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Stability Analysis Using AMMI and GGE Biplot Model for Terminal Heat Stress Tolerance in Wheat (*Triticum aestivum* L.)

Md Mahadi Hasan, Sidratul Muntaha, Yousuf Islam, Israt Jahan, Md. Sultan Ahmed, G H M Sagor ✉

Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

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Correspondence
G H M Sagor
✉: sagorgpb@bau.edu.bd

ABSTRACT

Terminal heat stress poses a significant challenge to wheat production in Bangladesh, especially under late-sown conditions. The rise in temperatures during this period leads to a shortened grain-filling phase, ultimately resulting in decreased yields. This research investigated to assess and pinpoint heat-tolerant wheat genotypes by employing multi-environment trials, which were analyzed using the AMMI and GGE biplot models. A total of twelve wheat varieties were evaluated across three distinct environments: optimum conditions (E1), moderate stress (E2), and terminal heat stress (E3) and the assessment focused on eight agronomic traits. Significant genotype × environment interactions were detected for all traits, highlighting the need for stability assessment. The AMMI 1 biplot effectively distinguished between the main effects of genotypes and their interaction effects, allowing for the identification of genotypes that exhibit both high average performance and adaptability. A better way to differentiate stable performers was possible by AMMI 2, which further clarified the interaction patterns. Among the varieties, BARI Gom 25 (G2), BARI Gom 33 (G6), BARI Gom 26 (G3), and Pavon (G12) were best, according to the Stress Tolerance and Susceptibility index, AMMI, GGE biplot (Which-won-where), Mean vs. stability, Ranking genotype approaches, since they combined above-average yields with stability in both stress and non-stress conditions. The inverse was also true for several genotypes; they showed excellent performance but bad stability. The consistent performance, especially under terminal heat stress, shows that BARI Gom 25 (G2), BARI Gom 33 (G6), BARI Gom 26 (G3), and Pavon (G12) might be used as breeding parents for climate-resilient wheat varieties that are suitable for agro-ecologies in South Asia. A strong framework for choosing stable, high-yielding genotypes in the face of climate change is provided by this combined AMMI-GGE strategy.

1. Introduction

Wheat (*Triticum aestivum L.*) remains one of the world's most important cereal crops. In 2011, global harvests were estimated at about 704 million tons, supplying a large proportion of daily calories for the dense populations of South Asia (Dhyani *et al.*, 2013). In Bangladesh, this cool-season cereal is not merely a commodity; it contributes substantially to food security and rural livelihoods. Consequently, any factor that constrains wheat production has implications far beyond the farm gate. Change of seasonal patterns and rise of temperature now threaten this crop. Elevated heat shortens phenological phases, particularly the grain filling period, and markedly reduces both yield and quality (Kumar *et al.*, 2023). Empirical studies indicate that increasing air temperatures by only 3–4 °C above the optimum during grain filling can lead to yield losses ranging from 10% to 50% in Asian wheat, while each additional degree Celsius reduces yields by roughly 6% worldwide and by 3–17% in South Asia (Khan *et al.*, 2020). Climate modelling suggests that a warming of 2–3 °C could reduce wheat output in developing countries by 20–30 %, and the favorable wheat growing zone of the Indo Gangetic Plain is projected to contract sharply by mid-century (Hossain & Teixeira da Silva, 2013). These general warnings are particularly relevant to Bangladesh. Field experiments in the region corroborate the threat: delayed sowing that exposes crops to terminal heat stress can depress yields by 20–57 % (Anwar *et al.*, 2024). For this reason, improving genotypes that can be productive under terminal heat stress is important nowadays. To understand genetic improvement for heat tolerance properly, it demands an understanding of genotype × environment interaction (GEI), because the performance of a genotype can vary dramatically across environmental conditions. Performance differences

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among genotypes cannot be interpreted adequately from mean yields alone because interactions between genetic backgrounds and environmental factors contribute significantly to variation. The additive main effect and multiplicative interaction (AMMI) model offers a solution by combining analysis of variance with principal component analysis to separate additive effects from interaction effects (Dang *et al.*, 2024). Likewise, the genotype plus genotype by environment (GGE) biplot focuses on the combined influence of genotypic main effects and their interactions, providing a graphical means to evaluate mean performance, stability, and the discriminating ability of test environments (Dang *et al.*, 2024). When used alongside stress tolerance indices, these tools allow breeders to identify lines that perform well across a range of conditions and to select environments that effectively reveal heat tolerance (Anwar *et al.*, 2024). The present study evaluates twelve wheat varieties under three sowing dates corresponding to normal, late, and very late planting to find out the stable genotypes over the different environmental conditions. The varietal characteristics were declared at release, but these are generally based on single-environment evaluations. This assess the multi-environment stability using AMMI and GGE models, which reveal genotype × environment interaction patterns under normal, late, and terminal heat stress conditions. This provides new insights on which varieties maintain consistent stability and adaptability across environments. These identified genotypes can be prioritized as parents for breeding programs aimed at climate resilience. Finally, through this approach, our objective was to identify heat-tolerant, high-yielding genotypes and to identify key physiological and morphological traits associated with heat tolerance using multivariate approaches.

2. Materials and Methods

2.1. Experimental site and environments

The study was conducted at the Genetics and Plant Breeding Farm, Bangladesh Agricultural University, Mymensingh, Bangladesh, during the 2023–2024 rabi season. According to UNDP and FAO (1988), the site lies in the Old Brahmaputra Floodplain (AEZ 9), characterized by a subtropical monsoon climate with distinct dry and wet seasons, making it ideal for evaluating heat stress response in wheat. For considering terminal heat stress, the genotypes were planted under three different environments. Those were Environment 1 (E1) – Optimum sowing on 12 November 2023, Environment 2 (E2) – Late sowing on 16 December 2023, Environment 3 (E3) – Very late sowing on 27 December 2023. Weather Data from November to April was collected from the Weather Yard under the Department of Irrigation and Water Management in Mymensingh. During this period, mean monthly air temperature ranged from 15.5 °C in December 2023 to 35.1 °C in April 2024. The critical reproductive and grain-filling phases (March–April) were characterized by high maximum temperatures (up to 35 °C), creating terminal heat stress conditions. Relative humidity (RH) varied from 47.7% (November 2023) to 93.9% (April 2024). Total rainfall was negligible during most of the season, indicating that heat stress was the dominant abiotic factor affecting the crop. Average soil temperature at 10 cm depth ranged between 19.3 °C in January 2024 and 30.8 °C in April 2024, confirming progressive soil warming across the season.

2.2. Plant materials

A total of 12 wheat varieties were evaluated for the study (Table 1). Among the 12 varieties, BWMRI Gom 4 (G10) was designated as the susceptible check for terminal heat stress for exhibiting extremely low STI (0.033) together with consistently poor yield and unstable performance across environments, as confirmed by biplot analyses.

2.3. Experimental design

The experiment was done in a Randomized Complete Block Design (RCBD) having three replications in every environment. Each genotype was sown under all three conditions to assess both genotypic performance and genotype × environment interactions.

