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## Comparative Assessment of AMMI, BLUP, and WAASB Models for Identifying Stable Wheat Genotypes across Diverse Agroclimatic Conditions in Pakistan

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### Abstract

The interaction between genotype and environment (GEI) complicates the identification of stable, high-yielding genotypes in multi-environment trials. This study evaluates three statistical methods—AMMI, BLUP, and WAASB—to identify stable wheat genotypes. AMMI and WAASB consistently identified genotypes with superior mean yield and phenotypic stability, with G2 showing the highest yield and remarkable stability across environments. BLUP stability indices identified G1 as a stable and high-yielding variety. AMMI also helped identify "which-won-where" genotypes and mega-environments with discriminating powers useful for genotype evaluation. BLUP provided insights into genetic parameters and heritability, while WAASB was effective in clustering genotypes based on yield and stability. These methods offer valuable tools for selecting stable wheat genotypes suitable for diverse environments.

**Keywords:** Accessions, Best linear unbiased prediction, Environment, Phenotypic stability

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## Introduction

Breeders often utilize multi-environment trials (METs) to assess advanced genotypes, group similar environments, and identify the most suitable genotype for each environment and all environments. (Yan et al., 2000). METs help to identify genotypes that exhibit low variability and show consistent response across various environments (Fox et al., 1997). Identifying stable and high-yielding genotypes in multi-environment trials (METs) is challenging due to the combined additive effects of genotype (G) and environment (E), as well as the multiplicative influence of genotype  $\times$  environment interaction (GEI) (Aarthi et al., 2020). Genotype  $\times$  environment interaction (GEI) refers to the differential response of a genotype when exposed to varying environmental conditions (De Leon et al., 2016). The GEI is grouped into two main types: non-crossover, or quantitative interaction, and crossover, or qualitative interaction. When the ranks of genotypes shift, crossover interaction is used to explain how different genotypes react to multiple environments as their rankings change. Non-crossover interaction refers to differences in the level of genotype performance in different contexts without altering the order of the genotypes. From this perspective, a genotype is regarded as stable if its performance remains consistent and comparable across a range of different environmental conditions (Yan & Tinker, 2006).

Selecting and introducing stable, high-yielding genotypes in various locations is one way to reduce GEI and enhance production (Malosetti et al., 2013). Various techniques have been utilized to identify stable genotypes, including both univariate and multivariate approaches. These methods encompass parametric and non-parametric stability analyses, each offering different ways to assess the consistency of genotypic performance across diverse environments (Sitaresmi et al., 2019; Yaghotipoor et al., 2017). However, univariate statistical approaches, both parametric and non-parametric, are often unable to accurately interpret complex and multidimensional interactions (Yaghotipoor et al., 2017). Multivariate methods, such as AMMI, BLUP, integrated AMMI and BLUP, and GGE Biplot, were found to be more effective in overcoming this issue. (Ahakpaz et al., 2021; Kindie et al., 2022; Santos & Marza, 2020). WAASB is a new stability metric that was created to address GEI by uniting the characteristics of both AMMI and BLUP models (Olivoto et al., 2019).

From a practical perspective, AMMI, BLUP, and WAASB are distinct methodologies that aim to achieve the same objective of evaluating genotype stability. To enhance accuracy and robustness, multiple approaches and methods have been utilized to forecast the stability of genotypes across different crops, rather than relying on a single method alone (Kindie et al., 2022; Olivoto et al., 2019; Santos & Marza, 2020; Verma & Singh, 2021). In the current study, three widely recognized statistical methods —AMMI, BLUP, and WAASB —were utilized to assess and identify wheat genotypes with high productivity and stability. These methods were applied to evaluate the performance of genotypes across ten distinct environmental conditions in Pakistan. By combining these approaches, the study aimed to gain a comprehensive understanding of genotype  $\times$  environment interactions and select genotypes that exhibit consistent high-yield performance and stability under diverse growing conditions.

## Materials and Methods

*Stability analysis of wheat genotypes*

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Fifty wheat genotypes, along with two check varieties (Akbar-19 (G03) and Pak 13 (G35)), were evaluated in the present study (Table 1). Multi-environment trials (METs) were conducted across ten distinct regions in Punjab, Pakistan, during the 2021-2022 cropping seasons (Table 2).

