust relationship of these traits to produce heat tolerance. While minimum positive load was detected in root dry weight (0.416) and shoot dry weight (0.610).

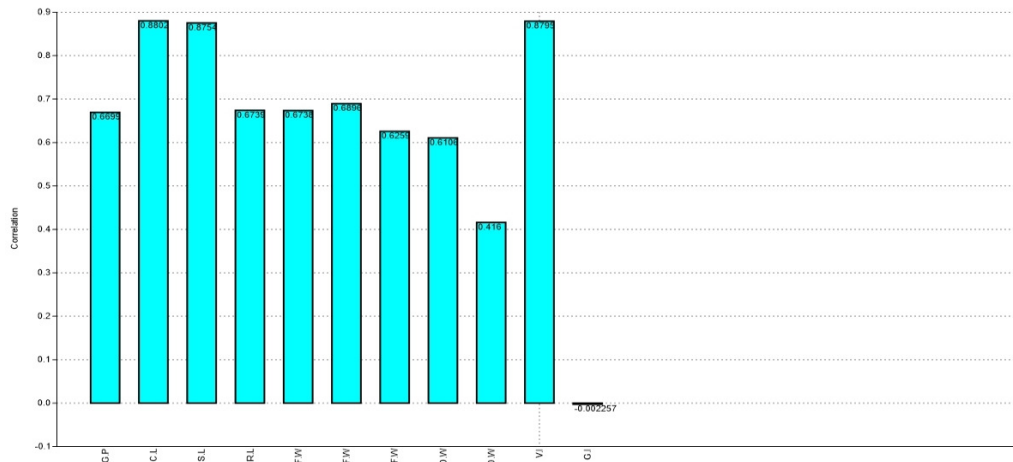


Figure 7. Loading for factor 1 in 20 wheat genotypes for different seedlings traits

Loading for factor 2

Figure 8 showed loading for factor two. Maximum positive load was noticed for root dry weight (0.809) and shoot dry weight (0.535). Minimum positive load was noted in germination index (0.282) preceded by root fresh weight (0.463). Whereas maximum negative load was found in germination percentage (-0.53) and vigor index (-0.395). Coleoptile length showed minimum negative load (-0.145) followed by root length (-0.155).

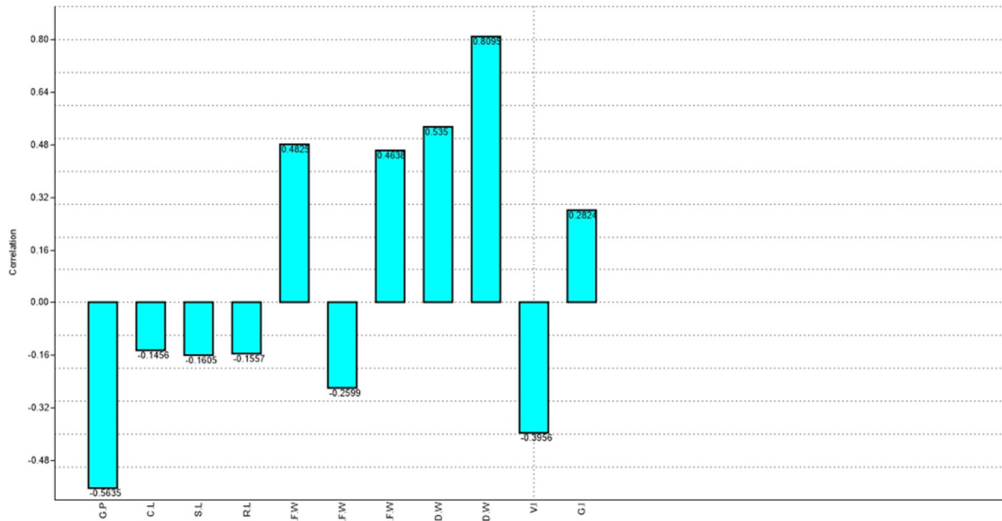


Figure 8. Loading for factor 2 in 20 wheat genotypes for different seedlings traits

Loading for factor 3

Figure 9 showed loading for factor three. Maximum positive load was indicated by germination index (0.934) followed by shoot length (0.245) and minimum positive load was noted in total fresh weight (0.006) preceded by root dry weight (0.007). Maximum negative load was confirmed by shoot dry weight (-0.398) preceded by root fresh weight (-0.210). While minimum negative load was observed in coleoptile length (-0.017) preceded by root fresh weight (-0.188).