2.4. Data collection

At the time of harvest, the data that have direct effect to the yield and yield components were collected. They were as follows: Yield per plant (YPP); Number of effective tillers per plant (NTP); Spikelets per spike (SPS); Seeds per spike (SDS); Seeds dry weight per spike (SWS); Plant height (PH); Hundred seed weight (HSW), and Harvest index (HI).

2.5. Statistical analysis

Data analysis was done with different software. The majority of the part was done with R statistical software (RStudio 4.4.1) with the help of different packages for AMMI model, and GGE biplot construction. PBTools v1.4.0 (IRRI, Philippines) was used for genotype × environment interaction analysis. Microsoft Excel was used for data management and formatting as well.

Table 1. The list of twelve wheat genotypes with their special feature

Genotype with legends	Special feature	Genotype with legends	Special feature
BARI Gom 20 (G1)	Early maturing	BWMRI Gom 1 (G7)	Early heat tolerant
BARI Gom 25 (G2)	Salt tolerant	BWMRI Gom 2 (G8)	Heat tolerant
BARI Gom 26 (G3)	Salt tolerant	BWMRI Gom 3 (G9)	Heat tolerant
BARI Gom 30 (G4)	Short duration	BWMRI Gom 4 (G10)†	Early maturing
BARI Gom 32 (G5)	Short duration	BWMRI Gom 5 (G11)	Resistant to blast and leaf rust diseases
BARI Gom 33 (G6)	High grain filling capacity	Pavon (G12)	Susceptible to heat stress

† Susceptible check

2.6. Stress tolerance index

To evaluate genotypic performance under heat stress, the following index were calculated using yield data under optimum (Yp) and stress (Ys) conditions:

1) Tolerance Index (TOL) = Yp−Ys

   (Rosielle and Hamblin, 1981)

2) Stress Susceptibility Index (SSI) = $\frac{1-(\mathbf{Ys/Yp})}{1-(\mathbf{Xs/Xp})}$

   (Fischer and Maurer, 1978)

3) Yield Stability Index (YSI) = $\frac{\mathrm{Ys}}{\mathrm{Yp}}$

   (Bouslama and Schapaugh, 1984)

4) Mean Productivity (MP) = $\frac{(\mathrm{Yp+Ys})}{2}$

   (Rosielle and Hamblin, 1981)

5) Geometric Mean Productivity (GMP) = $\sqrt{(\mathrm{Yp\ x\ Ys})}$

   (Ramirez and Kelly, 1998)

6) Stress Tolerance Index (STI) = $\frac{\mathrm{Yp{\times}Ys}}{X2\mathrm{p}}$

   (Fernandez, 1992)

Here, Yp = Grain yield of a genotype under normal (non-stress) conditions; Ys = Grain yield of the same genotype under stress conditions; $\bar{X}$p = Mean yield of all genotypes under normal conditions;$\bar{X}$s = Mean yield of all genotypes under stress conditions.

These indices have been widely used in stress-tolerance studies (e.g., Gupta et al., 2023) to identify high-yielding and stable genotypes under stress and non-stress environments.

3. Results

3.1. ANOVA of the AMMI model

The environmental (ENV) effect was significant ($p \le 0.01$) for SWS and HI, non-significant for SDS, and highly significant ($p \le 0.001$) for NTP, SPS, PH, HSW, CC, CT and YPP, according to the combined ANOVA (Table 2, Supplementary Table 1). This suggests that some traits were significantly impacted by the environment, while others showed more stability across the stress conditions. Significant differences ($p < 0.001$) between genotypes (GEN) were observed for NTP, SPS, SDS, SWS, PH, HSW, HI, and YPP, suggesting a high level of genetic diversity for these variables. For SPS, SWS, HSW, HI, and YPP, the genotype × environment interaction (GEN: ENV) was highly significant ($p < 0.001$), significant ($p \le 0.01$) for SDS and PH, and not significant for NTP. This indicates that the interaction effects on characteristics vary depending on the genotype and testing environment. Principal Component 1 (PC1) explained a significant portion of the GEI for all traits. However, PC2 showed non-significance for all traits, confirming that PC1 captured the majority of interaction variance. The study found that both environment and genotype had a significant impact on key yield parameters, with a particularly strong genotype × environment interaction for SPS, SWS, HSW, HI, and YPP. This highlights the need for multi-environment testing to find stable and productive genotypes.

3.2. Performance of Genotypes based on STI and SSI

The yield performance of twelve wheat genotypes was assessed under terminal heat stress utilizing the Stress Susceptibility Index and the Stress Tolerance Index. The calculations (Table 3) were conducted for both late sowing (LS) and very late sowing (VLS), with their averages to rank genotypes based on productivity and stress stability. G2 had the highest STI average (0.63), which means it was the most productive in both stressful situations. G11 and G3 were next in line, and they too had outstanding production potential. G12, G1 and G8 had intermediate STI values, which means they did well under stress, but they did not produce as much as G2. G10 and G7 had low STI values, so they did not adapt or produce well in the heat. The results of SSI for G8 and G9 were less than 1 (0.83 and 0.93), which means they were less susceptible to heat and had more steady yields. G4, G2 and G6 likewise had excellent stability, with SSI values that were close to 1. G1 and G3, on the other hand, were more sensitive to stress and had higher SSI values (1.16), even though they were productive. G7 and G10 had SSI negative values. This is probably because the yields were so low in all situations that those numbers cannot be trusted. In short, G2 was the best overall since it had a high yield and could handle stress well. G6 was more stable in the heat, even though it did not produce the most. G10 and G7 did poorly in all areas and may not be suitable for places. High CC under stress is associated with better canopy cooling and delayed leaf senescence, which supports grain filling. G1, G3, G12, and G7 had lower CC values, indicating earlier senescence and potentially reduced photosynthate availability during critical reproductive stages.

3.3. Additive main effects and multiplicative interaction 1 (AMMI 1) biplot

AMMI 1 (Figure 1) shows the genotype × environment (G × E) interaction between 12 top wheat genotypes produced in three sowing environments: E1 (optimal), E2 (late), and E3 (very late, terminal heat stress). The x-axis shows the main effects (average performance), and the y-axis shows the interaction effects (PC1). This shows that PC1 can appropriately describe G × E interactions when there is stress. The YPP biplot indicates that PC1 accounts for 99% of the interaction, positioning G10, G7, G5, and G4 as the highest yielders under terminal heat (E3) and mild heat stress (E2), whereas G3, G1, and G11 excelled in E1 but exhibited a significant fall in stress conditions. Genotypes such as G8 and G6 were positioned near the origin, indicating extensive adaptability and consistency over all planting periods. In SPS, G6, G1, G8 and G2 exhibited more stability across settings and demonstrated robust performance in E3, signifying stress-specific adaptation. In PH, genotypes G12, G4, G10, and G9 were close to E3, which meant they were very resistant to stress. Genotypes like G7 and G2 were close to E2, which meant they were somewhat adaptable to stress. In terms of the SDS, G11 and G1

Table 2. Mean squares of the combined analysis of variance of twelve wheat varieties, for yield and yield components in three different environments