**Table 1.** List of Wheat Genotypes Evaluated in the 2021-2022 METs

Sr. No.	Genotype	Genotype code	Sr. No.	Genotype	Genotype code
1	V-20337	G1	26	NW-74	G26
2	V-19532	G2	27	V-19080	G27
3	AKBAR-19	G3	28	AZP-21	G28
4	V-19559	G4	29	19C166	G29
5	V-20395	G5	30	NW-103	G30
6	V-20355	G6	31	EV-19103	G31
7	HYT-10-95	G7	32	19BT022	G32
8	V-20418	G8	33	BF-7799	G33
9	BF-20105	G9	34	INDUS-21	G34
10	TWS-1907	G10	35	PAK-13	G35
11	V-19590	G11	36	IS-18363	G36
12	20C207	G12	37	V-20241	G37
13	PGMB-20-43	G13	38	AZRI-8	G38
14	TWS-1902	G14	39	V-19261	G39
15	IS-18565	G15	40	NR-561	G40
16	10141	G16	41	NR-564	G41
17	180007	G17	42	WV-1196	G42
18	NR-559	G18	43	NR-560	G43
19	BF-7792	G19	44	19C160	G44
20	TWS-1926	G20	45	WVH-1214	G45
21	V-20352	G21	46	191272	G46
22	10HP-428	G22	47	JM-1683	G47
23	V-20330	G23	48	JM-1215	G48
24	PGMB-20-48	G24	49	18FJ01	G49
25	RUSTAM-21	G25	50	GOLDEN-100	G50

**Table 2.** Geographical and Environmental ID of the Ten Regions used in MET.

Sr. No.	Location Name	Location ID	Coordinates
1	Wheat Research Institute, Faisalabad, Punjab, Pakistan	E1	31° 29' 09" N 73° 02' 20" E
2	Barani Agricultural Research Station, Fateh Jang, Punjab, Pakistan	E2	33° 39' 20" N 72° 36' 53" E
3	Groundnut Research Station, Attock, Punjab, Pakistan	E3	34° 05' 36" N 72° 18' 25" E
4	Barani Agricultural Research Institute, Chakwal, Punjab, Pakistan	E4	33° 14' 02" N 72° 40' 50" E
5	National Agricultural Research Center, Islamabad, Pakistan	E5	34° 02' 19" N 73° 09' 50" E
6	Arid Zone Institute of Research, Bhakkar, Punjab, Pakistan	E6	31° 43' 59" N 71° 08' 38" E
7	Karor, Layyah, Punjab, Pakistan	E7	31° 18' 08" N 70° 57' 06" E
8	Regional Agricultural Research Centre, Bahawalpur, Punjab, Pakistan	E8	32° 34' 11" N 71° 07' 14" E
9	University of Agriculture, Faisalabad, Punjab, Pakistan	E9	31° 33' 01" N 73° 08' 12" E
10	Kallarkot, Sialkot, Punjab, Pakistan	E10	32° 54' 09" N 74° 17' 05" E

An experiment was conducted using a randomized complete block design (RCBD) with two replications to ensure statistical reliability. The experimental plots consisted of four rows, each 5 meters in length, with a 30-centimeter spacing between rows. Grain yield (GY) was then estimated by converting the data from each experimental unit into kilograms per hectare, providing a standardized measure of productivity across the trials.

The grain yield data obtained from all the METs were analyzed using the AMMI model, following the statistical methodology outlined by Zobel et al. (. To check the significance of GEI, an AMMI ANOVA was performed, and subsequently, the GEI was split into different AMMI PCs, with the significance of principal component axes (PCA) contributing to GEI being checked. Further, the WAAS stability index was calculated from the significant PCAs of  $G \times E$  shown in AMMI ANOVA. Traditional AMMI biplots 1 and 2 were plotted using mean performance and PC1 and PC1 and PC2, respectively. A view of the AMMI was created by using nominal yield and PC1.  $Y \times WAAS$  Biplot was plotted by replacing the PC1 of the traditional AMMI biplot with WAAS (Olivoto et al., 2019).

For BLUP analysis, a linear mixed model was used, keeping genotype and  $G \times E$  interaction effects as random effects. The significance of the random effects was assessed using the Likelihood Ratio Test (LRT). This statistical method compares the goodness of fit between models with and without the random effects. Variance components for the

random effects were estimated using Restricted Maximum Likelihood (REML), which provides unbiased estimates of variance in mixed-effects models (Dempster et al., 1977). HMGV, RPGV, and HMRPGV stability indices were computed as described by Filho et al. (). WASSB analysis was performed according to the model proposed by Olivato et al. (). WAASB stability index was also calculated to rank the genotypes regarding yield and stability. The analyses of the studied stability models were performed in R software (R 4.5.1) using the metan multi-environment trial analysis Package developed by Olivato & Lúcio.