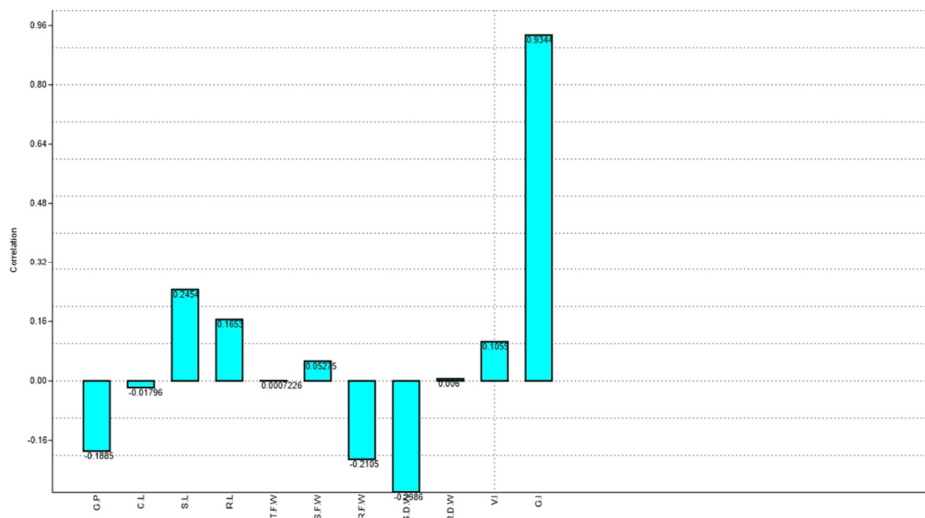


Figure 9. Loading for factor 3 in 20 wheat genotypes for different seedlings traits

Loading for factor 4

Figure 10 showed loading for factor 4. Maximum positive load was noted for shoot fresh weight (0.519) proceeded by total fresh weight (0.180). These values indicating a strong bonding between these parameters to produce genetic variability. Minimum positive load was observed for shoot length (0.001) and germination index (0.038). Root length (-0.618) and dry shoot weight (-0.227) showed maximum positive load. Whereas root fresh weight (-0.033) and vigor index (-0.154) showed minimum negative load.

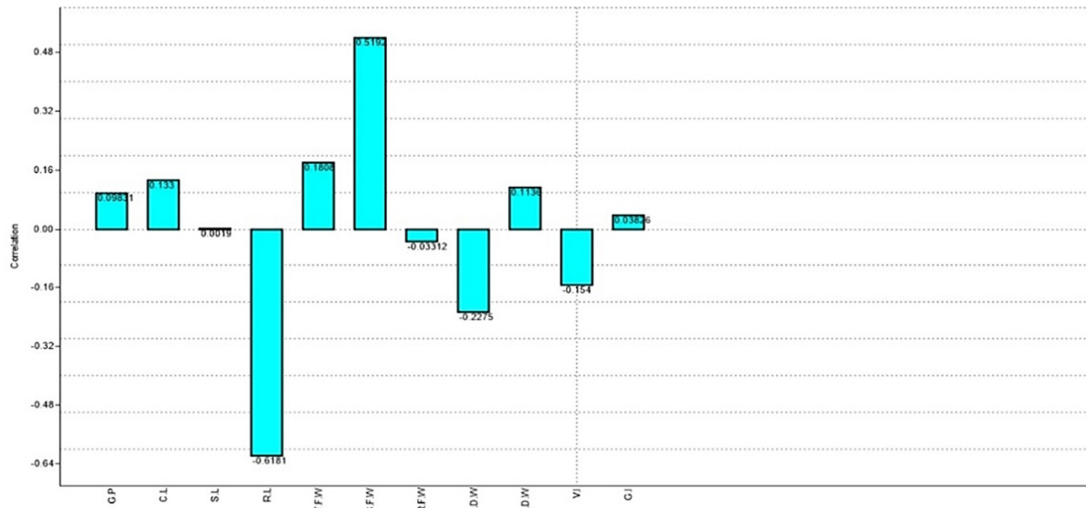


Figure 10. Loading for factor 4 in 20 wheat genotypes for different seedlings traits