Source	df	NTP	SPS	PH	SDS	SWS	YPP	HSW	HI
		MS	MS	MS	MS	MS	MS	MS	MS
ENV	2	179.11 ***	25.73 ***	1799.71 ***	357.51 ns	5.26 **	1818.32 **	15.45 ***	0.09 **
REP(ENV)	6	0.91 ns	0.69 ns	28.55 ns	91.81 ns	0.25 ns	85.27 *	0.36 ns	0.00 ns
GEN	11	9.65 ***	49.61 ***	660.99 ***	986.04 ***	0.93 ***	111.51 ***	4.97 ***	0.04 ***
GEN: ENV	22	1.70 ns	10.09 ***	93.98 **	240.91 **	0.65 ***	101.75 ***	1.00 ***	0.04 ***
PC1	12	2.28 ns	16.06 ***	113.38 **	381.91 ***	1.10 ***	184.69 ***	1.60 ***	0.05 ***
PC2	10	1.00 ns	2.92 ns	70.70 ns	71.72 ns	0.12 ns	2.23 ns	0.27 ns	0.02 ***
Residuals	66	1.63 ns	3.41 ns	40.38 ns	99.34 ns	0.24 ns	32.15 ns	0.31 ns	0.01 ns

*, ** and *** Indicates significance at 5%, 1% and 0.1% level of significance. Yield per plant (YPP), Spikelets per spike (SPS), Plant height (PH), Seeds per spike (SDS), Seeds dry weight per spike (SWS), Number of effective tillers per plant (NTP), Hundred seed weight (HSW), Harvest index (HI), and Mean of square (MS)

Table 3. Performance of twelve wheat genotypes for Stress Susceptibility Index (SSI) and Stress Tolerance Index (STI) under terminal heat stress

Genotype	TS	LS	VLS	STI_LS	STI_VLS	SSI_LS	SSI_VLS	STI_Avg	SSI_Avg
G1	27.76	3.46	3.50	0.37	0.37	1.24	1.08	0.37	1.16
G2	28.60	7.56	3.85	0.83	0.42	1.04	1.07	0.63	1.06
G3	28.45	4.05	2.79	0.44	0.30	1.21	1.11	0.37	1.16
G4	9.31	2.83	2.02	0.10	0.07	0.98	0.97	0.09	0.98
G5	8.88	1.84	1.21	0.06	0.04	1.12	1.07	0.05	1.09
G6	11.66	3.24	3.33	0.15	0.15	1.02	0.88	0.15	0.95
G7	1.44	6.30	4.26	0.03	0.02	-4.79	-2.43	0.03	-3.61
G8	13.25	6.20	3.61	0.32	0.18	0.75	0.90	0.25	0.83
G9	14.22	5.10	3.34	0.28	0.18	0.91	0.95	0.23	0.93
G10	1.92	5.77	3.19	0.04	0.02	-2.83	-0.82	0.03	-1.82
G11	23.47	5.44	2.94	0.49	0.27	1.09	1.08	0.38	1.08
G12	24.61	5.00	2.88	0.47	0.27	1.13	1.09	0.37	1.11

were close to E3, while G7 was close to E2. On the other hand, G5, G10, and G12, did not do well and did not adapt well. The SWS showed that G10 and G7 were going toward E3. When it came to NTP, genotypes G4 and G5 were the closest to E3, and G6 was positioned near E2. Regarding the HSW, G7 were close to E3, while G6 and G4 were near E2. In terms of the HI, G7 were close to E3, and G12 were positioned near E2. The results show that G4, G7, G5, and G6 do well when planted in terminal heat stress conditions. G3 appeared central, showing general adaptability without extreme response. G1 and G11 were variably located near E3 and E1, pointing to genotype-specific canopy behavior across environments (Figure 1).

3.4. Additive main effects and multiplicative interaction 2 (AMMI 2) biplot

AMMI 2 biplots (Figure 2) show the scores of principal components 1 and 2 for both types of plants and types of settings. This strategy helps us study in greater detail how genotypes and environments work together when plants are planted at the best time, late, or extremely late. PC1 and PC2 together made up 100% of the G + G × E interaction for all eight agronomic characteristics. The YPP, which made up 99% of the overall interaction through PC1, significantly separated heat-tolerant genotypes from heat-sensitive ones. G6, G5, and G4 were all significantly linked to E3, which means they did better and were more stable during terminal heat stress. Their site, close to E3, with short vectors, showed that there was very little crossover interaction and that the yield expression was consistent even when planting late. G8 and G10 were productive under E2, but they moved away from E3, which means that they will have lower yields in extreme hot weather. G3 and G11, which were far away from E3, were the most sensitive to terminal stress. Genotypes G2, G10, and G6 exhibited a good inclination toward E3 for SPS. This suggests that their spike growth was less affected by terminal heat.

For PH, genotypes G6 and G10 were the closest to E3. This suggests that they may be able to keep their height better and avoid heat by having a compact structure. G4 also stayed stable around E3, while G7 was closer to E2. The SDS biplot showed that G11, G10, and G1 were genotypes close to E3. This means that they can keep their spike fertility even when it becomes really hot.These genotypes look good for keeping yields high even when planting is late. In the pattern of SWS, G10, G1, and G2 stood out as heat-resistant genotypes. They were near E3 with short vectors, which showed that they could adapt and stay stable. G7, which was close to E2, was shown to be stable at moderate heat stress levels. For the NTP, genotypes G5 and G8 were close to E3. G2 and G11 were moved closer to E2. Talking about the HSW, G10 were very close to E3, and G6 were near E2. In terms of the HI, G7 were comparatively near to E3, and G8 and G9 were close to E2. Overall, the AMMI 2 data showed that G6 and G2 were the best genotypes for tolerating terminal heat stress. They were strongly linked to the very late planting environment (E3) and late planting environment (E2) across several parameters. G10 and G6 aligned with E3 demonstrated adaptability in maintaining chlorophyl under harsher conditions. G7 and G9 near the origin showed stable chlorophyll development (Figure 2).

3.5. GGE biplot (which won where)

The principal components PC1 and PC2 explained 96.8%, 91.54%, 94.82%, 93.45%, 95.82%, 95.26%, 96.34%, and 99.78% of the total variation for the yield contributing traits HSW, HI, NTP, PH, SDS, SWS, SPS, and YPP, respectively which was indicated by the "Which-won-Where" model (Figure 3) of GGE Biplot. The environments were allocated among several sectors in the polygon views, indicating the existence of mega-environments for each attribute. E2 and E3 consistently occupied distinct sectors from E1 across most variables, hence affirming the influence of terminal heat stress on genotype performance. The GGE biplot for YPP accounted for 99.78% of the total variation, indicating high model precision. The vertex genotypes comprised G2, G7, and G6. G2 excelled in both E1 and E3, whereas G7 demonstrated supremacy in E2. The GGE biplot for SPS resulted in PC1: 75.93% and PC2: 20.41%, combinedly 96.34% of the overall variation. Genotype G12 exhibited superior performance under E3. G7 and G10 excelled under E2, while genotypes close to the origin, like G2, G3, and G6, had moderate and consistent performance across several environments. The GGE biplot for PH resulted in 78.88% for PC1 and 14.57% for PC2. G3, G5, G7 and G10 were the vertex genotypes. G2 and G7 were superior in E2, whereas G3 prevailed in E3. Genotypes G6, G11, and G1 concentrated near the origin, indicating a steady performance for plant height across many settings. The GGE biplot for SDS accounted for 95.82% of the overall variation. The vertex genotypes comprised G5, G4, G6, G12, and G7. G7 exhibited optimal performance under E2, but G3, G12, and G9 approached near the E3, signifying consistent seed production under terminal heat stress conditions.