## **Results**

The AMMI AONVA showed that the main effects of genotype and environment, along with GEI, were significant, with a p-value less than 0.001 (Table 3). In the total variation, the environmental effect of 65% was observed while the share of GEI was 17%. Further, the sum square was found to be 8.5 times more influential than the genotype effect, indicating that the variation due to GEI played a significantly larger role in determining grain yield than the genotypic differences alone. The GEI was further partitioned into 9 PCs by AMMI analysis, which significantly contributed to G×E. Principal component 1 (PC 1) was highly significant ( $P < 0.01$ ) and accounted for 30.2% of the variability in GEI. In the current study, the top two IPCAs explained approximately 51.1% of the total sum of squares of GEI.

In the AMMI1 biplot, PC1 values and mean yields were used for plotting environments and genotypes (Figure 1). The genotypes G2 and G40, which had a yield value higher than the mean and comparable PC1 scores, were identified as the most adapted genotypes with relatively less yield variation across environments. The environment E6 with PC1 scores close to zero indicated minimal interaction effects, suggesting that all genotypes exhibited consistent performance in this environment. Hence, these were considered favorable environments for all the tested genotypes. The environments E2, E9, E1, E5, and E8, farthest from the origin, had the most significant effect of the G×E. So, the relative ranking of genotypes was unstable in these tested environments (Figure 1).

In the AMMI2 biplot, the first two IPCs, which contributed more to G×E, were used for plotting genotypes and environments. Genotypes G50, G47, G32, and G8, located far from the origin, exhibited the most significant fluctuations in response to environmental changes, suggesting a higher degree of genotype × environment interaction (G×E). On the other hand, genotypes G2, situated within the origin, and G13, positioned close to the biplot origin, demonstrated a smaller degree of G×E interaction, reflecting their greater stability and consistent performance across all environments. Environment E3, E4, E7, and E10 near the origin were showing low GEI, while the environment E9, E2, E6, E1, E5, and E8 far from the origin had high GEI (Figure 2).

**Table 3.** AMMI Analysis of variance of wheat grain yield along with significant IPCA

Sov	Df	SS	MS	F value	P Value	SS%
ENV	9	873484186.8	97053799	6079.162	0**	65
REP(ENV)	10	159649.96	15965	14.52204	1.1E-22 <sup>NS</sup>	0
GEN	49	28196855.52	575446	523.4357	0**	2
GEN×ENV/(GEI)	441	222556970.2	504664.3	459.0514	0**	17
The proportion of PCs in GEI						
PC1	57	67101096.77	1177212	1070.81	0**	30.2
PC2	55	46520070.74	845819.5	769.37	0**	20.9
PC3	53	35081964.92	661923.9	602.1	0**	15.8
PC4	51	26806732.71	525622.2	478.12	0**	12
PC5	49	21925637.55	447462	407.02	0**	9.9
PC6	47	11327680.38	241014.5	219.23	0**	5.1
PC7	45	7972271.046	177161.6	161.15	0**	3.6
PC8	43	3168619.094	73688.82	67.03	0**	1.4
PC9	41	2652896.98	64704.8	58.86	0**	1.2
Residuals	490	538688.04	1099.363			
<b>Total</b>	1440	1347493321	935759.3			

