

Genotypic variation and yield stability of bread wheat under induced field heat stress during grain-filling for climate resilience in arid regions

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Abstract

Climate change causes frequent periods of heat stress that threaten global wheat production and food security. High temperatures, particularly during grain-filling stage, shorten filling duration and reduce grain yield. Therefore, this study aimed to evaluate the performance, heat stress tolerance, and yield stability of 35 bread wheat genotypes under normal and heat stress conditions during two consecutive growing seasons. Field experiments were conducted under six environments created by varying heat stress duration using plastic tunnel covers (two and four weeks) compared to control plots. Yield components, including number of grains per spike, 1000-grain weight, grain weight per spike, and grain yield, were measured. Heat stress significantly reduced all yield traits, with the greatest relative losses occurring for grain yield and grain weight per spike. Substantial genotypic variability was observed, and genotype-by-environment interaction effects were highly significant. Advanced statistical models, including AMMI1, AMMI2, GGE dendrogram, and hierarchical clustering, were employed to dissect genotype performance and stability across heat treatments and seasons, revealing significant variations in tolerance and adaptation. Dendrogram, heatmaps, and stability analyses identified genotypes with broad adaptability and yield stability under thermal stress. The advanced lines G11, G13, G15, G16, G29, and G25, and G32 exhibited superior heat tolerance and stable high yields. Significant positive correlations among grain yield traits were observed under normal and short-term heat stress. However, prolonged heat stress disrupted these relationships, weakening the association among yield components, which indicates detrimental impact of extended thermal stress on wheat yield formation. The results of this study provide valuable insights into wheat genotypic responses to heat stress and identify promising genotypes for breeding, aiming to improve wheat productivity and sustainability under increasing temperature pressures in arid agroecological zones.

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Keywords: AMMI biplot; boxplot; bread wheat; cluster analysis; genotype by environment interaction; heat stress tolerance; heatmap

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important staple crops worldwide, providing significant portion of daily caloric intake for large segment of the global population (Hossain *et al.*, 2021). It is cultivated on approximately 220.4 million hectares worldwide with an annual production of 799.0 million tons. In Egypt, wheat plays a central role in national food security, covering 1.35 million hectares and producing around 9.7 million tons annually (FAOSTAT, 2025). Despite this substantial production, Egypt still relies heavily on imports to meet 45-50% of its wheat consumption (Abdalla *et al.*, 2022). This considerable production-consumption gap indicates the urgent need to enhance wheat productivity, particularly in the face of increasing climate variability pressures. Given its economic and nutritional importance, breeding and developing resilient wheat genotypes are vital for sustaining and enhancing wheat production in Egypt.

Climate change is characterized by several factors, with rising temperatures being one of the most impactful (Fan *et al.*, 2025). Climate models predict that heat stress events will become more frequent, intense, and prolonged, posing major challenges to agricultural systems worldwide (Verma *et al.*, 2025). High temperatures present a significant influence, as even brief exposure to elevated temperatures during sensitive reproductive stages can cause substantial yield reductions and impair wheat productivity (Qian *et al.*, 2025). Optimal temperatures for wheat during critical growth phases, such as grain filling, range from 19 °C to 22 °C (Djanaguiraman *et al.*, 2020). Heat events exceeding 30 °C during anthesis and grain filling adversely affect pollen viability and fertilization, resulting in reduced seed set, lowered grain numbers, and decreased grain productivity (Lohani *et al.*, 2025). Moreover, heat stress after anthesis accelerates leaf senescence and shortens the grain-filling period, which leads to diminished grain weight and overall grain quality (Schittenhelm *et al.*, 2020; Fazaa *et al.*, 2025). The long-term consequences of climate change for wheat remain complex and region-specific (Han *et al.*, 2023).

The interaction between genotype and environment (G×E) is a fundamental consideration in the selection and breeding of wheat genotypes (Mansour *et al.*, 2018). This interaction determines how genotypes perform under varying environmental conditions, impacting traits such as yield stability and overall adaptability (Gracia *et al.*, 2012). Studying G×E is essential to identify high-yielding genotypes and exhibit consistent performance across diverse environments, particularly under current fluctuations in climatic variables (Wondaferew *et al.*, 2024). Neglecting to account for G×E can result in selecting genotypes that perform well in specific environments but poorly in others, limiting their broader applicability (Galal *et al.*, 2025). To effectively evaluate and interpret G×E interactions, various statistical models are employed. Classic linear models partition the phenotypic variance into genotype, environment, and G×E components, providing insights into the relative contribution of each factor (Elias *et al.*, 2016). More advanced approaches, including additive main effects and multiplicative interaction (AMMI) models and genotype plus genotype-by-environment (GGE) biplots, facilitate visual exploration and ranking of genotypes based on their stability and adaptability (Mansour *et al.*, 2021). These modeling techniques are crucial for interpreting G×E, supporting the selection of genotypes with desirable performance profiles, and ultimately accelerating the development of resilient wheat varieties for climate-challenged regions.

Previously published studies employed different parameters to identify heat-tolerant genotypes. Empirical indices such as the Stress Tolerance Index (STI), Heat Susceptibility Index (HSI), and Mean Productivity have been extensively applied to quantify genotype performance stability under heat stress conditions (Poudel *et al.*, 2021; Megahed *et al.*, 2022; Lamba *et al.*, 2023; Devi *et al.*, 2024). Additionally,

genotype-by-environment interaction analysis through multi-environment trials and AMMI modeling provides robust insights into genotype adaptability and yield stability across variable heat stress environments (Kamara *et al.*, 2021; El-Abssi *et al.*, 2024; Mullualem *et al.*, 2024). Integrating these approaches could enable researchers to accurately classify genotypes based on their tolerance levels and select candidates with high yield potential under heat stress for breeding programs. This study adopts similar screening strategies to evaluate bread wheat genotypes for heat tolerance and stability under natural and experimentally induced field heat stress conditions. By identifying genotypes with heat tolerance, this research supports breeding efforts to enhance wheat resilience to climate-induced heat stress, thereby helping to mitigate the adverse effects of climate change on wheat production.

Materials and Methods

Plant materials

Thirty-five bread wheat (*Triticum aestivum* L.) genotypes were selected for assessing heat stress tolerance. The pedigree and codes of these genotypes are presented in Table A1. These genotypes comprised a diverse set of elite genetic materials representing variation in agronomic performance and adaptation. This set included high-yielding commercial cultivars currently listed among the Egyptian recommended wheat varieties and genotypes developed by the International Maize and Wheat Improvement Center, CIMMYT. This selection ensured broad genetic base encompassing locally adapted and internationally recognized germplasm.

Experimental site

The study was conducted during the growing seasons of 2022/2023 and 2023/2024 at the Agricultural Experimental Station at Ghazala belongs to Zagazig University, Egypt (30° 34' 7" N, 31° 34' 16 E). Meteorological data of the experimental site provided by the Egyptian Meteorological Authority, as presented in Table A2. Throughout both seasons, the site exhibited variations in temperature, humidity, and precipitation. Maximum temperatures in the winter season ranged from 16.84 °C to 31.39 °C, with the lower values observed during the months of December to February and higher readings occurring from late spring in April and May. Minimum daily temperatures varied between 10.16 °C and 19.35 °C over the same period, indicating generally mild winter nights and a gradual warming trend into spring. These climatic conditions, characterized by moderately high temperatures during spring which are adequate for assessing wheat genotypes under natural and elevated heat stress conditions during critical growth stages. Sampling soil to a depth of 0–30 cm was collected before sowing in both seasons. Particle size distribution was analyzed using the pipette method according to Pansu and Gautheyrou (2006). Soil pH was determined using a soil-to-distilled water suspension (H₂O) as the extraction solution following McLean (1982). Soluble cations and anions were measured following the procedures described by Jackson (2005). Available phosphorus was extracted by the Olsen method and quantified calorimetrically (Olsen, 1954). Available nitrogen was assessed using the approach outlined by Black and Evans (1965). Based on the analysis, the soil is classified as clay, with clay content of 59.85% in 2022/2023 and 57.4% in 2023/2024. The soil was slightly alkaline, with pH values of 7.23 and 7.37 for both seasons in the same order. Total nitrogen levels were low, ranging from 0.08% to 0.09%. Available phosphorus was moderate, varying from 7.20 to 9.08 ppm. Detailed soil physical and chemical properties are presented in Table A3. The agrotechnical measures were implemented to ensure optimal growth conditions for the evaluated wheat genotypes. The land was carefully prepared through plowing and harrowing to create a fine seedbed suitable for seed germination. The sowing date was on 21 November in both seasons. The evaluated genotypes were sown at density of 400 seeds per square meter. Recommended fertilizer doses were applied as follows: phosphorus at 60 kg P₂O₅/ha, potassium at 90 kg K₂O/ha, and nitrogen at 200 kg N/ha. Surface irrigation was used according to the standard practice in the study region with a total water of approximately 450 mm throughout the growing season.

Experimental design and applied treatments

The experiment was conducted using split-plot design in three replications, temperature treatments were applied in the main plots, and genotypes were randomized within the sub-plots. Each plot consisted of six rows spaced 20 cm apart and 3 meters long. Two heat stress treatments were applied during the grain-filling stage by covering plots with plastic tunnels to increase the temperature for two and four weeks, respectively. A control treatment was maintained outdoors adjacent to the tunnels. Simple high tunnel structures made of transparent polyethylene covers supported by a frame. The tunnels were installed over the plots 90 days after sowing to induce elevated temperature conditions by trapping solar radiation. Temperature measurements inside the tunnels showed average increases of approximately 4-6 °C compared to outside ambient conditions during the stress periods. The tunnels are sealed at the edges to retain heat but allow for ventilation openings to prevent overheating and excessive humidity. This design enables controlled simulation of heat stress while maintaining other environmental variables consistent with field conditions.

The tested environments are presented in Table 1.