Loading for factor 5

Figure 11 showed loading for factor 5. Root fresh weight (0.535) and root length (0.117) showed maximum positive load for factor 5, representing the strong relationship between these traits to create genetic variability among various wheat genotypes. Minimum positive load was showed in germination index (0.013) proceeded by shoot fresh weight (0.078) whereas shoot dry weight (-0.368) and root dry weight (-0.187) had maximum negative load. Minimum negative load was showed in germination percentage (-0.025) and coleoptile length (-0.069).

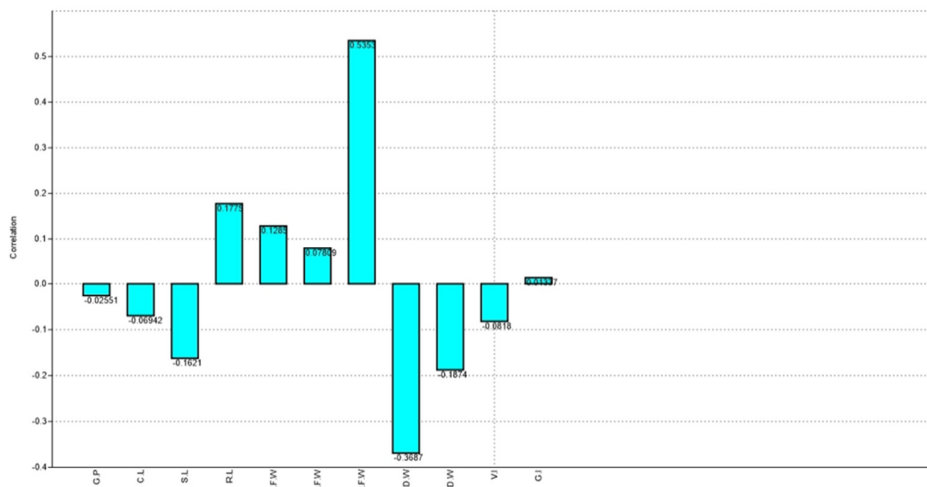


Figure 11. Loading for factor 5 in 20 wheat genotypes for different seedlings traits

Loading for factor 6

Figure 12 showed loading for factor 6. Total fresh weight (0.451) and germination percentage (0.290) had maximum positive load for factor 6. Minimum positive load observed in vigor index (0.033) preceded by dry shoot weight (0.057) whereas shoot fresh weight (-0.211) and dry root weight (0.002 g) had maximum negative load. Minimum negative load was observed in root length (-0.072) and coleoptile length (-0.102).