\*\*= Significant at 1% and 5% of P value, NS results are not significant

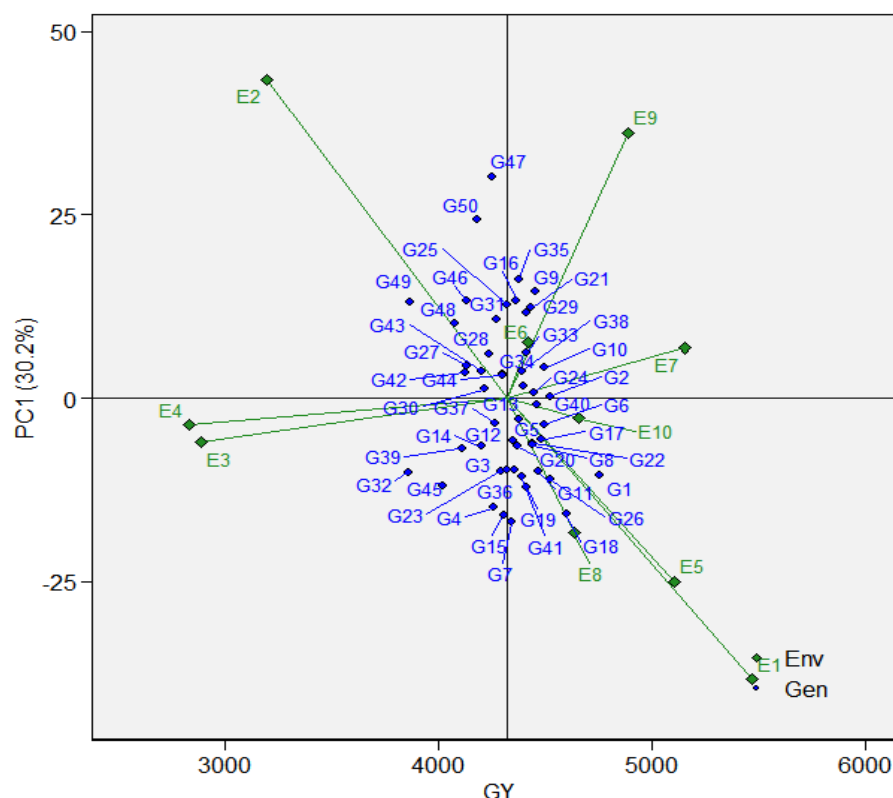


Figure 1 AMMI Biplot of yield and PC1

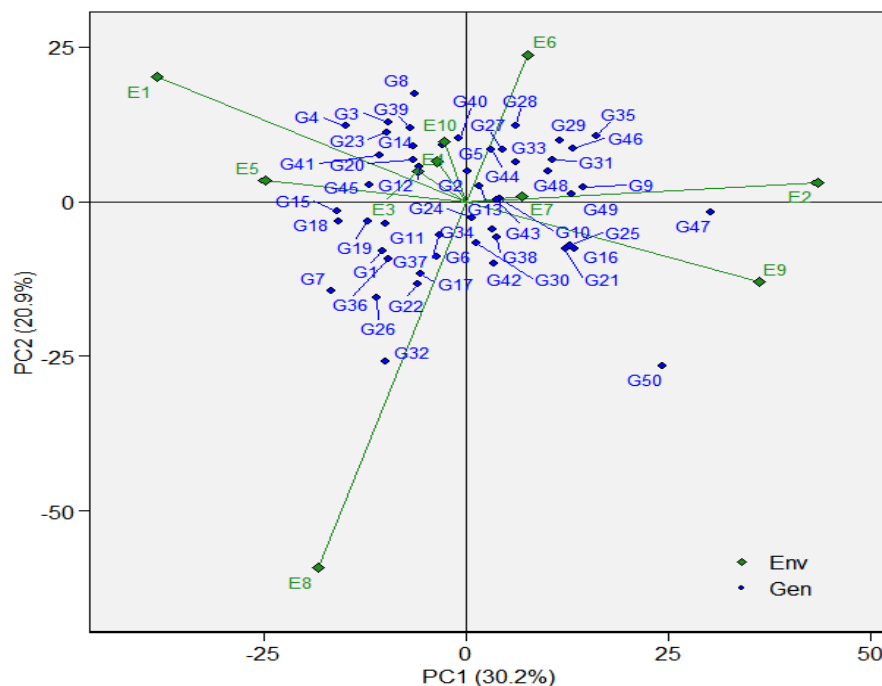
### Which-won-were

The "which-won-where" genotype, according to their forecasted yield, was illustrated, along with the identification of mega-environments, in Figure 3. Genotypes G18, G1, G7, G26, G35, G48, G46, G49, G50, and G47 exhibit a highly variable response across all environments. The genotype G-18 topped in environments E1 regarding nominal yield. The genotype G-47 performed well in environments E9 and E2, while yielding less in E1. From the environment, PC1 scores identified seven mega-environments. Regarding mega environments, E1, E5, E8, E9, and E2 fall into five separate mega environments, while E3, E4, and E10 form a single mega environment, and E7 and E6 collectively form another mega environment.