Table 1. Description of normal and heat stress treatments applied to evaluated wheat genotypes during two growing seasons

Code	Description
E1	Control treatment with no heat stress, the plots were maintained outdoors, adjacent to tunnels, during the first season
E2	The plots were covered with plastic tunnels for two weeks during the first season
E3	The plots were covered with plastic tunnels for four weeks during the first season
E4	Control treatment with no heat stress, the plots were maintained outdoors, adjacent to tunnels, during the second season
E5	The plots were covered with plastic tunnels for two weeks during the second season
E6	The plots were covered with plastic tunnels for four weeks during the second season

Data recorded

To evaluate the agronomic performance of wheat genotypes under both heat stress and control conditions at harvest, key yield-related traits were measured. The number of grains per spike was determined by randomly collecting a sample of 15 spikes from each plot, followed by grain counting after threshing. The weight of grains per spike was measured by weighing the grains harvested from these sampled spikes, and an average grain weight per spike was calculated for each plot. Thousand-grain weight (g) was obtained by randomly collecting two samples of 1000 clean and undamaged grains as a representative sample from the bulk harvested grain of each plot. These samples were weighed using a calibrated digital balance. Grain yield was assessed by harvesting the central four rows of each plot. The harvested grain was threshed and cleaned, weighed, and then converted to yield expressed in tons per hectare ($t\ ha^{-1}$).

Statistical analysis

A combined Analysis of Variance was performed to identify significant differences among genotypes, environments, and their interactions. Genotype-by-environment interactions (GEI) were further explored using the Additive Main Effects and Multiplicative Interaction (AMMI) model using Genstat 19 (Payne *et al.*, 2009). Boxplot was carried out using R programming, employing packages of tidyverse, rstatix, and ggpubr (Team, 2016). While the dendrogram used ggplot2, factoextra, and FactoMineR, and the heatmap used gplots. To distinguish heat-tolerant wheat genotypes from sensitive ones, three tolerance indices were employed. The following indices were calculated for each genotype:

Mean Productivity = $(Y_s + Y_p) / 2$ (Hossain *et al.*, 1990) Stress Tolerance Index = $(Y_s \times Y_p) / (\bar{Y}_p)^2$ (Fernandez, 1992) Yield Index = Y_s / \bar{Y}_s (Gavuzzi *et al.*, 1997)

where Y_s is the grain yield under prolonged heat stress (four-week treatment), Y_p is the grain yield under normal (non-stressed) conditions, \bar{Y}_p is the mean yield of all genotypes under normal conditions, and \bar{Y}_s is the average grain yield of all genotypes in the treatments of prolonged heat stress (four-week treatment).

Results

Analysis of variance

The analysis of variance indicated highly significant effects of environment, year, heat treatment, genotype, and their interactions on all yield traits studied (Table 2). The environment was the predominant source of variation, contributing significantly to all traits. The mean square values for the environment were higher, indicating substantial influence of environmental conditions across seasons and treatments. Moreover, year significantly affected all traits, although the variation explained was lower compared to the environment. The year significantly influenced all measured traits, reflecting the impact of seasonal climatic variations on wheat performance. Although the variation attributed to year was lower than heat treatment, it remains an important factor influencing trait expression. This emphasizes the need to consider year-to-year environmental fluctuations in multi-season trials when evaluating genotype stability and stress responses. Heat treatment exhibited highly significant effects, particularly evident in the increased mean squares for studied traits, indicating critical role of heat stress on wheat performance. The interaction between year and heat treatment was significant for all traits, revealing that the response to heat treatment differed across years. The genotypes contributed significantly to the detected variation, indicating significant genetic diversity among the evaluated genotypes for heat tolerance and yield-related traits. Significant interaction was detected between genotype with year and heat treatment, and also the three-way interaction (genotype, year, and heat treatment) for all traits. These interactions indicate that genotype performance varied across years and under different heat treatments, revealing the complexity of genotype responses to combined environmental and heat stress factors.

Table 2. Means of squares of the ANOVA analyses for Number of grains per spike, 1000-grain weight, weight of grains per spike, and grain yield of 35 wheat genotypes were evaluated under normal and heat stress conditions across two growing seasons

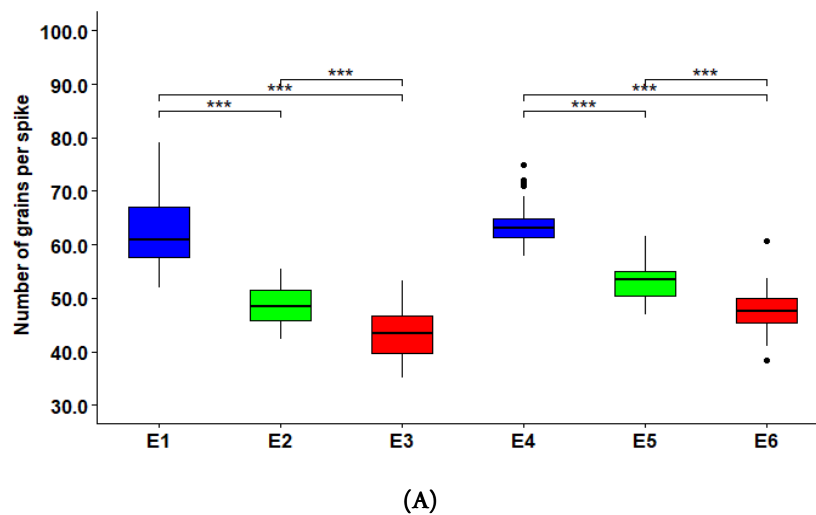
Source of variance	df	Number of grains per spike	1000 grains weight (g)	Weight of grains per spike (g)	Grain yield (t ha ⁻¹)
Environment (E)	5	7291**	11767**	52.10**	961.8**
Year (Y)	1	1951.4**	2479**	1.70**	23.25**
Heat treatment (H)	2	17120.2**	27994**	128.8**	2345**
Y × H	2	131.4**	183.8**	0.680**	47.57**
Genotype (G)	34	108.8**	81.20**	0.530**	3.312**
G × E	170	55.4**	47.60**	0.340**	2.453**
G × Y	34	113.1**	28.30**	0.890**	2.856**
G × H	68	42.7**	59.70**	0.220**	2.687**
G × Y × H	68	39.2**	45.20**	0.190**	2.017**
Error	418	11.859	19.78	0.127	0.450
Total	629	86.70	124.2	0.62	8.78

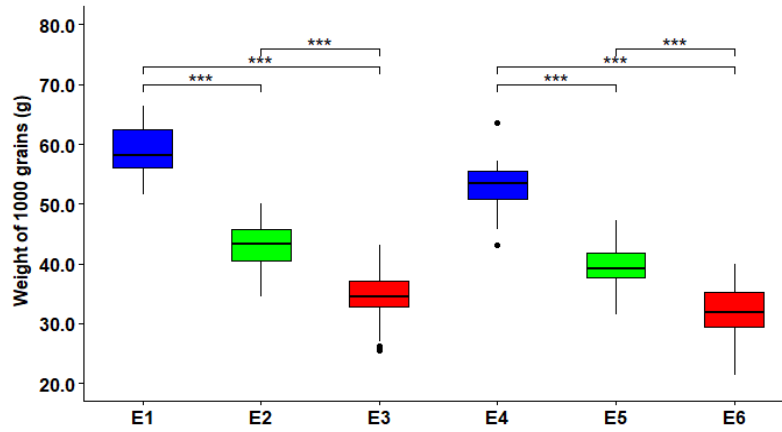
df: degrees of freedom, * : p-value < 0.05, and ** : p-value < 0.01

Mean performance

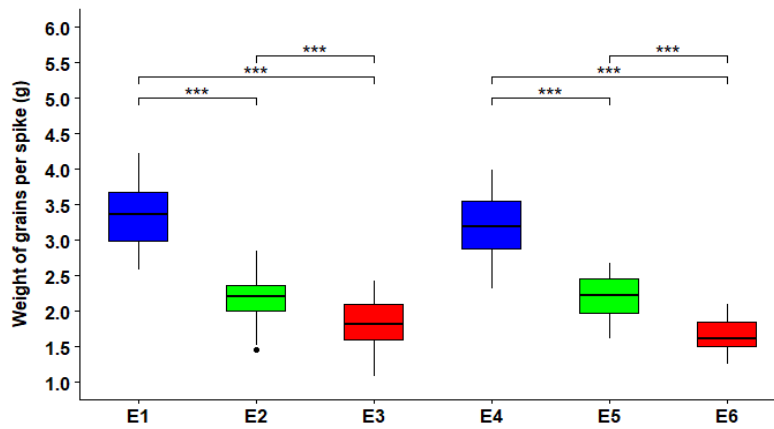
The key yield components for 35 bread wheat genotypes were evaluated under three heat stress treatments across two growing seasons. The treatments consisted of control (E1, E4), heat stress induced by plastic tunnel coverage for two weeks (E2, E5), and heat stress by tunnel coverage for four weeks (E3, E6). The duration of heat stress negatively affected yield traits in the wheat genotypes in both seasons, as presented in