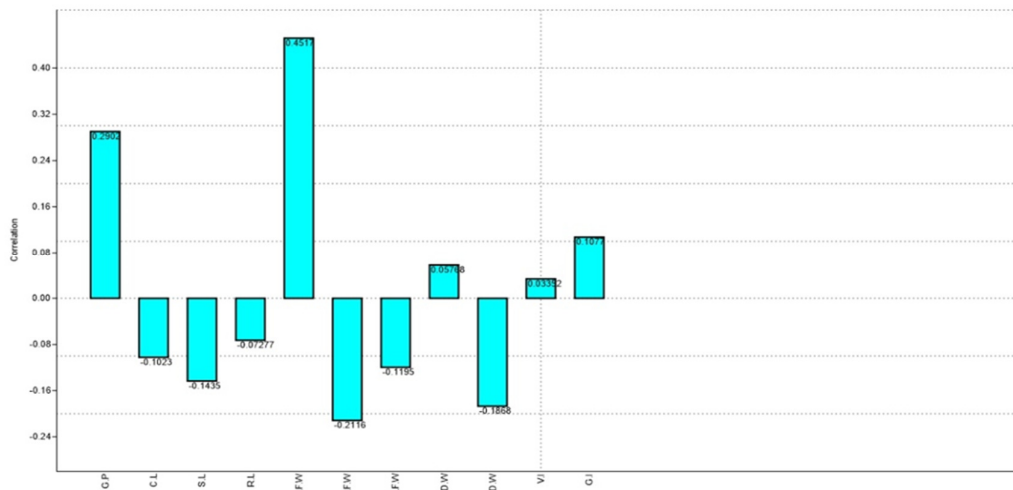


Figure 12. Factor loading in 20 wheat genotypes for different seedlings traits

Simple correlation coefficient for different seedling traits in 20 wheat genotypes at 35 °C

Simple correlation coefficient of 20 wheat genotypes for seedling traits was showed in Table 4, which showed highly significant correlation of germination % with coleoptile length, fresh shoot weight and vigor index and significant correlation with shoot length. Germination percentage showed positive non-significant correlation with root length, total fresh weight, root fresh weight, shoot dry weight and proline. It showed negative correlation with root dry weight, germination index and glycine betaine. Coleoptile length had highly significant correlation with shoot length, shoot fresh weight and vigor index. Also showed significant correlation with root length, total fresh weight, root fresh weight and shoot dry weight. Coleoptile length had positive non-significant correlation with root dry weight and proline and negative correlation with germination index and glycine betaine.

Shoot length showed a highly significant correlation with root length, shoot fresh weight, and vigor index and a significant correlation with total fresh weight. While showing a positive non-significant correlation with fresh root weight, fresh shoot weight, total fresh weight, shoot, and root dry weight, and germination index and a negative correlation with glycine betaine. Root length showed a highly significant correlation with vigor index and positive non-significant with total fresh weight, shoot and root fresh weight, shoot and root dry weight, and germination index. It had a negative correlation with proline and glycine betaine. Total fresh weight had a highly significant correlation with shoot and root dry weight and a significant correlation with root dry weight. It showed a positive non-significant correlation with shoot fresh weight, vigor index, germination index and proline. Total fresh weight showed a negative correlation with glycine betaine. Shoot fresh weight revealed highly significant correlation with vigor index and a positive non-significant correlation with root fresh weight, shoot and root dry weight, vigor index, proline and glycine betaine. It showed negative correlation with germination index. Root fresh weight showed positive significant correlation with shoot and root dry weight and glycine betaine. It also had positive non-significant correlation with germination index.

Shoot dry weight had highly significant correlation with root dry weight but positive non-significant correlation with vigor index and proline. It showed negative correlation with germination index and glycine

betaine. Root dry weight had positive non-significant correlation with vigor index, germination index and proline. It showed negative correlation with glycine betaine. Vigor index showed positive non-significant correlation with proline and negative correlation with germination index and glycine betaine. Germination index had positive non-significant correlation with proline and negative correlation with glycine betaine. Proline showed positive non-significant correlation with glycine betaine. Glycine betaine had a positive non-significant correlation with root fresh weight and showed a negative non-significant correlation with germination index, vigor index, shoot and root dry weight, total fresh weight, root and shoot length, coleoptile length, and germination percentage.

Table 4. Simple correlation coefficients for different seedling traits in 20 wheat genotypes at 35 °C

	GP	CL	SL	RL	TFW	SFW	RFW	SDW	RDW	VI	GI	PRO	GB
GP	1												
CL	0.591**	1											
SL	0.561**	0.827**	1										
RL	0.407	0.477*	0.591**	1									
TFW	0.258	0.502*	0.458*	0.287	1								
SFW	0.582*	0.656*	0.598*	0.314	0.365	1							
RFW	0.181	0.478*	0.365	0.385	0.613**	0.294	1						
SDW	0.165	0.467*	0.367	0.370	0.591**	0.161	0.494*	1					
RDW	-0.144	0.206	0.295	0.072	0.558*	0.159	0.558*	0.679**	1				
VI	0.819**	0.788**	0.885**	0.722**	0.358	0.591**	0.325	0.342	0.066	1			
GI	-0.268	-0.056	0.126	0.067	0.153	-0.025	-0.050	-0.107	0.212	-0.015	1		
PRO	0.024	0.211	0.074	-0.156	0.017	0.020	0.000	0.248	0.180	0.026	0.305	1	
GB	-0.082	-0.277	-0.074	-0.146	-0.296	0.007	-0.525*	-0.281	-0.221	-0.118	-0.117	0.140	1