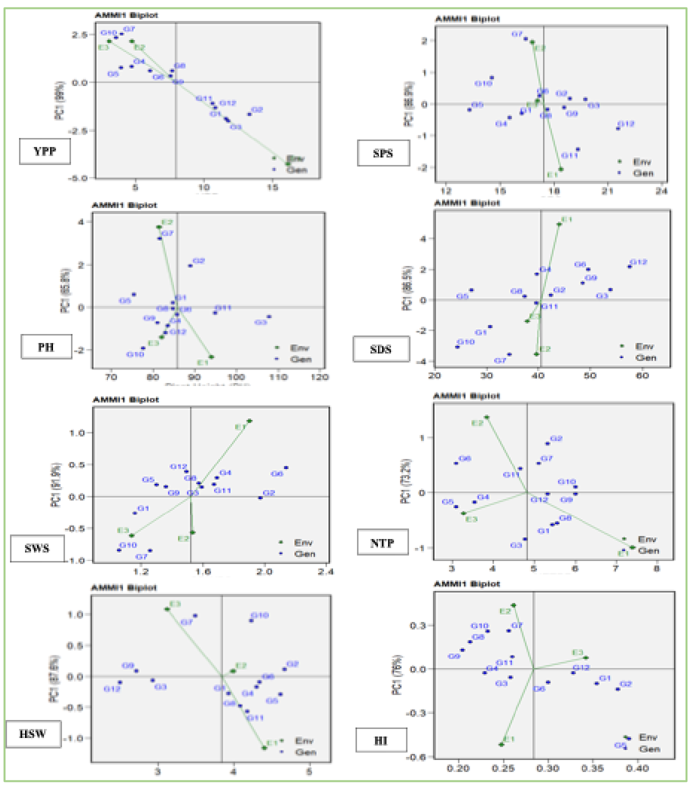

Figure 1. Additive main effects and multiplicative interaction 1 (AMMI 1) biplots illustrating G × E interactions of the twelve wheat genotypes under three environments based on Yield per plant (YPP), Spikelets per spike (SPS), Plant height (PH), Seeds per spike (SDS), Seeds dry weight per spike (SWS), Number of effective tillers per plant (NTP), Hundred seed weight (HSW), and Harvest index (HI)

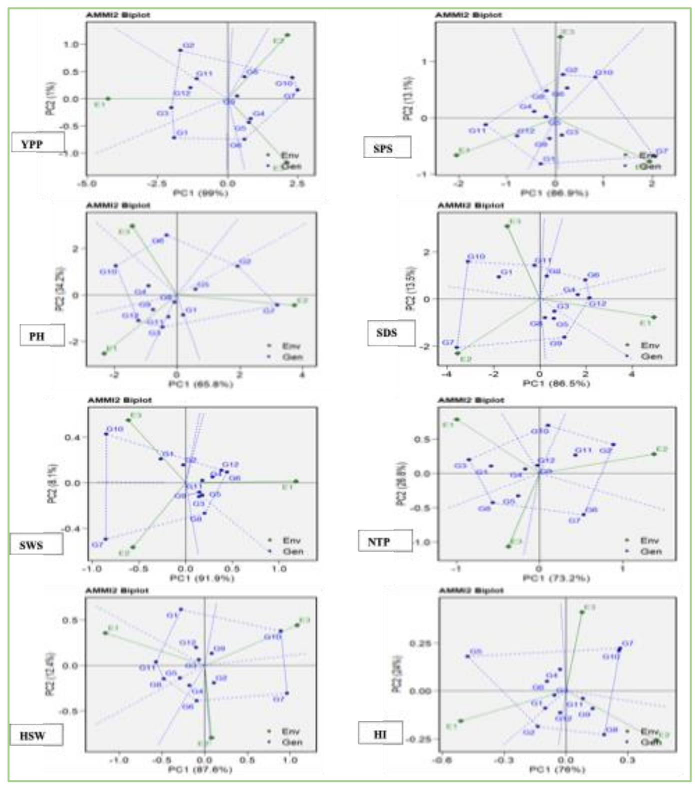

Figure 2. Additive main effects and multiplicative interaction 2 (AMMI 2) biplots derived from PC1 and PC2, depicting G × E interactions of twelve wheat genotypes across three environments based on Yield per plant (YPP), Spikelets per spike (SPS), Plant height (PH), Seeds per spike (SDS), Seeds dry weight per spike (SWS), Number of effective tillers per plant (NTP), Hundred seed weight (HSW), and Harvest index (HI)

The SWS explained 95.26% of the variation. Genotypes G2 and G6 performed better under terminal heat stress (E2 and E3). The NTP showed 94.82% of the total variation. G2, G10, G9, and G8 performed exceptionally well in those environments during terminal heat stress because they inhabited vertex sites corresponding to E2 and E3 habitats. The biplot for HSW showed that G10, G2, and G6 were positioned near the E2 and E3 environments and regarding the HI, G5 were near the E3, while G8 and G2 were positioned close to E2. Overall, G2, G9, and G8 performed exceptionally well in those environments during terminal heat stress. G3 showed a strong ability to adjust to even late planting. G1 and G5 showed good performance under non-stress (E1) but declined in stress conditions. Stable performers like G3 and G7, positioned near the origin, maintained moderate chlorophyll levels across environments, indicating potential for consistent canopy function (Figure 3).

3.6. Genotypic variation in mean performance across traits

The estimated mean performance values (Figure 4) describe the average trait value, which is calculated across multiple environments. The genotypes designated with blue circles outperformed the grand mean, while those marked in red were below average. The 95% confidence intervals for the estimated trait values under terminal heat stress conditions are denoted by horizontal error bars. The genotypes G2, G3, G1, G12, and G11 exhibited the highest mean performances in terms of YPP, while genotypes G10, G5, G7, and G4 exhibited the lowest yields. The best genotypes for SPS were G12, G3, G11, and G2. The worst were G5, G10, and G4. In terms of PH, G3, G11, G2, and G6 were much taller than usual. On the other hand, G5, G10, and G9 had the smallest plants. In SDS, G12, G3, G6, G9, and G2 were above average, while G10, G5, G1, and G7 performed below the mean. For SWS, G6, G2, G4, G11, and G3 did the best, whereas G10, G1, G7, and G5 did the worst. For NTP, G9, G10, G8, G1, G2, and G12 did better than average, whereas G5, G4, and G6 had the lowest tiller counts when it was under heat stress. For HSW, G2, G5, G6, G4, G10, G11, G8, and G1 were above average, while G7, G3, G9, and G12 performed below the mean. Finally, the HI, G5, G2, G1, G12 and G6 were above, while the rest were below the average. These results clearly show that G2, G3, and G6 are the best and most consistent genotypes for several parameters. This means that they have a good chance of being stable and adapting to stress in stressful environments. CC serves as a proxy for photosynthetic activity and the plant's ability to delay senescence under stress. G6, G5, G4, and G9 recorded above-average chlorophyll levels, suggesting prolonged green leaf area duration and sustained carbon assimilation under heat. Breeding for high CC under terminal stress can significantly improve yield stability, as chlorophyll retention directly influences biomass accumulation and assimilate supply to developing grains (Figure 4).