Figure 1A-D. Significant reduction in number of grains per spike was observed under both heat stress treatments compared to the control in both seasons (Figure 1A). In the first season, the mean number of grains per spike among all genotypes decreased from 62.38 (E1, control) to 48.75 under two weeks of heat stress (E2; 21.85% reduction) and further to 43.23 under four weeks of heat stress (E3; 30.70% reduction) (Table 3). A similar trend was observed in the second season, where the general mean decreased from 63.96 (E4, control) to 53.18 (E5; 16.86% reduction) and 47.61 (E6; 25.57% reduction). Similarly, heat stress significantly reduced 1000-grain weight in all genotypes during both seasons, with the most severe reduction under four-week stress (Figure 1B). In the first season, the general mean of 1000-grain weight decreased from 59.09 g in control (E1) to 42.75 g after two weeks (E2), representing 27.64% reduction. This reduction increased under the four-week heat stress treatment (E3), where mean 1000-grain weight decreased by 41.49% compared to the control (Table 4). In the second season, 25.96% reduction was recorded under two-week heat stress (E5) compared to the control (E4), where the mean 1000-grain weight was 53.05 g in E4 and 39.28 g in E5. The four-week heat stress (E6) caused further decrease to 32.18 g (39.34% reduction) relative to the control. Grain weight per spike decreased significantly when heat stress increased (Figure 1C). In the first season, mean grain weight per spike decreased from 3.35 g (E1, control) to 2.18 g after two weeks of heat stress (E2), by 34.86% reduction. Under four weeks of heat stress (E3), the grain weight per spike further decreased to 1.82 g, representing 45.77% reduction compared to the control (Table 5). In the second season, grain weight per spike averaged 3.20 g under control (E4) but decreased to 2.21 g under E5 (two-week heat stress) by 30.88% reduction and 1.66 g under E6 (two-week heat stress) by 48.06% reduction compared to control treatments. Furthermore, heat stress induced by covering plots with plastic tunnels significantly reduced grain yield in both seasons (Figure 1D). Grain yield presented the greatest decline among the treatments studied with more prolonged heat exposure. In the first season, the mean grain yield was 10.07 t ha⁻¹ in control (E1), which reduced significantly to 4.03 t ha⁻¹ under two-week (E2) and to 3.01 t ha⁻¹ under four-week (E3) heat stress treatments, corresponding to reductions of 60.02% and 70.10%, respectively (Table 6). Similarly, in the second season, grain yield declined from 8.60 t ha⁻¹ in control (E4) to 4.04 t ha⁻¹ (E5 by 53.04% reduction) and 3.32 t ha⁻¹ (E6 by 61.44% reduction) under the two- and four-week heat treatments.

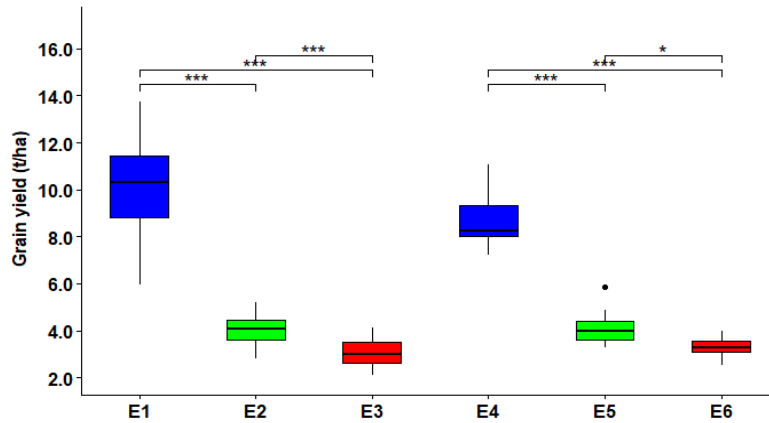




(B)



(C)



(D)

Figure 1. Boxplots for number of grains per spike (A), 1000-grain weight (B), weight of grains per spike (C), and grain yield (D) of 35 wheat genotypes were evaluated under normal and heat stress conditions across two growing seasons. E1-E6 are the tested environments as presented in Table 1. The colors of the boxplots represent the different heat stress treatments applied across both growing seasons: blue corresponds to the control treatment with no heat stress in both seasons, green indicates two weeks of heat stress, and red denotes four weeks of heat stress. The rectangles represent the interquartile range, the horizontal line inside each box marks the median.

Table 3. Number of grains per spike for 35 bread wheat genotypes under normal and heat stress conditions across two growing seasons. E1-E6 are the tested environments as presented in Table 1

Genotype	E1	E2	E3	E4	E5	E6	Average
G1	57.50	45.90	39.00	62.70	51.70	48.00	50.80
G2	55.80	50.90	40.00	57.80	53.70	50.00	51.37
G3	62.90	48.60	35.50	61.30	54.90	48.10	51.88
G4	60.90	48.00	43.50	63.50	53.40	42.30	51.93
G5	59.50	52.50	47.00	71.50	60.90	42.50	55.65
G6	61.40	45.20	42.00	66.00	51.70	45.30	51.93
G7	75.10	52.40	47.60	71.50	53.50	47.00	57.85
G8	72.00	43.00	35.00	61.40	48.00	41.40	50.13
G9	55.50	49.50	46.50	59.50	50.10	46.00	51.18
G10	53.10	47.00	44.00	61.50	54.90	45.50	51.00
G11	70.90	48.40	46.50	58.90	52.50	51.80	54.83
G12	70.00	50.50	48.00	64.90	49.60	38.40	53.57
G13	57.60	45.00	43.40	61.70	53.90	49.50	51.85
G14	65.00	47.50	45.00	63.50	50.50	46.50	53.00
G15	67.50	55.50	49.50	64.00	55.30	53.50	57.55
G16	53.60	51.90	37.00	62.20	47.00	42.50	49.03
G17	67.40	54.80	47.50	59.00	49.80	45.00	53.92
G18	79.00	49.30	38.00	60.60	48.90	47.50	53.88
G19	60.90	46.30	48.00	60.00	52.00	51.00	53.03
G20	64.70	42.20	41.00	71.50	59.50	50.50	54.90
G21	70.70	49.90	45.00	59.00	48.60	41.00	52.37
G22	52.50	45.70	39.50	63.70	53.50	46.50	50.23
G23	53.50	44.10	38.50	69.10	53.70	46.90	50.97
G24	60.30	48.60	38.60	64.00	55.50	48.00	52.50
G25	61.50	54.90	47.40	63.00	49.40	45.00	53.53
G26	64.70	52.70	48.00	63.50	54.53	49.00	55.41
G27	66.50	48.40	46.00	65.00	51.90	51.00	54.80
G28	58.00	44.20	36.00	62.70	53.40	50.00	50.72
G29	65.40	54.60	53.30	60.00	50.00	48.00	55.22
G30	60.80	49.50	46.50	61.50	51.40	45.50	52.53
G31	59.90	45.50	41.50	63.00	55.50	46.50	51.98
G32	60.90	44.50	40.00	71.00	57.20	53.60	54.53
G33	57.20	47.10	43.00	72.00	55.60	53.00	54.65
G34	52.00	47.10	42.00	63.20	57.70	49.40	51.90
G35	69.00	54.90	43.60	75.00	61.50	60.60	60.77
Mean	62.38	48.75	43.23	63.96	53.18	47.61	-
Reduction (%)		21.85	30.70	-	16.86	25.57	-
LSD g×e 0.05				5.53			

Table 4. Weight of 1000 grains (g) for 35 bread wheat genotypes under normal and heat stress conditions across two growing seasons. E1-E6 are the tested environments as presented in Table 1

Genotype	E1	E2	E3	E4	E5	E6	Average
G1	53.05	49.84	34.20	50.34	39.81	24.95	42.03
G2	51.54	41.42	34.16	56.75	40.34	27.98	42.03
G3	64.32	40.79	37.61	45.83	38.91	34.56	43.67
G4	62.47	47.58	34.54	55.06	45.67	28.78	45.68
G5	60.29	34.52	32.91	53.48	33.67	31.78	41.11
G6	55.39	45.63	27.00	49.65	46.11	21.33	40.85
G7	61.12	44.53	36.48	50.52	40.25	39.35	45.38
G8	53.78	44.01	33.43	52.78	31.53	29.59	40.85
G9	55.85	43.52	25.96	47.88	37.42	36.79	41.24
G10	59.55	48.71	32.25	52.21	45.90	34.66	45.55
G11	66.32	40.68	35.44	55.70	37.84	31.15	44.52
G12	63.78	49.99	43.19	52.28	37.89	35.08	47.04
G13	58.07	39.08	34.31	55.01	32.91	29.41	41.47
G14	56.52	40.51	27.81	48.43	33.34	31.02	39.61
G15	60.04	46.86	41.41	63.62	40.14	35.22	47.88
G16	57.42	46.12	40.68	57.23	41.90	39.85	47.20
G17	57.90	44.70	30.73	47.75	40.32	36.76	43.03
G18	60.56	44.57	34.47	55.59	43.77	31.44	45.07
G19	63.24	45.10	32.27	53.29	47.16	23.22	44.05
G20	64.44	40.33	38.62	43.04	38.47	38.91	43.97
G21	64.53	43.39	35.81	55.08	42.76	34.07	45.94
G22	64.51	36.73	32.89	54.49	38.49	28.79	42.65
G23	57.84	41.63	35.63	50.44	37.58	35.23	43.06
G24	57.53	47.03	26.17	52.60	39.09	35.22	42.94
G25	60.76	36.42	35.16	56.49	31.48	29.28	41.60
G26	55.49	41.99	35.56	54.54	41.89	31.78	43.54
G27	53.66	39.72	38.42	52.75	32.70	26.94	40.70
G28	65.78	44.57	25.51	53.68	39.09	30.51	43.19
G29	62.57	47.41	36.59	51.08	41.62	37.14	46.07
G30	55.12	43.10	42.25	56.65	33.19	28.75	43.18
G31	54.29	45.98	40.32	55.83	44.54	39.35	46.72
G32	56.26	37.56	32.95	54.34	39.90	30.82	41.97
G33	59.62	40.92	38.09	50.93	42.47	32.28	44.05
G34	57.42	35.29	32.63	56.01	38.47	29.85	41.61
G35	56.99	36.08	34.59	55.43	38.04	34.49	42.60
Mean	59.09	42.75	34.57	53.05	39.28	32.18	-
Reduction (%)		27.64	41.49	-	25.96	39.34	-
LSD $g \times e_{0.05}$	6.14						

Table 5. Weight of grains per spike (g) for 35 bread wheat genotypes under normal and heat stress conditions across two growing seasons. E1-E6 are the tested environments as presented in Table 1