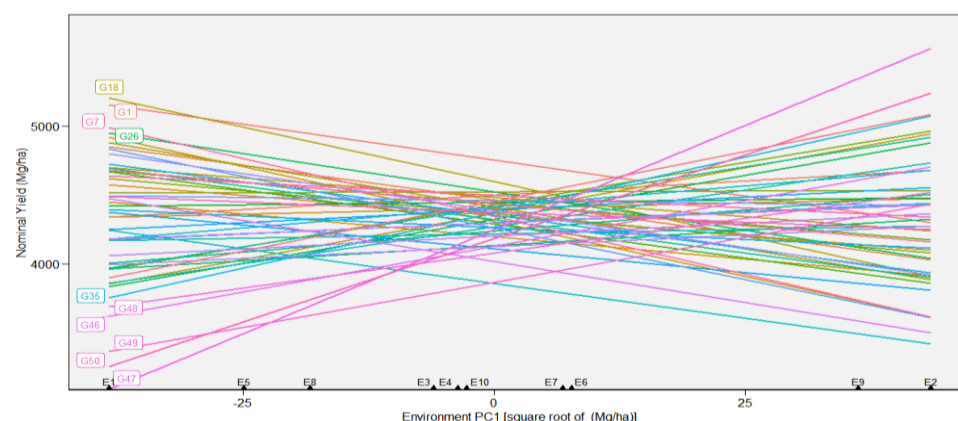
### $Y \times$ WAAS biplot

In AMMI analysis, only two PCs were used, whereas nine PCs were significant for the current data set. Significant PCAs of  $G \times E$  obtained the WAAS values. Furthermore, the average yield performance and stability of the genotypes and environments were demonstrated by plotting a graph between Y and the WAAS value. The  $Y \times$  WAAS biplot

was divided into four quadrants, which represented the four classifications of genotypes and environments.



**Figure 2.** AMMI2 Biplot among the first two IPCAs contributing more to G  $\times$  E

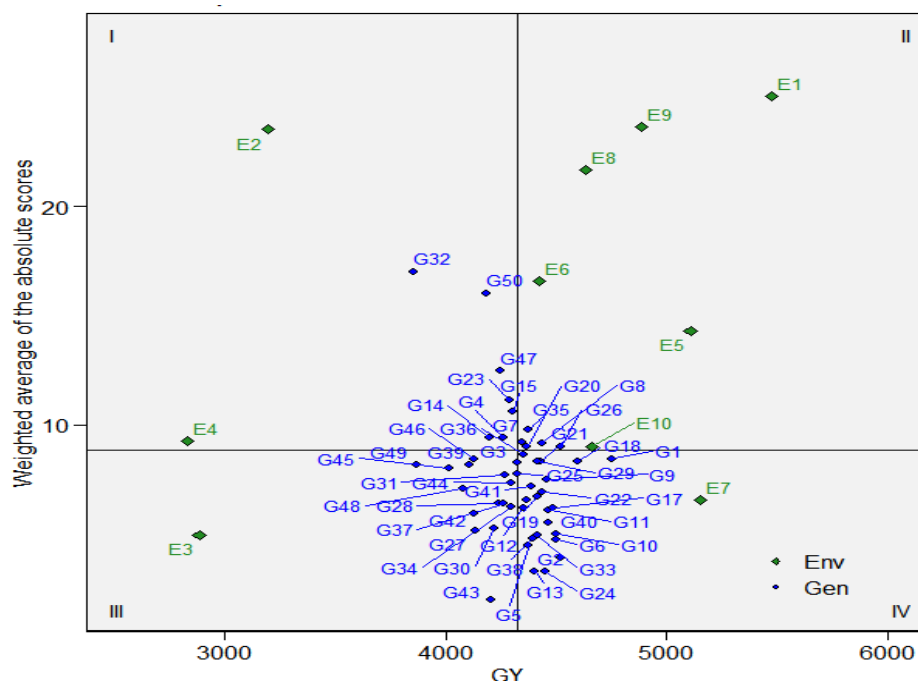


**Figure 3.** Nominal yield plot showing Which-won-were and mega environments.

Genotypes grouped in the I quadrant, i.e., G32, G50, G47, and G23, were unstable genotypes and had lower productivity than the grand mean, while environments E2 and E4 present in quadrant I were identified as highly discriminating. In quadrant II, G35, G20,