3.7. Ranking genotypes

The GGE biplot ranking (Figure 5) was imposed to recognized genotypes that exhibit both elevated mean performance and stability across various environments. In these biplots, genotypes situated nearer to the centre of the concentric circles and the average environment vector were deemed more optimal, indicating both superior performance and stability. Genotypes situated distantly from the centre or in contrast to the stress environments (E2 and E3) demonstrated inadequate adaptability to hot conditions. In the YPP, G6 came closest to the best under terminal stress, followed by G11 and G12. These three showed strong and sustained yield performance, especially under E3. Genotypes G7 and G10, which were far from the centre, were consistently positioned near the optimal range, indicating stable growth across different heat intensities. Conversely, G5 and G10 had the lowest duration and the least stability across various situations. In SDS, G12, G3, and G6 were always the best-performing and most stable genotypes while they were under stress. However, G5, G7, and G10 were not as good at adapting to terminal heat. Genotypes G6, G2, and G4 had the best stability and performance when it came to SWS under E2 and E3 conditions, which were moderate and terminal heat stress, respectively. G9 and G8 were the closest to the best genotype for NTP. Regarding the HSW, G2, and G6 were the best performers. In the end, G2, G5, G1, and G12 were close to the centre of the concentric circles, meaning top-ranked genotypes for HI. In conclusion, genotypes G6, G2, G3, and G12 consistently demonstrated superior performance and stability under terminal and moderate heat stress, making them exemplary candidates for cultivation in heat-affected areas or for breeding programs focused on improving heat resilience. For CC and CT, Genotype G9 also performed relatively well, positioned nearer to the optimal axis, suggesting a tendency toward heat tolerance (Figure 5).

3.8. Mean performance vs. stability

The GGE biplot analysis in the "mean vs. stability" (Figure 6) perspective offered a comprehensive evaluation of mean performance and stability. In this perspective, the average environment coordinate (AEC) arrow indicates ascending mean performance, while the projection length from the AEC axis signifies genotype stability; a shorter projection denotes greater genotype stability. The AEC abscissa line (the horizontal line with the arrow) indicates the direction of increasing trait mean value. In terms of YPP, Genotypes G6 and G4 had advantageous mean performance under E2 and E3, demonstrating adequate stability, although G8 and G9 ranked among the most stable genotypes, yielding over the average mean yield. In terms of SPS, G3, G2, and G9 had superior mean performance, with G2 also demonstrating notable stability, rendering it a plausible choice under terminal stress conditions. Conversely, G7 and G11 had the most extended projections, signifying G7, and G10 exhibited instability with increased projection distances, whereas G6, G8, G2, and G4 showed more stability. In the SDS examination, G12, G3, and G6 had superior mean performance under E2 and E3, although G2 showed notable stability in performance. In terms of SWS, G6, G2, and G4 exhibited superior mean performance. Among these, G2 again demonstrated remarkable stability, underscoring its reliability in generating robust seeds under stress conditions. The least preferable options were G1, G5, and G10 in terms of both average and consistency. Under heat-stressed conditions, G6, G5, and G4 demonstrated superior performance in terms of NTP, while G10, G9, and G8 exhibited enhanced stability but reduced average tiller output. G2 and G6 consistently exhibited stability and good performance across various attributes, especially during terminal heat stress (E3). Genotypes G4, G3, and G5 exhibited favorable mean performance alongside moderate to good stability, rendering them strong prospects for heat-resilient wheat breeding projects. The superior performance of G6 and G5 for CC and CT suggests that they can serve as valuable donors for heat tolerance, while genotypes like G10 and G8 require improvement to avoid rapid senescence under heat-stressed environments (Figure 6).

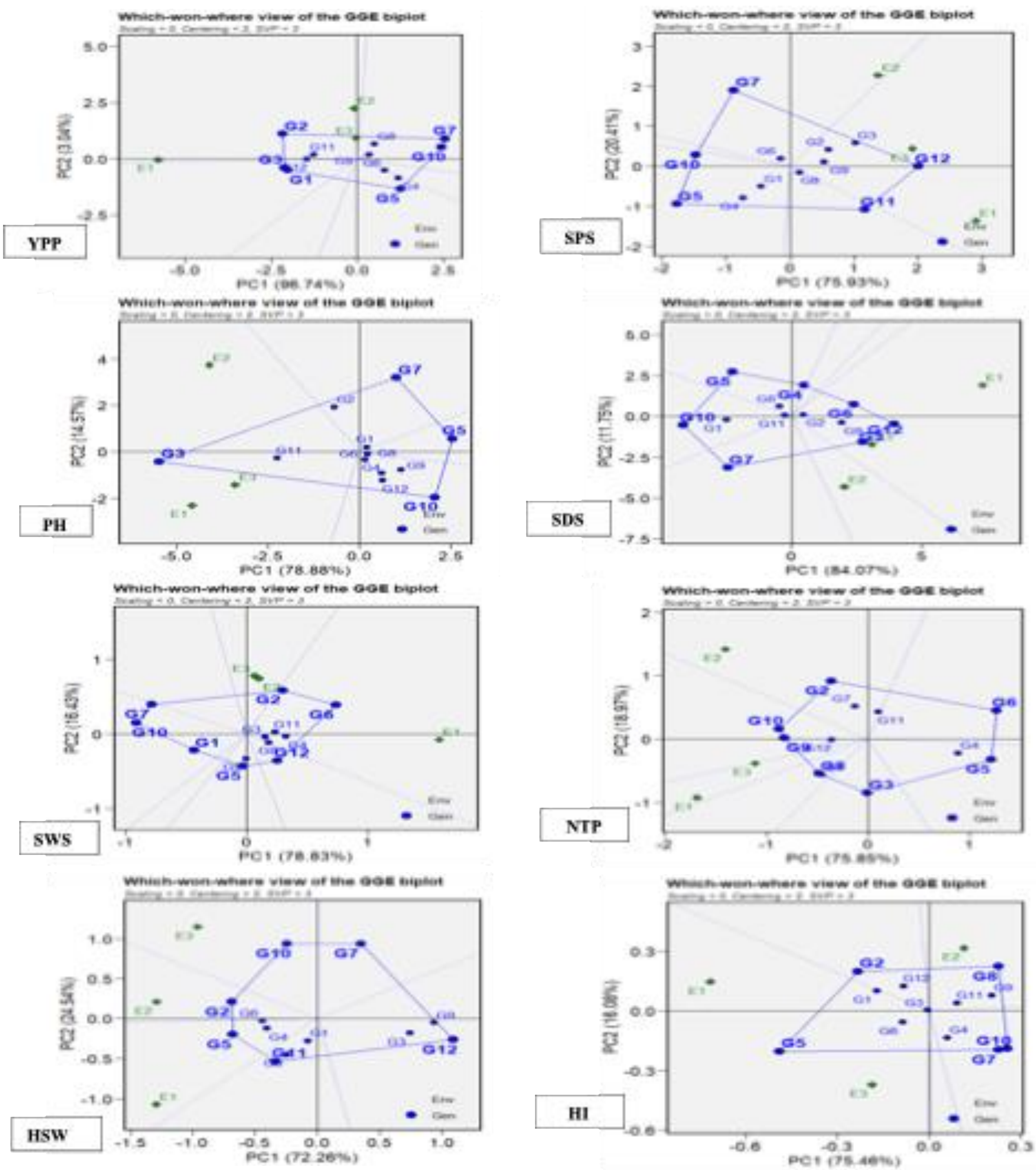

Figure 3. GGE biplots (Which-won-where) illustrating the performance of twelve wheat genotypes across three environments based on Yield per plant (YPP), Spikelets per spike (SPS), Plant height (PH), Seeds per spike (SDS), Seeds dry weight per spike (SWS), Number of effective tillers per plant (NTP), Hundred seed weight (HSW), and Harvest index (HI).