Genotype	E1	E2	E3	E4	E5	E6	Average
G1	3.19	1.85	1.26	3.17	2.43	1.53	2.24
G2	2.59	1.55	1.37	3.77	2.62	1.91	2.30
G3	3.64	1.45	1.08	3.06	2.41	1.67	2.22
G4	2.91	1.52	1.39	3.5	2.29	1.81	2.24
G5	3.14	1.76	1.19	3.42	2.17	1.49	2.20
G6	3.48	2.26	2.24	3.01	1.97	1.27	2.37
G7	4.10	2.28	1.71	2.74	1.97	1.53	2.39
G8	2.71	2.09	1.60	2.69	1.96	1.34	2.07
G9	3.33	2.85	2.18	2.57	1.62	1.30	2.31
G10	3.11	2.36	2.17	3.48	2.33	2.01	2.58
G11	3.79	2.18	1.86	3.2	2.22	1.36	2.44
G12	4.15	2.39	1.77	2.58	1.86	1.60	2.39
G13	3.35	1.88	1.68	2.94	1.81	1.52	2.20
G14	2.83	2.75	2.43	2.32	1.66	1.25	2.21
G15	3.59	2.61	2.28	2.81	2.44	1.82	2.59
G16	3.01	2.15	1.83	3.35	2.37	1.89	2.43
G17	3.70	2.49	2.03	2.97	2.47	1.91	2.60
G18	3.95	1.93	1.81	3.62	2.56	1.34	2.54
G19	3.89	1.95	1.59	3.84	2.40	1.88	2.59
G20	3.70	2.10	1.75	3.01	1.96	1.62	2.36
G21	3.06	2.27	1.54	2.49	2.03	1.68	2.18
G22	2.71	2.21	1.56	3.99	2.67	2.08	2.54
G23	2.95	2.07	1.88	3.60	2.51	1.97	2.50
G24	3.47	2.49	2.35	3.31	2.41	1.78	2.64
G25	3.95	2.29	2.10	3.16	2.05	1.59	2.52
G26	2.96	2.20	1.60	2.52	1.82	1.40	2.08
G27	3.01	2.14	2.09	3.62	2.19	1.71	2.46
G28	4.23	2.36	1.84	3.36	1.93	1.54	2.54
G29	3.38	2.85	2.15	3.33	2.50	1.61	2.64
G30	3.45	2.01	1.75	3.14	2.07	1.59	2.34
G31	3.36	2.40	2.23	3.64	2.47	1.85	2.66
G32	3.49	1.99	1.79	3.86	2.54	2.10	2.63
G33	2.91	2.27	1.96	3.6	2.63	1.86	2.54
G34	2.78	2.29	1.59	2.82	1.93	1.50	2.15
G35	3.47	2.20	1.98	3.45	2.10	1.83	2.51
Mean	3.35	2.18	1.82	3.20	2.21	1.66	-
Reduction (%)		34.86	45.77	-	30.88	48.06	-
LSD $g \times e$ $_{0.05}$	1.08						

Table 6. Grain yield ($t\ ha^{-1}$) for 35 bread wheat genotypes under normal and heat stress conditions across two growing seasons. E1-E6 are the tested environments as presented in Table 1

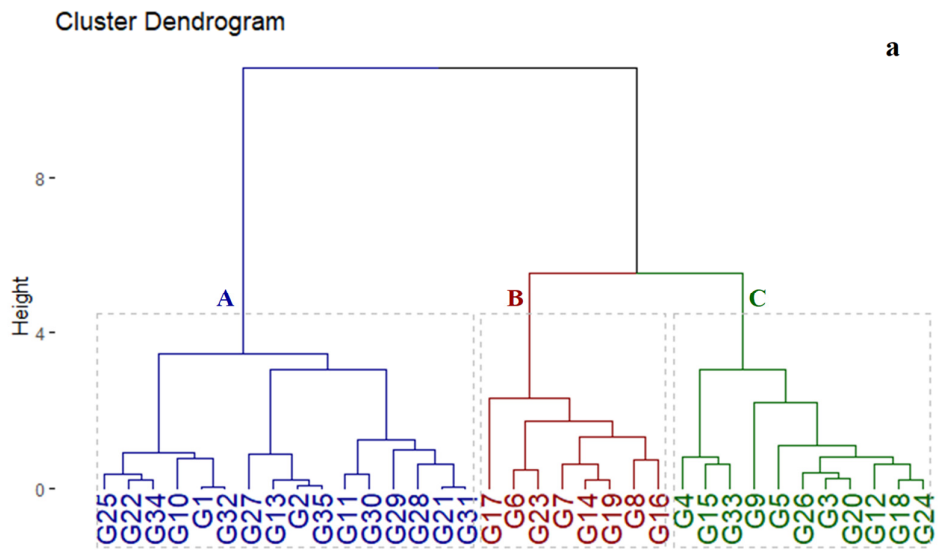
Genotype	E1	E2	E3	E4	E5	E6	Average
G1	11.88	4.75	3.22	7.30	3.76	2.90	5.64
G2	9.60	3.83	3.52	9.89	4.01	3.43	5.71
G3	8.75	3.45	2.50	7.42	3.92	3.28	4.89
G4	7.00	3.65	2.82	8.96	4.30	3.82	5.09
G5	9.83	3.23	2.10	7.55	3.37	2.54	4.77
G6	10.45	2.98	2.47	8.05	4.44	2.93	5.22
G7	11.30	2.88	2.62	7.43	3.51	3.41	5.19
G8	9.00	4.40	3.00	8.38	4.48	3.80	5.51
G9	8.35	3.65	1.75	7.85	4.86	3.28	4.96
G10	13.05	3.40	2.95	7.21	4.00	3.64	5.71
G11	10.30	4.37	3.65	9.40	4.10	3.23	5.84
G12	9.65	2.80	2.45	8.01	4.26	3.73	5.15
G13	9.50	4.22	3.63	8.89	3.80	3.17	5.54
G14	10.93	5.03	2.88	8.35	4.16	3.78	5.86
G15	5.95	4.22	3.28	9.24	4.50	3.73	5.15
G16	9.80	4.05	3.15	8.13	4.78	3.98	5.65
G17	13.75	3.55	2.23	11.04	3.53	3.40	6.25
G18	8.85	4.00	2.60	8.79	3.57	2.68	5.08
G19	10.55	3.68	2.92	8.31	4.36	3.85	5.61
G20	8.30	3.78	2.52	7.99	4.41	2.96	4.99
G21	11.65	4.90	3.73	8.24	4.41	3.48	6.07
G22	11.95	3.30	3.02	9.97	5.85	3.81	6.32
G23	11.35	3.93	2.33	9.43	3.38	2.90	5.55
G24	8.45	3.60	2.67	10.72	3.59	3.45	5.41
G25	11.68	3.88	2.95	8.85	3.28	3.11	5.63
G26	8.25	4.72	2.40	8.23	3.62	3.23	5.08
G27	8.40	4.37	3.83	8.03	3.74	3.48	5.31
G28	11.30	4.52	3.58	8.25	3.90	3.36	5.82
G29	10.63	4.47	4.12	9.64	4.46	3.39	6.12
G30	10.40	4.60	3.80	8.10	4.17	3.17	5.71
G31	11.60	4.70	3.75	10.06	3.36	2.64	6.02
G32	11.90	4.35	3.23	10.05	4.52	3.16	6.20
G33	7.00	4.05	3.13	7.38	3.74	3.24	4.76
G34	11.55	4.47	3.12	8.20	3.50	3.08	5.65
G35	9.70	5.20	3.52	7.82	3.81	3.09	5.52
Mean	10.07	4.03	3.01	8.60	4.04	3.32	-
Reduction (%)		60.02	70.10	-	53.04	61.44	-
LSD $g \times e_{0.05}$				1.06			

Substantial differences among genotypes were detected for number of grains per spike (Table 3). The genotypes G7, G15, G18, G32, G33, and G35 produced higher number of grains per spike under both normal and stressed conditions across both seasons. Under the most severe stress (four-week tunnel coverage), the genotypes G15, G29, G32, G33, and G35 maintained relatively high grain numbers. In addition, significant genotypic differences were observed in 1000-grain weight under both normal and stressed conditions (Table 4). Genotype G11 recorded the highest 1000-grain weight (66.32 g), followed by G28 (65.78 g), under normal conditions in the first season. Under heat stress, the genotypes G15, G16, and G29 displayed better tolerance by maintaining higher values of 1000-grain weight. Likewise, the assessed genotypes exhibited significant

differences in their potential for grain weight per spike under both conditions (Table 5). The genotypes G11 and G7 produced higher grain weight per spike values under both normal and heat stress compared to genotypes such as G3 and G5. Furthermore, significant genotypic variation was detected for grain yield under normal and heat stress treatments in both seasons (Table 6). The genotypes, G10, G17, G21, G22, G25, and G32 produced the highest grain yield under control conditions. These genotypes under heat stress showed significant yield reduction but still surpassed several genotypes. Moreover, the genotype G29 demonstrated better grain yield stability with values of 4.12 t ha⁻¹ (E3) and 3.39 t ha⁻¹ (E6), while genotypes like G5 displayed sharper reductions. The genotype G16 showed moderate resilience to heat stress, with yields close to or higher than the mean in both stressed treatments, suggesting potential tolerance mechanisms.