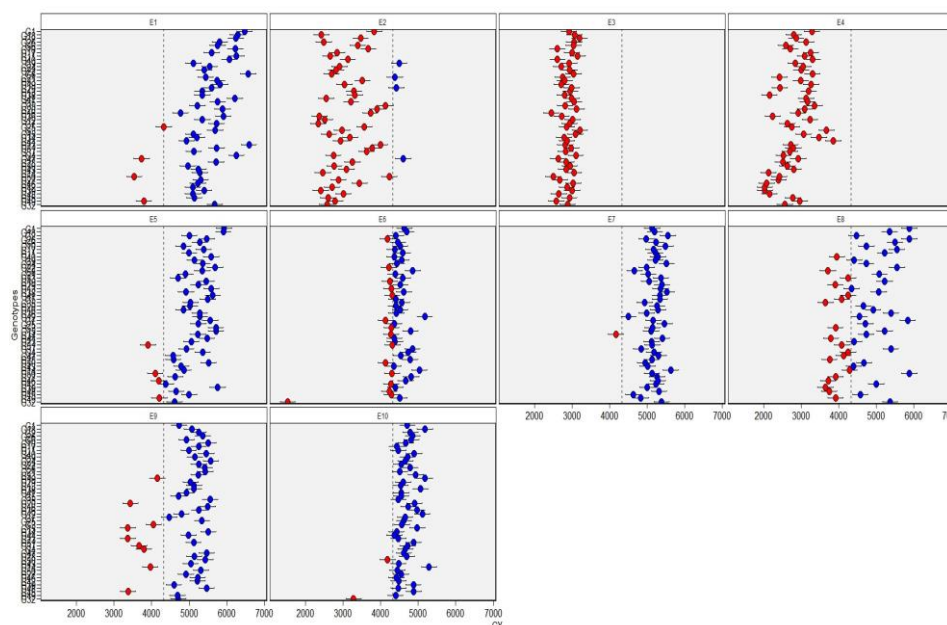
G21, and G26 were grouped, which were also poorly stable and had grain yields above the average value. The environments E1, E9, E8, E6, and E5, included in the II quadrant, provided high grain yield with good discrimination ability, as they have a high WAAS value. Genotypes, i.e., G45, G39, G42, and G43, included in the III quadrant had low grain yield, hence are less productive, but are stable, as they have a low value of WAAS. The environments E3 within quadrant III were poorly productive and had low discrimination power. The genotypes present in the IV quadrant, i.e., G1, G18, G25, G29, G13, G23, G24, G6, G10, G40, G41, and G9, were broadly adapted as they had high productivity as well as greater stability performance (lesser values of WAAS). G13 and G24 present in the IV quadrant are more stable than the rest of the genotype, as they have low WAAS values, but their performance regarding yield was just near the mean. The environment E7, included in this quadrant, was highly productive but had low discrimination aptitude (Figure 4).



**Figure 4.** Y  $\times$  WAAS biplot showing genotypes and environmental distribution into different quarters according to stability and yield

### BLUP Analysis

LRT verifies the random effects significance. GEI was significant as the probability value for the Likelihood Ratio Test value was  $p < 0.01$  (Table 4). The interaction was of a qualitative or crossover type, as the ranking of the genotypes changed across environments (Figure 5).



**Figure 5.** BLUP plot showing qualitative or crossover type GEI.

Variance components, along with different genetic parameters, were presented in Table 4. Proximally, 98.19% of the  $\sigma^2_p$  was found due to the  $\sigma^2_{ge}$ .  $\sigma^2_g$  contribution in  $\sigma^2_p$  was only 1.38%. Furthermore, fewer estimates of broad-sense heritability ( $h^2_g$ ) of 0.014 were detected, which was due to the presence of a high  $\sigma^2_{ge}$ . The genotypic accuracy of selection was also low, 0.357. The genotypic coefficient of variance was (1.376), found to be high as compared to the residual CV (0.767%) (Table 4).

BLUP-based stability indexes, i.e., the harmonic mean of genotypic values (HMGV), the relative performance of genotypic values (RPGV), and harmonic mean of relative performance of genotypic values (HMRPGV), along with their ranking, are described in Table 5. G1 was ranked 1<sup>st</sup> regarding all three stability indices of BLUP (Table 5), while the rest of the genotypes showed variation concerning ranking regarding these parameters.

**Table 4.** BLUP base variance components and genetic parameters

Statistics	Likelihood ratio test	
	G	GEI
$\chi^2$	0.393	2309.88
p value	0.530	0.0000
REML	Variance components	
	Estimates	

*Stability analysis of wheat genotypes*

Genetic variance ( $\sigma^2_g$ )	3539.75(1.38%)
GEI-induced variance ( $\sigma^2_{ge}$ )	251782.2(98.19%)
Error variance ( $\sigma^2_e$ )	1099.362(0.42%)
Variance observed in the phenotype ( $\sigma^2_p$ )	256421.27
Heritability in the broad sense ( $h^2_g$ )	0.014
Coefficient of determination of the Interaction effects ( $R^2_{ge}$ )	0.981
Mean-based heritability ( $h^2_{gm}$ )	0.123
Selection accuracy ( $A_c$ )	0.357
GE correlation ( $r_{ge}$ )	0.996
Genotypic coefficient of variation ( $CV_g$ )	1.376
Error coefficient of variation ( $CV_r$ )	0.767
$CV_g/CV_r$	1.794