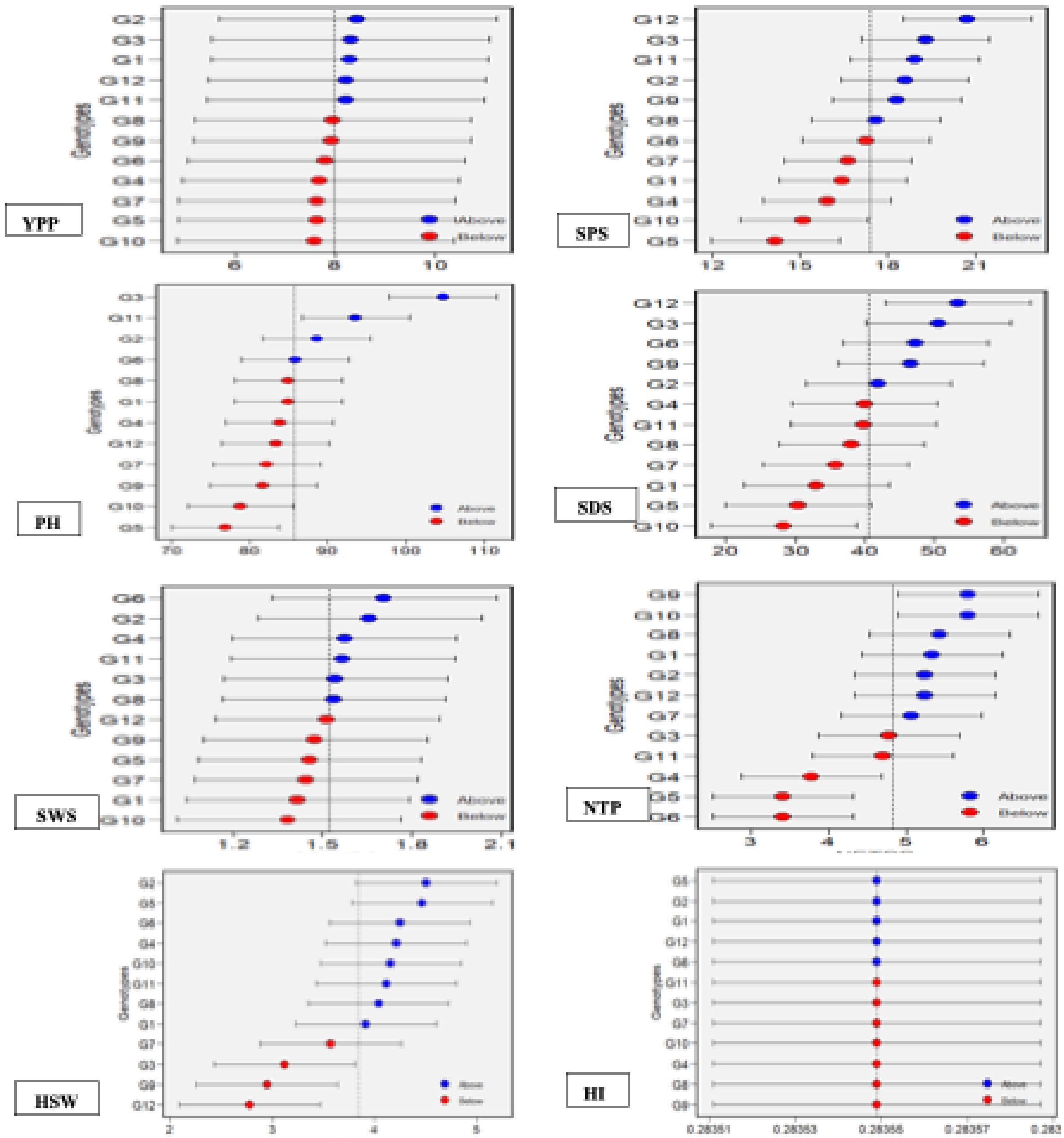

Figure 4. Genotypic variation in mean performance across traits of the twelve wheat genotypes under three conditions based on Yield per plant (YPP), Spikelets per spike (SPS), Plant height (PH), Seeds per spike (SDS), Seeds dry weight per spike (SWS), Number of effective tillers per plant (NTP), and Hundred seed weight (HSW), and Harvest index (HI)

4. Discussion

In South and Central Asia, terminal heat stress is considered a barrier for wheat production because the crop frequently grows in hot, arid environments. The sensitivity of wheat to temperature is partially attributed to the limited optimal range for anthesis and grain filling; Physiological studies suggest optimal temperatures of approximately 22–23 °C for flowering and 20–22 °C for grain filling (Lamba et al., 2023; Mirosavljevic et al., 2024). Spike fertility decreases when the temperature rises. Apart from that, the acceleration of grain filling and advancement of canopy senescence are observed, which ultimately leads to lower yield for reduced grain size (Yadav et al., 2022; Mahdavi et al., 2022). Physiological injuries like destabilization of membranes, disruption of photosynthesis, and reduction of effective tiller number and grain are caused by heat stress, which is demonstrated by mechanistic investigations. Our late sowing experiment shows that these physiological impairments significantly reduce yields, highlighting the need for heat-tolerant cultivars.