Genotypic classification based on heat tolerance indices

The hierarchical cluster analysis of the evaluated 35 wheat genotypes based on the heat tolerance indices (Table A4) across two studied seasons is presented in Figure 2A-B. The cluster revealed distinct patterns of genetic divergence among assessed wheat genotypes based on mean productivity, stress tolerance index, and yield index. The genotypes were grouped into three main clusters in the first season (Figure 2A). Group A comprised the majority of high-performing (G25, G22, G34, G10, G1, G32, G27, G13, G2, G35, G11, G30, G29, G28, G21, and G31) which exhibited superior values for heat tolerance indices. Group B contained genotypes with moderate tolerance and intermediate performance under heat stress (G17, G6, G23, G7, G14, G19, G8, and G16). Group C included the most heat-sensitive genotypes with the lowest index values (G4, G15, G33, G9, G5, G26, G3, G20, G12, G18, and G24). In the second season (Figure 2B), similar grouping trend was observed, although some shifts in genotype placement. Cluster A comprised the genotypes G2, G29, G11, G32, G17, G24, G22, G4, and G15. Cluster B included G12, G16, G19, G8, and G14. Cluster C consisted of G13, G25, G23, G31, G10, G28, G21, G27, G30, G34, G9, G26, G3, and G33. Finally, cluster D contained G1, G5, G18, G35, G6, and G20. Certain genotypes demonstrated consistent tolerance across both seasons and were classified in the same Group A (highly tolerant genotypes), such as G2, G22, and G32. In contrast, some genotypes shifted from the highly tolerant group in the first season (Group A) to the moderately tolerant group in the second season (Group C), such as G25, G34, G10, G27, and G13. Otherwise, G1 and G35 moved from the highly tolerant group (Group A) in the first season to the sensitive group in the second season (Group D), demonstrating that GEI had a significant role in their performance.



(A)

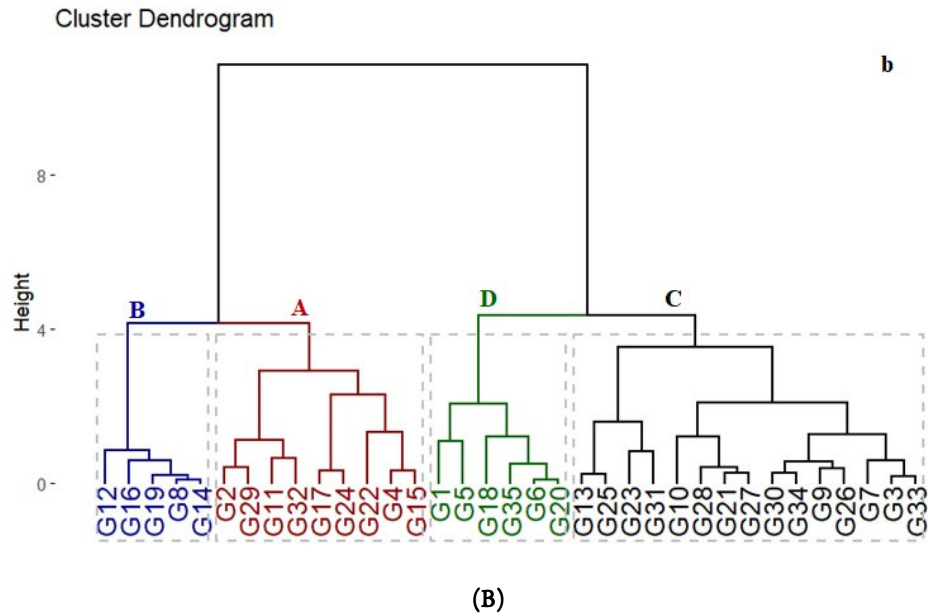


Figure 2. Dendrogram depicting phenotypic distances among 35 wheat genotypes based on heat tolerance indices under first (A) and second seasons (B).

Heatmap and hierarchical clustering

The heatmap presented in Figure 3 grain yield performance of 35 wheat genotypes (G1-G35) evaluated across six environments (E1-E6). The color scale reflects relative yield level, with blue color indicating higher performance and red color revealing lower yield values. A clear variation was observed between environments. Among the genotypes, G29, G32, G2, and G22 consistently exhibited blue color across most environments, suggesting stable and superior performance under both normal and heat stress conditions. In contrast, G5, G3, G20, G33, and G18 displayed red color across most environments. Certain genotypes, such as G23, displayed strong performance under normal conditions but showed marked decline under heat stress environments. Moreover, some genotypes, such as G17 and G21, maintained moderate performance levels across environments. Beyond the color gradient, cluster analysis reveals distinct groupings among both genotypes and environments. The dendrograms clearly show clusters of genotypes with similar response patterns, such as those grouped toward the top (e.g., G22, G17, and G24), indicating shared trait and performance profiles across environments. Similarly, the environments are clustered into groups with comparable responses in the genotypic data. This clustering offers valuable insight into genotype-environment interactions, highlighting which genotypes are most similar in their reaction to specific stress conditions, and allowing for the identification of groups with higher or lower performance under different environments.

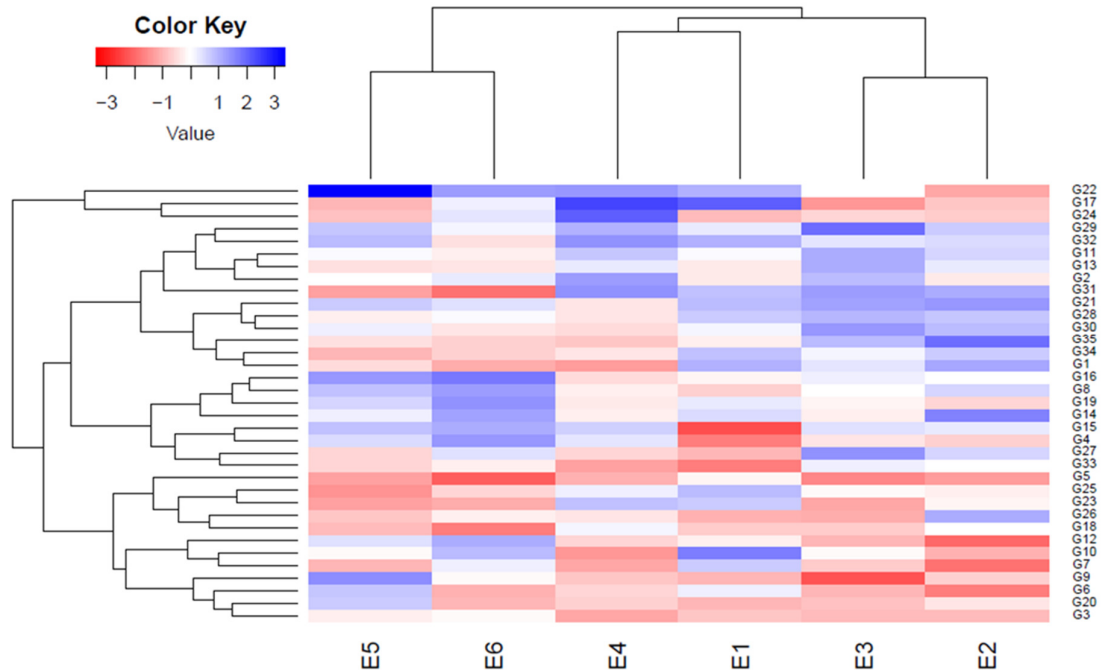


Figure 3. Heatmap of grain yield performance of 35 wheat genotypes across six environments under normal and heat stress conditions

Yield stability

The AMMI1 biplot for grain yield of 35 wheat genotypes (G1-G35) evaluated under six environments (E1-E6), which included normal and heat stress conditions across two growing seasons, is presented in Figure 4. The biplot displays the relationship between grain yield and the first principal component axis (PC1) for evaluated wheat genotypes and tested environments. Genotypes G17, G22, G32, G31, G21 and G29 exhibited high mean yields. The genotypes were positioned close to the origin, such as G12, G6, G19, and G30 displayed relatively stable performance across environments, meaning their yields were less influenced by environmental fluctuations. In contrast, the genotypes G17, G15, G10, G4, and G33 displayed substantial interaction effects on PC1, indicating sensitivity or specific adaptation to certain environments. Among the environments, E1 and E4 exhibited high mean yield and high interaction, indicating that these environments were favorable for wheat productivity and highly discriminating among genotypes. Conversely, environments E3, E6, E5, and E2 were located together with lower yields and less interaction effects. Furthermore, the AMMI2 biplot displays the relationship between the first two principal component axes (PC1 and PC2) for evaluated wheat genotypes and tested environments, as presented in Figure 5. AMMI2 biplot indicates significant interaction pattern between genotypes and environments, providing insights into genotype adaptability and stability in response to heat stress. Genotypes located close to the origin with low scores on the first two principal components exhibited overall stability. Conversely, genotypes with higher PC scores displayed larger responses to specific environments, indicating either high adaptability to particular conditions with positive contribution to yield traits under certain environments or instability. The biplot exhibits that the first two PCs explained 83.44% of the total variation (PC1 = 63.40%, PC2 = 20.04%). The genotypes G12, G5, G6, G19, G13, G29, G11, and G25 are positioned close to the origin. In contrast, genotypes such as G1, G4, G10, G15, G17, G24, and G33 are located farther away from the origin, each in a different direction relative to the environmental vectors (E1–E6). These genotypes displayed higher sensitivity to environmental conditions and instability depending on the environmental conditions. Certain of these genotypes indicate specific adaptability and potential for higher performance in particular environments that are aligned with their positions. The position of G24 on the very close right side of the environment E4 indicates specific adaptability and potential for higher performance in

this environment. The relative positioning of the tested environments (E1-E6) on the biplot further identifies which environments are most conducive to high yields and which impose greater stress. The environments E1 and E4 are distinctly separate from the other environments, indicating they have a significantly different interaction effect with the genotypes. These two environments, representing control conditions with no heat stress in different seasons, are generally more favorable for genotype performance. In contrast, the other environments (E2, E3, E5, E6), which correspond to heat stress treatments, display greater dispersion from E1 and E4, illustrating strong genotype-by-environment interactions caused by heat stress. This separation highlights the significant impact of heat stress on genotype performance and confirms the AMMI model ability to distinguish environments with contrasting stress levels and their effects on genotype stability. Furthermore, the GGE biplot in Figure 6 illustrates the relationship between the evaluated genotypes and tested environments. The biplot captured 78.89% of the total variation, with PC1 explaining 60.22% and PC2 accounting for 18.67% of the variability. The genotypes G17, G24, G15, G33, and G4 were positioned on the border of the biplot, indicating significant interactions and responses to specific environments. The genotype G17 and G10 indicated specific adaptability and potential for higher performance in E1. Genotypes G17 and G24 have specific adaptability and potential for higher performance in E4. The genotypes G16, G35, G12, G19, G30, G6, and G13 were grouped close to the origin, indicating higher stability across environments. The environments E1 and E4 were positioned distinctly apart, revealing their roles as discriminating conditions where genotypic performance varies most significantly.