GP= germination percentage; CL= coleoptile length; SL= Shoot length; RL= Root length; TFW= Total fresh weight; SFW= Shoot fresh weight; RFW= Root fresh weight; SDW= Shoot dry weight; RDW= Root dry weight; VI= Vigor index; GI= Germination index; PRO= Proline; GB= Glycine betaine; **Correlation is highly significant at 0.01 level; *Correlation is significant at 0.05 level.

Discussion

Wheat (*Triticum aestivum* L.) is the most widely grown crop and a major component of the human diet worldwide. This staple crop is one of the most important sources of energy, and on average provides 20% of the total protein and calories in human nutrition (El Hassouni *et al.*, 2023). The rise in Earth's temperature is one of the most alarming climatic issues in the field of agriculture and food production in the present context. Heat stress, a significant abiotic stress caused by the rise in temperature, has a significant negative impact on crop productivity. In addition to other crops, wheat suffers significantly from acute heat stress in terms of yield and overall productivity. Yet, wheat has further evolved built-in defenses against heat damage. The selection of cultivars with a higher degree of tolerance mechanism protects against thermal stress, which minimizes the risk of poor productivity to a greater extent (Kayastha *et al.*, 2023). The purpose of study was to investigate the effect of different temperature regimes viz., 25 °C, 30 °C, 35 °C and 40 °C, which were used as treatments on germination of wheat genotypes and early germination events. The optimum temperature for wheat crop germination is 15-25 °C which is used as control.

The genotypes were evaluated and data was recorded against several key seedling traits. Analysis of variance (ANOVA) for seedling and biochemical traits showed that the genotypes differed significantly for coleoptile length, shoot length, root length, fresh shoot weight, vigor index, glycine betaine, and proline content. The effect of treatments on genotypes was also significantly higher for vigor index. Some traits like shoot fresh weight showed a non-significant result as shown in Table 2. In another study, Analysis of variance (ANOVA) showed the presence of significant differences among parents and their crosses in wheat under optimal and heat stress conditions for all studied traits except starch, which showed non-significant differences among parents and their crosses under heat stress conditions (Riaz *et al.*, 2021).

Principal component analysis (PCA) is a criterion to select significantly important components. In this study, PCA revealed that four factors contributed 82.8% to total variability with the Eigen value greater than 0.7 at 35 °C as shown in Table 3. Maximum Eigen value was showed by PC1 which is 5.08 whereas PC4 showed a minimum Eigen value of 0.80. All the components contributed 82.8% variability among all genotypes. PC1 showed a maximum variance of 46% preceded by PC2 (19%) and PC3 (10%) of the entire variance described. The maximum variance was produced by the first 4 PCs used to construct a scatter biplot diagram for seedling traits of 20 wheat genotypes. For genotypes to be tested for heat tolerance, genetic diversity and appropriate selection criteria must be present. In order to comprehend the significance of the principal component analysis in reducing a large amount of highly correlated data into a small number of major principal components that account for the greatest amount of genetic variability, 60 advanced breeding lines and four standard checks were evaluated for morpho-physiological traits under late sown conditions. On the basis of eigenvalues and a scree plot, only three of the twenty major components were kept, contributing 67.10% to the overall variability. The first, second, and third major components had the highest positive loadings for grain yield (0.995), days to heading (0.912), and plant height (0.752), respectively. To retain the major components having largest contribution towards the total variability and clustering pattern, scree plot was used. On the basis of scree plot only first three components that contributed for 67.10% variability were used for further analysis (Bhatti *et al.*, 2023).