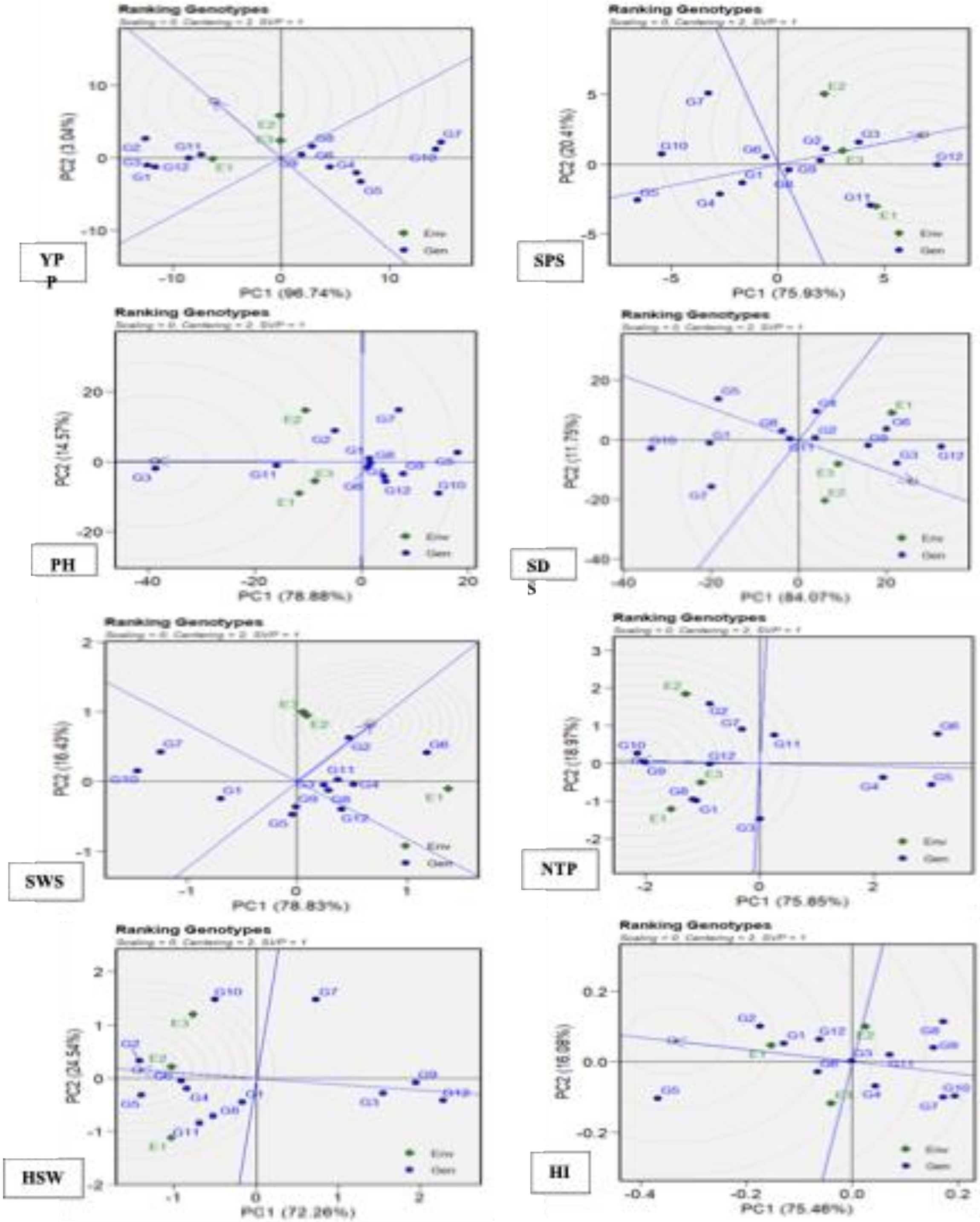

Figure 5. Ranking of twelve wheat genotypes under three environments based on Yield per plant (YPP), Spikelets per spike (SPS), Plant height (PH), Seeds per spike (SDS), Seeds dry weight per spike (SWS), Number of effective tillers per plant (NTP), Hundred seed weight (HSW), and Harvest Index (HI).

Bagherikia et al. (2025) observe that late sowing results in the grain filling period coinciding with elevated temperatures, thereby diminishing grain filling and yield. Their field studies throughout Iran revealed that increased temperatures during grain filling expedite maturity while diminishing grain size and yield, whereas augmenting protein content frequently compromises gluten strength. Our investigation revealed the same patterns: the late December sowing (E3) subjected genotypes to elevated temperatures during anthesis and grain filling, leading to significant reductions in grain yield and its components. In line with research showing that heat stress reduces starch accumulation but increases protein concentration, grain moisture, and seed weight decreased while grain protein levels tended to rise under terminal heat stress conditions (Mahdavi et al., 2022; Bagherikia et al., 2025).

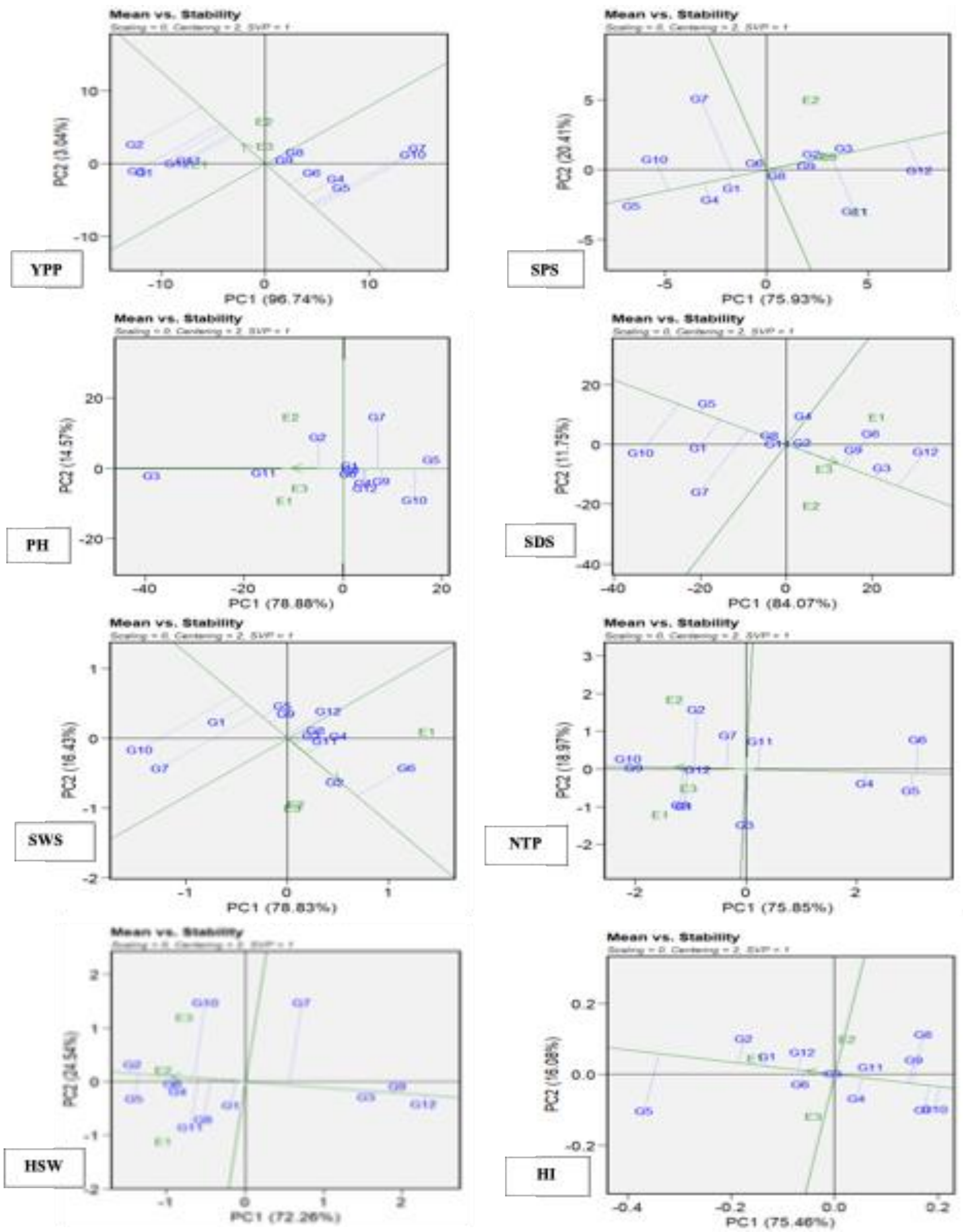

Figure 6. Mean Performance vs. Stability of twelve wheat genotypes under three environments based on Yield per plant (YPP), Spikelets per spike (SPS), Plant height (PH), Seeds per spike (SDS), Seeds dry weight per spike (SWS), Number of effective tillers per plant (NTP), and Hundred seed weight (HSW), and Harvest index (HI).