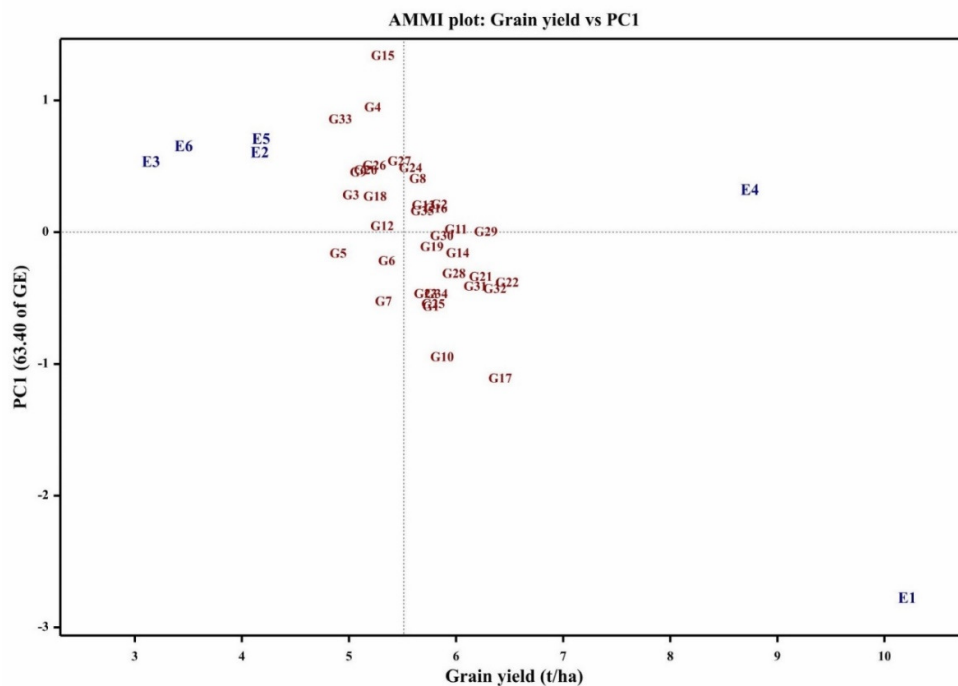


Figure 4. AMMI1 biplot for grain yield and interaction principal component (PC1) of 35 wheat genotypes (G1-G35) were tested in six environments, including control and heat stress conditions across two growing seasons. E1-E6 are the tested environments as presented in Table 1

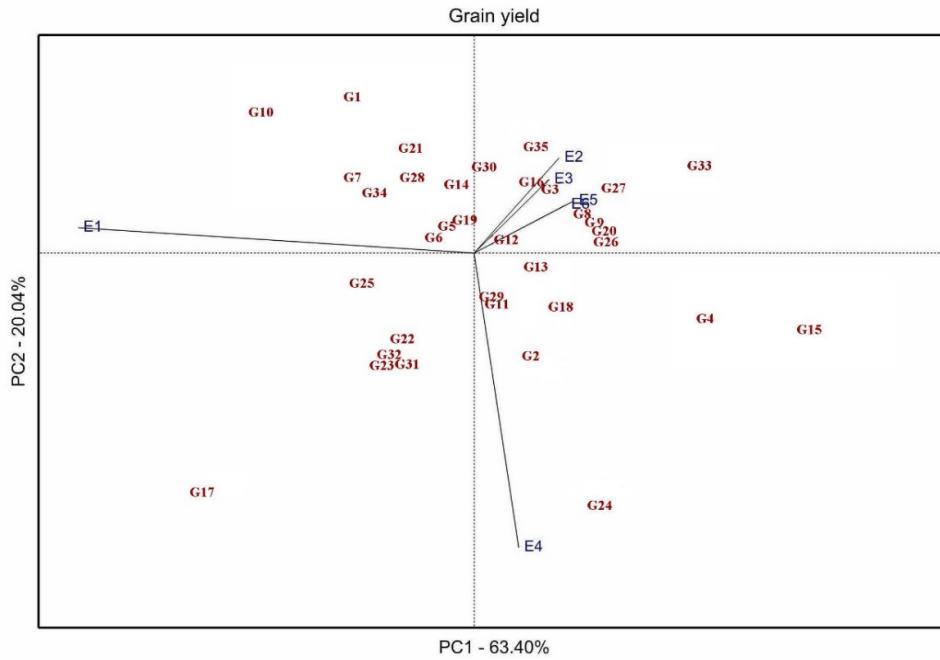


Figure 5. AMMI biplot for grain yield of 35 wheat genotypes (G1-G35) was tested in six environments, including control and heat stress conditions across two growing seasons. E1-E6 are the tested environments as presented in Table 1

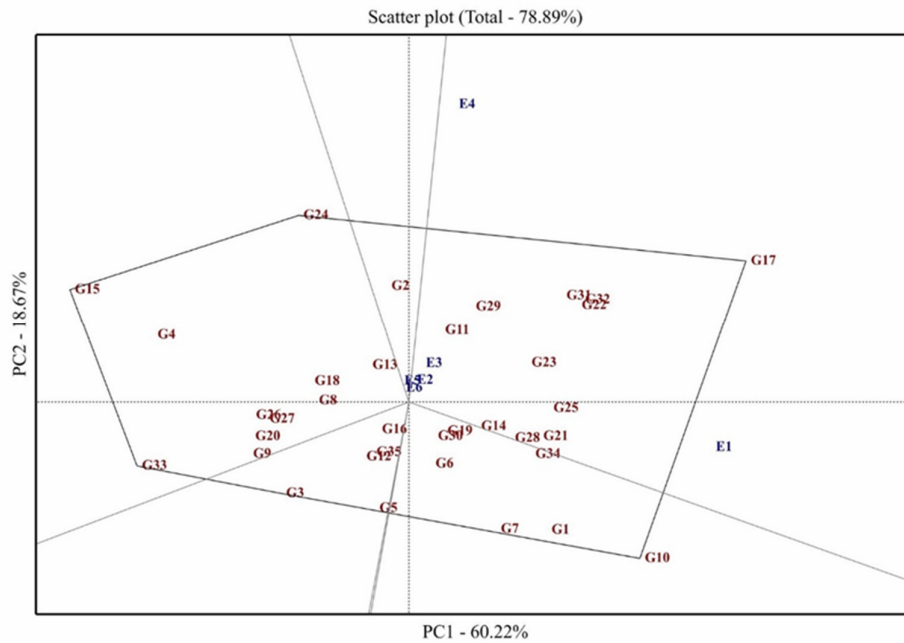


Figure 6. GGE biplot for grain yield of 35 wheat genotypes (G1-G35) was tested in six environments, including control and heat stress conditions across two growing seasons. E1-E6 are the tested environments as presented in Table 1

Correlation analysis of grain yield and its attributing traits under normal and heat stress conditions

Correlation coefficients among grain yield and its key components, number of grains per spike, 1000-grain weight, weight of grains per spike, under normal and heat stress conditions, are presented in Table 7. Under normal conditions without heat stress (E1 and E4), grain yield was negatively correlated with number of grains per spike, but positively associated with 1000-grain weight and weight of grains per spike. Significant positive correlations were observed between the weight of grains per spike and the number of grains per spike and 1000-grain weight. Under heat stress for two weeks (E2, E5), 1000-grain weight exhibited significant negative correlation with number of grains per spike, while its association with grain yield was not significant. The correlation between weight of grains per spike and 1000-grain weight remained significantly positive. Under more intensive heat stress for four weeks of heat stress (E3, E6), the correlations among all measured traits were weak and statistically non-significant, with the highest values observed between 1000-grain weight and grain yield. These results demonstrate that prolonged exposure to high temperatures disrupts the relationships among yield components.

Table 7. Correlation coefficients among grain yield and its components of the evaluated wheat genotypes were evaluated under six environments, including normal (E1-E4), heat stress for two weeks (E2, E5), and heat stress for four weeks (E3, E6)

Trait	Number of grains per spike	1000 grains weight	Weight of grains per spike
Under control treatment with no heat stress (E1 and E4)			
1000 grains weight	-0.04 ns	-	-
Weight of grains per spike	0.23**	0.32**	-
Grain yield	-0.21*	0.23**	0.13ns
Under heat stress for two weeks (E2, E5)			
1000 grains weight	-0.19*	-	-
Weight of grains per spike	0.08ns	0.28**	-
Grain yield	-0.06ns	-0.04ns	0.04ns
Under heat stress for four weeks (E3, E6)			
1000 grains weight	-0.05ns	-	-
Weight of grains per spike	0.08ns	0.02ns	-
Grain yield	0.03ns	0.09ns	-0.02ns

Discussion

Wheat production system faces the dual challenge of meeting rising global demand while coping with climate change-driven yield instability. The present study evaluated the performance of 35 bread wheat genotypes under normal and field-induced heat stress conditions across two growing seasons. The results highlighted significant genetic variability for grain yield and its components and significant GEI, indicating potential for breeding heat-resilient wheat cultivars. High temperature stress during grain filling significantly reduced number of grains per spike, 1000-grain weight, grain weight per spike, and grain yield. This is in agreement with studies of Fernie *et al.* (2022); Ullah *et al.* (2022); Singh *et al.* (2024); Li *et al.* (2025) who demonstrated that post-anthesis heat accelerates leaf senescence, shortens the grain-filling period, and disrupts carbohydrate translocation, ultimately decreasing yield potential and grain quality. In the present study, yield component reduction under the most severe treatment (four-week tunnel coverage) ranged from approximately 25% to 70%, depending on the trait, confirming the high sensitivity of wheat reproductive processes to sustained heat stress. Among yield components, 1000-grain weight and grain weight per spike were particularly sensitive, with several genotypes exhibiting marked declines under heat stress. This aligns with earlier observations of Wu *et al.* (2018); Jacott and Boden (2020); Chaubey *et al.* (2021); Han *et al.* (2025),

who reported that grain weight is one of the most temperature-sensitive traits in wheat, due to its dependence on the duration of grain filling.