Scatter biplot illustrated 8 wheat genotypes such as ‘Darabi’, ‘Borlaug-16’, ‘Faislabad-2008’, ‘Barsat’, ‘NARC-2009’, ‘Inqalab-91’, ‘Johar’ and ‘NARC-2011’ were shown significant differences from other wheat genotypes. These genotypes can perform best under heat stress condition than others. The genotypes ‘Raj 3765’ and ‘Raj 4027’, produced in Jaipur, were shown to be more stable across all settings according to the genotypes × environment interaction biplots for grain yield. They are now being suggested as possible germplasm sources for late-sown and/or warmer habitats because of their resilience to high-temperature situations. The genotypic response pattern in Varanasi and Jaipur is given varied, it is also proposed that any shared breeding approach should priorities on grain production stability while attempting to breed for high-temperature tolerance (Rane *et al.*, 2007).

The factor loading is the correlation between the item and the factor, a factor loading of values more than 0.30 usually indicates a moderate correlation between the item and the factor. In this study for some traits, maximum positive load was shown and for some maximum negative loads were shown for 6 factors discussed previous section as shown in Figure 7 to 12. In similar study factor analysis divided the thirteen traits into three factors. The cumulative variation for these factors was 0.76 and also its portions for factor one to three were 0.59, 0.16 and 0.06, respectively. In the first factor, the traits including GY, BY, SW, GWS, SPS, NGS and SSW had high factor loadings. The traits compromise HI, SSW and SRW had high factor loadings in second factor and also SPM, GW and PH had high values of factor loadings in third factor (Ebrahimnejad and Rameeh, 2016).

The strength of the association between two variables or factors is indicated by the correlation coefficient. It is useful in plant breeding because it can demonstrate an analytical association that can be used in practice and because it provides information about the connections between many desired features. It provides a fundamental understanding of the relationships between numerous yield-contributing features, which helps plant breeders to choose varieties with the required characteristics (Ali *et al.*, 2008; Ghafoor *et al.*, 2013). This investigation may assist enhance tactics for the selection of necessary types with desirable qualities by providing evidence of the association between seedling attributes under stress and non-stress settings. In this study correlation analysis concluded that the coleoptile length and germination percentage had positively correlated with shoot length, vigor index, and shoot fresh weight. Scientists have previously reported that simple correlation coefficients of root length were shown a positive and strong correlation with root/shoot ratio, fresh weight, dry weight, cell membrane thermo-stability, carotenoid, and chlorophyll a, as well as a negative association with shoot length and relative water content same as in this study (Ahmad *et al.*, 2013).

Conclusions

The tolerant genotypes identified in the current study can be used as reference material for the acceleration of wheat breeding. Interaction between genotypes and treatment was highly significant for vigor index, while it was significant for shoot length, glycine betaine, coleoptile, and proline. Principle component analysis (PCA) exhibited that, at 35 °C four factors added 82.8% of total diversity with the Eigen value greater than 0.7. Among the investigated 20 genotypes of wheat, four genotypes including 'Mundar', 'Fareed', 'Darabi' and 'Barsat' showed wide diversity to heat stress. For hydrogen peroxide (H₂O₂) and antioxidant activity, the genotypes, 'Fareed', 'Galaxy' and 'Johar' showed a significant diversity. On the other hand, the correlation analysis showed that coleoptile length and germination percentage had a positive association with shoot length, vigor index, and shoot fresh weight. Genotypes 'Johar', 'Inqalab', 'Galaxy', and 'Fareed' also showed positive results for radical scavenging activity (DPPH) and hydrogen peroxide content when grown under different treatments of temperatures. The two local landraces of wheat (i.e., 'Munder' and 'Desi') did not show a significant diversity under heat stress, therefore they could be replaced with the heat tolerance genotypes in order to get high yield and quality in regions where temperature is high.

Authors' Contributions

Y.M, A.R, and S.F equally contributed to the article regarding original draft preparation; S.F.A.G, and Z.X helped in review process, M.F.S, A.M.D, and H.G improved the manuscript for scientific language writing. W.T supervised the study. All authors have read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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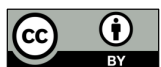
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