**Table 5.** Ranking of the genotypes according to BLUP and WAASB-based stability indexes.

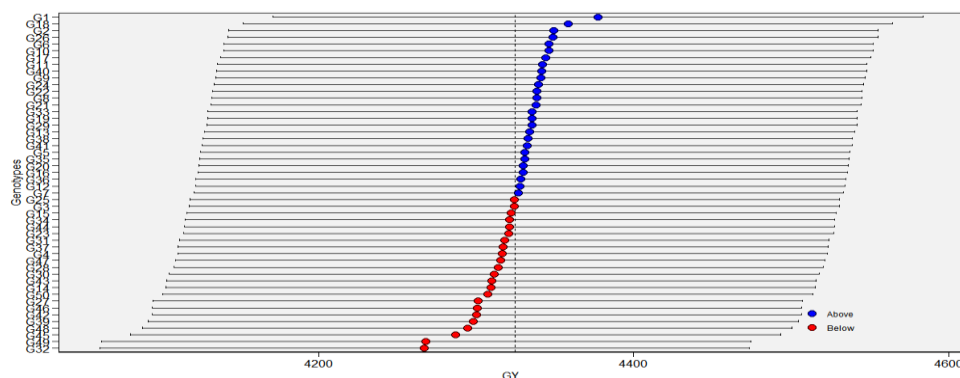
GE N	Yield	HMG V	HMG V Ranki ng	RPG V Yiel d	RPG V Ranki ng	HMRP GV Yield	HMRP GV Rankin g	WAA SB Value	WAA SB Ranki ng
<b>G1</b>	4753	4473	1	4760	1	4721	1	9	43
<b>G10</b>	4524	4163	12	4469	8	4442	7	5	7
<b>G11</b>	4322	4165	10	4454	9	4419	9	6	12
<b>G12</b>	4260	4074	27	4362	25	4306	25	6	18
<b>G13</b>	4377	4187	7	4419	19	4406	12	3	2
<b>G14</b>	4499	3775	44	4152	42	4062	42	9	40
<b>G15</b>	4344	4007	33	4318	31	4220	32	10	46
<b>G16</b>	4437	4095	25	4375	23	4335	22	6	15
<b>G17</b>	4457	4209	4	4484	6	4454	6	6	17
<b>G18</b>	4498	4155	14	4536	2	4473	3	9	35

<b>G1 9</b>	446 5	4135	16	4413	20	4375	17	7	21
<b>G2</b>	435 2	4272	2	4525	3	4513	2	4	6
<b>G2 0</b>	440 1	4174	9	4425	15	4344	21	9	38
<b>G2 1</b>	419 9	4128	21	4444	11	4380	15	7	27
<b>G2 2</b>	430 6	4135	17	4423	16	4393	14	7	23
<b>G2 3</b>	436 6	4010	32	4310	32	4212	34	11	47
<b>G2 4</b>	448 5	4131	20	4420	18	4403	13	3	3
<b>G2 5</b>	459 8	4092	26	4344	28	4308	24	7	25
<b>G2 6</b>	441 4	4156	13	4496	5	4431	8	9	42
<b>G2 7</b>	436 9	3818	42	4103	44	4078	41	6	11
<b>G2 8</b>	443 3	3954	36	4228	38	4197	36	6	16
<b>G2 9</b>	444 0	4132	18	4436	13	4366	18	8	29
<b>G3</b>	429 2	4132	19	4377	22	4306	26	8	31
<b>G3 0</b>	444 8	3929	37	4201	41	4176	40	5	9
<b>G3 1</b>	432 4	4062	28	4306	33	4254	30	8	30
<b>G3 2</b>	452 1	3268	50	3836	50	3438	50	14	49
<b>G3 3</b>	413 3	4175	8	4423	17	4407	11	5	8
<b>G3 4</b>	424 0	4104	23	4341	29	4294	28	6	14
<b>G3 5</b>	441 4	4193	6	4441	12	4365	19	10	44
<b>G3 6</b>	421 8	3850	41	4265	36	4189	38	8	32
<b>G3 7</b>	427 0	4028	31	4293	34	4233	31	6	19
<b>G3 8</b>	385 9	3998	34	4346	27	4302	27	4	5