Identification of genotypes combining high yield potential with heat tolerance is essential in breeding for heat resilience. Genotypes such as G7, G15, G18, G29, G32, G33, and G35 exhibited superior performance in maintaining a high number of grains per spike under both normal and heat stress conditions, indicating their potential for stable yield components under stress. Similarly, genotypes G15, G16, and G29 showed good stability in 1000-grain weight, producing higher grain weight under heat stress compared to sensitive genotypes, emphasizing their value in breeding programs for stability of grain index. Regarding weight of grains per spike, genotypes G7 and G11 performed well, maintaining higher grain weight per spike under both control and stress environments, whereas genotypes like G3 and G5 experienced significant reductions under stress. For grain yield, genotypes G10, G17, G21, G22, G25, and G32 recorded high yields under normal conditions but suffered considerable declines under heat stress. In contrast, genotypes G14, G16, and G29 demonstrated moderate to high grain yield performance under heat stress. This reflects their consistent ability to maintain acceptable grain yield under heat stress conditions, indicating their resilience to environmental fluctuations during grain filling. The stable performance of these genotypes under heat stress conditions aligns with Gupta *et al.* (2020); Bapela *et al.* (2022); Paux *et al.* (2022) who reported that wheat genotypes with the potential to sustain yield components under stress are promising candidates for breeding programs.

The dendrogram is a vital tool in hierarchical clustering analysis, offering a visual representation of the relationships and similarities among genotypes based on multiple traits or performance indices (Salem *et al.*, 2020; Hassanin *et al.*, 2025). It organized the assessed genotypes into clusters based on their tolerance profiles and performance across multiple indices (Omar *et al.*, 2022). Group A appeared as a distinct cluster containing genotypes that consistently demonstrated superior performance in heat tolerance. These genotypes exhibited a robust capacity to maintain performance under heat stress, reflecting strong genetic mechanisms conferring resilience. The clear separation of Group A genotypes from more sensitive clusters emphasizes their potential as elite candidates for breeding programs focused on enhancing heat tolerance in wheat. Therefore, the dendrogram illustrates relationships among assessed genotypes and also aids in selecting promising genotypes based on their clustering patterns, providing a powerful framework for understanding the structure of heat tolerance traits within the germplasm. The heatmap analysis provides further visual grouping of genotypes based on yield responses across environments (Mansour *et al.*, 2023; Abd-El-Aty *et al.*, 2024). Genotypes such as G29, G32, G2, and G22 consistently appeared in high-performance clusters with blue colors under both normal and stressed conditions, reflecting broad adaptability as reported by Al-Ashkar *et al.* (2022); Gupta *et al.* (2022); Ninou *et al.* (2023); Raffo and Jensen (2023). By contrast, G5, G3, G20, G33, and G18 were consistently low-performing across environments with red colors. Certain genotypes, such as G23, despite high performance under control conditions, were highly sensitive to prolonged heat stress, confirming the importance of screening for yield potential and stability, as elucidated by Liu *et al.* (2017); Gerard *et al.* (2020); Wang *et al.* (2023).

Stability under stress is an important breeding objective in heat-prone regions where inter-annual climatic variability is high (Li *et al.*, 2022). The detected variability revealed the complex mechanisms underlying heat tolerance in wheat and supports the importance of selecting genotypes exhibiting both component stability and yield resilience for effective breeding of heat-tolerant varieties (Langridge and Reynolds, 2021; Khanzada *et al.*, 2025). The highly significant GEI detected across all yield traits confirms that genotype performance under heat stress is strongly influenced by environmental conditions, revealing the environment-specific nature of heat tolerance (Aziz *et al.*, 2018; Al-Ashkar *et al.*, 2022). AMMI and GGE biplots provide comprehensive visualization of genotype-by-environment interactions for wheat yield performance under varying experimental conditions (Mansour *et al.*, 2018). The clear distinction observed between environments and genotypes in the plot indicates the pivotal role of both genetic potential and environmental influences in determining final yield. AMMI1 and AMMI2 identified E1 and E4 as favorable

environments, supporting high yields and contributing strongly to genotype differentiation. Genotype situation along the PC axes indicates yield and stability. AMMI1 displayed that G17, G22, G32, G31, G21 and G29, had high grain yields. In contrast, genotypes clustering close to the origin, including G12, G6, G19, and G30, displayed relative stability and general adaptability across environments. This differentiation is instrumental for breeding programs aiming to combine high yield potential with broad adaptation or, alternatively, to identify candidates for niche or stress-prone settings. Moreover, AMMI2 biplot identified certain genotypes such as G12, G5, G6, G19, G13, G29, G11, and G25 located close to the origin, reflecting their stability across environments. These genotypes combined high yield with stability across environments. In contrast, genotypes like G1, G4, G10, G15, G17, G24, and G33 showed higher interaction effects, indicating greater sensitivity and instability under varying conditions. The general instability observed for these genotypes was mainly due to their narrow adaptability to specific environmental conditions. These genotypes exhibit specific adaptation rather than broad adaptability, performing well under certain environments but showing sensitivity and poorer performance in others, which manifests as higher interaction effects and instability across the diverse conditions tested. Achieving stability under heat stress is a key breeding target in regions prone to frequent and unpredictable climatic fluctuations (Langridge and Reynolds, 2021; Arif *et al.*, 2025). Besides, the GGE biplot captured a substantial proportion of total variation (78.89%), indicating that the principal components effectively represent the dataset. Genotypes G17, G24, G15, G33, and G4, positioned at the vertices of the polygon, correspond to particular environments. This suggests potential suitability for specific environmental conditions, such as G17 and G10 in E1 and G17 and G24 in E4. Otherwise, genotypes located close to the origin, such as G16, G35, G12, G19, G30, G6, and G13, demonstrate more stable performance across the tested environments, exhibiting lower sensitivity to environmental variation. The differentiation of environments such as E1, E4 further highlights their role in discriminating among genotypes, which is crucial for identifying adaptive or broadly performing genotypes. Sector analysis of the polygon structure allows identification of winning genotypes within each environment or group of environments, supporting breeders in targeting genotypes for specific adaptation or general adaptability. The clear separation between genotypes and environments, as revealed by the GGE biplot, underscores the practical value of this approach in multi-environment trials and crop improvement programs. This analysis can guide the selection process by pinpointing stable, high-yielding genotypes and tailoring recommendations for wheat production in heterogeneous environments. Elbasyoni (2018); Al-Ashkar *et al.* (2023); Lamba *et al.* (2023); Ahmad *et al.* (2025) applied comparable approaches to identify promising stable genotypes under heat stress.

Correlation analysis is a fundamental statistical tool to explore relationships among traits, providing critical insights into how different yield components interact under varying environmental conditions (Gharib *et al.*, 2021; Zannat *et al.*, 2023). The correlation analysis revealed important differences in the relationships among grain yield and its key components under normal and heat stress conditions. Under normal and non-stressed conditions, positive and significant correlation between the weight of grains per spike and between the number of grains per spike and 1000-grain weight (Swailam *et al.*, 2021). This indicates efficient assimilate partitioning and coordinated development of yield components, consistent with optimal conditions favoring grain filling and yield formation (Alshaharni *et al.*, 2025). While the negative correlation between grain yield and number of grains per spike likely reflects trade-offs where increases in grain number may coincide with reductions in individual grain size or weight due to source-sink limitations (Zhang *et al.*, 2019). Under heat stress induced for two weeks, these relationships with the weight of grains per spike remained positively correlated with 1000-grain weight. This suggests that moderate heat stress allowed some preservation of grain filling processes despite stress-related limitations. However, the negative correlation of 1000-grain weight with number of grains per spike and the diminished association of grain yield with yield components indicate disruptions in reproductive and grain development caused by heat stress. Elevated temperatures during anthesis and grain filling reduce pollen viability, accelerate leaf senescence, and shorten the grain-filling duration, resulting in fewer grains and lower grain weight. These constraints explain the weakening of trait relationships under heat stress. Prolonged heat stress for four weeks led to further deterioration of the correlations, with

most associations becoming non-significant. These results indicate that heat stress reduces yield components and alters the fundamental relationships by disrupting developmental and physiological processes critical to yield formation (Sehgal *et al.*, 2018). This supports the concept that breeding for heat tolerance should target genotypes maintaining trait relationships under thermal stress, reflecting robust assimilate partitioning and reproductive resilience.

Conclusions

This study demonstrated significant genetic variation among 35 bread wheat genotypes in their responses to heat stress across two growing seasons. High temperature stress during the grain-filling stage caused significant reductions in grain number per spike, 1000-grain weight, grain weight per spike, and grain yield, with the magnitude of reduction depending on genotype and stress duration. Integrating stability analyses from AMMI biplots and hierarchical clustering allowed the identification of genotypes with broad adaptability. Certain genotypes, such as G11, G13, G15, G16, G29, and G25, and G32, exhibited high yield potential and stability under both normal and heat stress conditions, indicating their adaptability could be exploited to sustain productivity under high temperatures. Conversely, other genotypes exhibited high performance under normal conditions, such as G13 and G23, but were more sensitive to prolonged heat stress. The identified high-performing and stable genotypes are promising candidates for direct cultivation in heat-prone regions and can be exploited as valuable parents in breeding programs aiming to develop heat-tolerant wheat cultivars. The pronounced disruption of correlations among key yield components under prolonged heat stress indicates the complex challenges wheat faces. Developing wheat genotypes that can preserve strong, positive inter-trait relationships under heat stress is essential for breeding resilient cultivars capable of sustaining stable yields amid increasing global temperatures.