Heat stress adversely impacts not only reproductive phases but also impairs vegetative growth. Gudi et al. (2025) indicated that exposure of a global panel of wheat seedlings to 36 °C resulted in an 85.6% reduction in root length and a 15.4% decrease in coleoptile length, demonstrating significant genetic diversity in seedling heat tolerance. High temperatures induce pollen sterility; subjecting wheat to 35 °C during anthesis diminished floret fertility from 85% to 0%, resulting in total pollen abortion. These findings underscore the necessity of assessing heat tolerance across developmental stages.

Our findings reflect the preeminence of environmental influences documented in other studies. In the comprehensive analysis of variance (ANOVA), the environment contributed significantly to the variation in grain yield and associated variables. Bishwas et al. (2021) reported a comparable partitioning of variation, revealing that environment, genotype, and genotype × environment interaction accounted for 75.66%, 17.25%, and 7.08% of yield variation, respectively, in a cohort of wheat lines. The multi-environment studies conducted by Khare et al. (2024) revealed significant effects of environment, genotype, and genotype-environment interaction (GEI). They found that yields decreased by 6-10% for every degree Celsius increase. Mahdavi et al. (2022) noted that postponed planting and thermal stress diminish 1000-grain weight by lowering starch content.

In contrast, heat-tolerant genotypes preserve greater grain weight, starch, and moisture content, in contrast to sensitive genotypes, which exhibit elevated protein. Bagherikia et al. (2025) additionally indicated that heat stress is more detrimental than other abiotic conditions, such as salt or nutritional deficiency. Environmental factors during grain filling significantly influence both production and quality. These studies collectively corroborate our result that heat stress predominantly diminishes grain yield by truncating the grain filling period and reducing effective tillers, seeds per spike, and seed weight, while occasionally elevating grain protein content.

The use of stress tolerance indices provided a quantitative framework for ranking genotypes under heat stress. Several indices—stress tolerance index (STI), mean productivity (MP), geometric mean productivity (GMP), harmonic mean (HM), yield stability index (YSI), yield index (YI), stress susceptibility index (SSI), tolerance index (TOL), and relative stress index (RSI)—have been proposed for selecting heat-tolerant genotypes (Lamba et al., 2023; Redhu et al., 2025). There are strong positive associations between grain yield under stress and indices like STI, MP, GMP, and HM. They suggested choosing genotypes with high values for MP, STI, GMP, HM, YSI, and YI, and low values for TOL, SSI, RSI, and percent yield reduction (PYR) (Lamba et al., 2023). Our results corroborate these findings: G2, G3 and G12 as top performers across stress environments STI, whereas G7 and G10 exhibited low STI and high SSI values. The broad array of parameters enabled the identification of genotypes that exhibit both elevated mean yield and stability. This strategy is increasingly promoted in breeding programs focused on heat stress.

Multivariate models were essential for dissecting genotype × environment interactions. The additive main effect and multiplicative interaction (AMMI) model partitions GEI into additive and multiplicative components, and our analysis revealed that the first principal component (PC1) explained more than 80% of the GEI variation for most traits. This parallels findings from wheat trials in Nepal, where PC1 constituted the predominant portion of interaction variance. (Bishwas et al., 2021). The genotype plus genotype by environment (GGE) biplot focuses on genotype main effects plus GEI and graphically presents mean performance and stability. The use of GGE biplots to assess the representativeness of test conditions, adaptability, discriminative capacity, and productivity across various crops was highlighted by Dang et al. (2024). The ability of GGE biplots to rank genotypes in both optimum and elevated temperature settings, as well as to identify mega habitats, was demonstrated by Khare et al. (2024). In this study, we used AMMI1 and AMMI2 biplots to identify G6, G2, and G4, which exhibited straightforward heat adaptation, positioned around the terminal stress environment.

On the other hand, the clustering of G3, G1, and G11 near the typical habitat proved their sensitivity. The GGE biplots showed a stable and good mean yield in G6, G2, G3, and G12. Also, they demonstrated that the late planting environments (E2 and E3) were noteworthy and informative, in contrast to the conventional sowing environment (E1), which offered less unique data. According to Anwar et al. (2024), which was supported by these data, planting crops too late in the growing season in Bangladesh reduced yields by 20-57%. The study also identified SA 8, Chyria 3, and Pavan as stable, high-yielding genotypes.

Our results align with studies on experimental design and the heritability of heat stress indices. Redhu et al. (2025) advocated the use of alpha lattice designs for extensive heat stress experiments, noting that they provide greater precision and flexibility than randomized complete block designs. Lamba et al. (2023) predicted that global temperatures are likely to rise by 1–4 °C, potentially decreasing wheat yields by 4.1–6.4% and exposing crops to 25–32 °C during anthesis and grain filling. They emphasized that breeding for heat tolerance should exploit the existing genetic variation and use multivariate methods to select parents. Our identification of genotypes G2, G6, G3 and G12 as heat-tolerant and stable across environments suggests that such genetic variation exists within Bangladeshi breeding materials. At the same time, the poor performance of G7 and G10 highlights the need to remove susceptible lines from breeding pools.

In many places, heat stress is already an issue, and it gets considerably worse when coupled with drought. Terminal heat accelerates assimilate remobilization as a result of early senescence, resulting in reduced grain size and inferior quality. Furthermore, selecting genotypes with a mix of physiological and agronomic features is essential since, in comparison to heat alone, the combined effects of drought stress and heat significantly lower yield. These findings imply that heat-tolerant genotypes may be resistant to a range of stresses, which is a fascinating notion that requires more research, even though we only evaluated heat stress in this experiment. Finally, other factors, including soil moisture and nutrient availability, might impact some of our results.

5. Conclusion

In this study, delayed wheat sowing into late December placed plants squarely in the path of late-season heat waves, and the effect was unavoidable: grain filling shortened, tiller numbers dropped and overall yield declined. Not all lines responded the same way. However, a small group of genotypes—BARI Gom-25, BARI Gom-33, BARI Gom-26 and Pavon—managed to remain productive and stable across the normal, late and very late sowing conditions. Others, such as BARI Gom-4 and BARI Gom-1, were unable to cope and consistently produced low yields. Our biplot analyses helped to make sense of these patterns by showing that the very late sowing environment accentuates genotypic differences yet still mimics the stress conditions farmers face. Taken together, these results point breeders toward a handful of promising parents for developing heat-tolerant cultivars and suggest that carefully choosing sowing dates can help growers reduce the risk of yield loss in a warming climate.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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