Authors' Contributions

Conceptualization: EA, ASME, NQ, AME, MMAA, ASME, NQ, AME, MMAA, FAS, ASJ, SMA, EM. Validation: EA, ASME, NQ, AME, MMAA, FAS, ASJ, SMA, EM. Formal analysis: EA, ASME, NQ, AME, MMAA, FAS, ASJ, SMA, EM. Investigation: EA, ASME, NQ, AME, MMAA, EM. Resources: EA, ASME, NQ, AME, MMAA, FAS, ASJ, SMA, EM. Data curation: EA, ASME, NQ, AME, MMAA, FAS, ASJ, SMA, EM. Writing original draft preparation: EA, ASME, NQ, AME, MMAA, FAS, ASJ, SMA, EM. Writing review and editing: EA, ASME, NQ, AME, MMAA, FAS, ASJ, SMA, EM. Supervision EA, ASME, NQ, AME, MMAA, EM. Funding acquisition EA, ASME, NQ, AME, MMAA, FAS, ASJ, SMA, EM.

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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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Appendix

Table A1. Pedigree of the evaluated 35 bread wheat genotypes

Code	Name	Pedigree
G1	'Misr 1'	Oasis/SKAUZ//4*BCN/3/2*PASTOR.CMss00Y01881T-050M-030Y-030M-030WGY-33M-0Y-0S
G 2	'Misr2'	SKAUZ / BAV92.CMss96M03611S-1M-0105Y-010M-010SY-8M-0Y-0S.
G 3	'Shandaweel 1'	SITE//MO/4/NAC/TH.AC//3*PVN/3MIRLO/BUC.CMSS93B00567S-72Y-010M-010Y-010M-0HTY-0SH.
G 4	'Sids 12'	BUC//7C/ALD/5/MAYA74/ON//1160.47/3/BB/GLL/4/CHAT"S"/6/MAYA/VU L//CMH74A.630/4/*5X.
G 5	'Sids 14'	SW8488*2/ KUKUNA- CGSS01Y00081T-099M-099Y-099M-099B-9Y-0B-0SD
G 6	'Gemmeiza 9'	ALD "S" HUAC "S" // CMH74A.630/5XCGM4583-5GM-1GM 0GM
G 7	'Gemmeiza 11'	Bow"s"/Kz"s"//7C/aeri 82/3/Giza 168/Sakha 61. GM78922-GM-1GM-2GM-1GM-0GM.
G 8	'Gemmeiza 12'	OTUS/3/SARA/THB//VEE.CCMSS97Y00227S-5Y-010M-010Y-010M-2Y-1M-0Y-0GM
G 9	'Giza 168'	MIL/BUC//Seri:CM93046-8m-oy-om-2y-OB.
G 10	'Giza 171'	SAKHA 93 / GEMMIEZA 9 S.6-1GZ-4GZ-1GZ-2GZ-0S N.S.732/Pim/Vee"S"
G 11	'Line 4'	BABAX/LR42//BABAX*2/3/BRAMBLING/
G 12	'Line 15'	WBLLI*2/BRAMBLING
G 13	'Line 18'	WBLLI*2/KIRITATI
G 14	'Line 27'	ICB91-0539-7APP-0AP-3AP-0AP
G 15	'Line 29'	ICB97-0905-0AP
G 16	'Line 1'	BABAX/LR42//BABAX*2/3/BRAMBLING/
G 17	'Line 2'	BABAX/LR42//BABAX*2/3/TUKURU/
G 18	'Line 3'	BABAX/LR42//BABAX*2/3/TUKURU/
G 19	'Line 5'	D67,2/P66,270//AE, SQUARROSA (320)/3/
G 20	'Line 6'	CROC_1/AE,SQUARROSA(224)//OPATA/3/PASTRO
G 21	'Line 7'	FRET2*2/KIRITATI
G 22	'Line 8'	KIRITATI/WBLL1
G 23	'Line 9'	QT6581/4/PASTOR//SITE/MO/3/CHEN/,
G 24	'Line 10'	T,DICOCCON P194614/AE,SQUARROSA (409)//BCN
G 25	'Line 11'	TC870344/GYI//TEMPORALERA M87/AGR/3/TOBA97
G 26	'Line 12'	WAXWING*2/4/SNI/TRAP#1/3/KAUZ*2TRAP//KAUZ
G 27	'Line 13'	WAXWING*2/KUKUNA
G 28	'Line 14'	WAXWING*2/KUKUNA
G 29	'Line 16'	WBLLI*2/BRAMBLING
G 30	'Line 17'	WBLLI*2/KIRITATI
G 31	'Line 19'	YANAC/3/PRL/SARA//TSI/VEE#5/4/CROC_1/,
G 32	'Line 20'	TEMPORALERA M 87*2/KONK
G 33	'Line 21'	TEMPORALERA M 87*2/CHOS
G 34	'Line 22'	KINGBIRD
G 35	'Line 23'	ICB97-0727-0AP

Table A2. Monthly average minimum temperature, maximum temperature, humidity and precipitation during the two growing seasons

Month	Minimum temperature (°C)	Maximum temperature (°C)	Humidity (%)	Precipitation (mm)
Season 2022-2023				
November	18.00	26.37	62.57	9.57
December	12.35	19.48	59.79	15.54
January	10.16	16.84	56.96	10.29
February	10.96	19.14	58.33	10.51
March	11.42	20.26	50.60	16.93
April	15.07	30.60	43.72	0.00
May	17.94	31.35	42.24	0.00
Season 2023-2024				
November	16.93	24.63	58.24	11.46
December	12.94	22.26	61.94	12.58
January	11.71	20.29	58.81	19.06
February	10.79	18.54	58.16	15.06
March	14.90	24.77	48.32	15.50
April	16.81	27.90	44.15	11.97
May	19.35	31.39	45.95	1.01

Table A3. Physical and chemical analysis of the experimental soil during both growing seasons

Soil properties	2022/2023	2023/2024
<u>Mechanical analysis</u>		
Sand (%)	17.45	22.65
Silt (%)	22.7	19.95
Clay (%)	59.85	57.4
Soil texture	Clay	Clay
<u>Chemical analysis</u>		
pH	7.23	7.37
Total Nitrogen (%)	0.08	0.09
Available phosphorus (ppm)	9.0835	7.199
<u>Soluble cations (meq /L.)</u>		
Na ⁺	7	4.8
K ⁺	0.1	0.1
Ca ⁺⁺	5	4.5
Mg ⁺⁺	5.5	3
<u>Soluble onions (meq /100 g)</u>		
Cl ⁻	6	4
CO ₃ ⁻⁻	-	-
HCO ₃ ⁻	0.5	0.5
SO ₄ ⁻⁻	11.1	7.9

Table A4. Heat tolerance indices for 35 wheat genotypes were evaluated under high levels of heat stress (E3 and E6) compared to normal (E1 and E4) conditions during two growing seasons

Genotype	First season			Second season		
	Mean Productivity	Stress Tolerance Index	Yield Index	Mean Productivity	Stress Tolerance Index	Yield Index
G1	7.55	0.38	1.07	5.10	0.21	0.96
G2	6.56	0.33	1.17	6.66	0.33	1.14
G3	5.63	0.22	0.83	5.35	0.24	1.09
G4	4.91	0.19	0.94	6.39	0.34	1.27
G5	5.97	0.20	0.70	5.05	0.19	0.84
G6	6.46	0.25	0.82	5.49	0.23	0.97
G7	6.96	0.29	0.87	5.42	0.25	1.13
G8	6.00	0.27	1.00	6.09	0.31	1.26
G9	5.05	0.14	0.58	5.57	0.25	1.09
G10	8.00	0.38	0.98	5.43	0.26	1.21
G11	6.98	0.37	1.21	6.32	0.30	1.07
G12	6.05	0.23	0.81	5.87	0.29	1.24
G13	6.57	0.34	1.20	6.03	0.28	1.05
G14	6.91	0.31	0.96	6.07	0.31	1.25
G15	4.62	0.19	1.09	6.49	0.34	1.24
G16	6.48	0.30	1.05	6.06	0.32	1.32
G17	7.99	0.30	0.74	7.22	0.37	1.13
G18	5.73	0.23	0.86	5.74	0.23	0.89
G19	6.74	0.30	0.97	6.08	0.32	1.28
G20	5.41	0.21	0.84	5.48	0.23	0.98
G21	7.69	0.43	1.24	5.86	0.28	1.16
G22	7.49	0.36	1.00	6.89	0.37	1.26
G23	6.84	0.26	0.77	6.17	0.27	0.96
G24	5.56	0.22	0.89	7.09	0.36	1.15
G25	7.32	0.34	0.98	5.98	0.27	1.03
G26	5.33	0.20	0.80	5.73	0.26	1.07
G27	6.12	0.32	1.27	5.76	0.28	1.16
G28	7.44	0.40	1.19	5.81	0.27	1.12
G29	7.38	0.43	1.37	6.52	0.32	1.13
G30	7.10	0.39	1.26	5.64	0.25	1.05
G31	7.68	0.43	1.24	6.35	0.26	0.88
G32	7.57	0.38	1.07	6.61	0.31	1.05
G33	5.07	0.22	1.04	5.31	0.24	1.08
G34	7.34	0.36	1.04	5.64	0.25	1.02
G35	6.61	0.34	1.17	5.46	0.24	1.03



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