*Stability analysis of wheat genotypes*

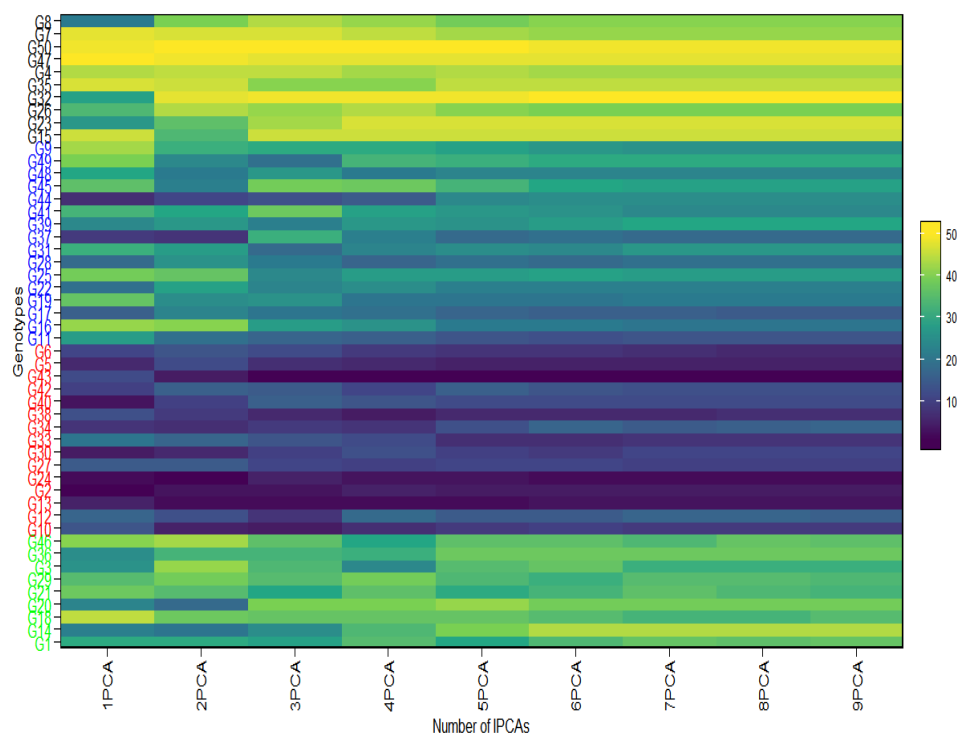
<b>G3</b> <b>9</b>	441 4	3670	49	4048	46	3963	48	8	28
<b>G4</b>	429 9	3928	38	4234	37	4179	39	9	39
<b>G4</b> <b>0</b>	437 4	4151	15	4447	10	4408	10	6	13
<b>G4</b> <b>1</b>	435 4	4061	29	4363	24	4321	23	7	24
<b>G4</b> <b>2</b>	426 4	3739	46	4081	45	4026	45	7	20
<b>G4</b> <b>3</b>	439 1	3982	35	4209	40	4207	35	3	1
<b>G4</b> <b>4</b>	410 9	4095	24	4349	26	4278	29	7	22
<b>G4</b> <b>5</b>	446 3	3775	45	4031	48	3975	47	9	36
<b>G4</b> <b>6</b>	438 8	3815	43	4126	43	4056	44	9	34
<b>G4</b> <b>7</b>	412 8	4036	30	4331	30	4191	37	12	48
<b>G4</b> <b>8</b>	420 4	3727	47	4039	47	3996	46	8	33
<b>G4</b> <b>9</b>	429 7	3691	48	3908	49	3859	49	10	45
<b>G5</b>	401 8	4164	11	4396	21	4378	16	4	4
<b>G5</b> <b>0</b>	412 9	3865	40	4226	39	4056	43	15	50
<b>G6</b>	425 0	4198	5	4483	7	4466	4	5	10
<b>G7</b>	407 9	3924	39	4291	35	4217	33	9	37
<b>G8</b>	386 7	4120	22	4431	14	4357	20	9	41
<b>G9</b>	418 4	4256	3	4509	4	4458	5	7	26

BLUP-based predicted grain yield for the 50 genotypes is shown in Figure 6. Blue circles signify the genotypes that had above-average BLUP, and red circles signify the genotypes that had below-average BLUP. Hence, genotype G1 followed by G18 had the highest BLUP (Figure 6).



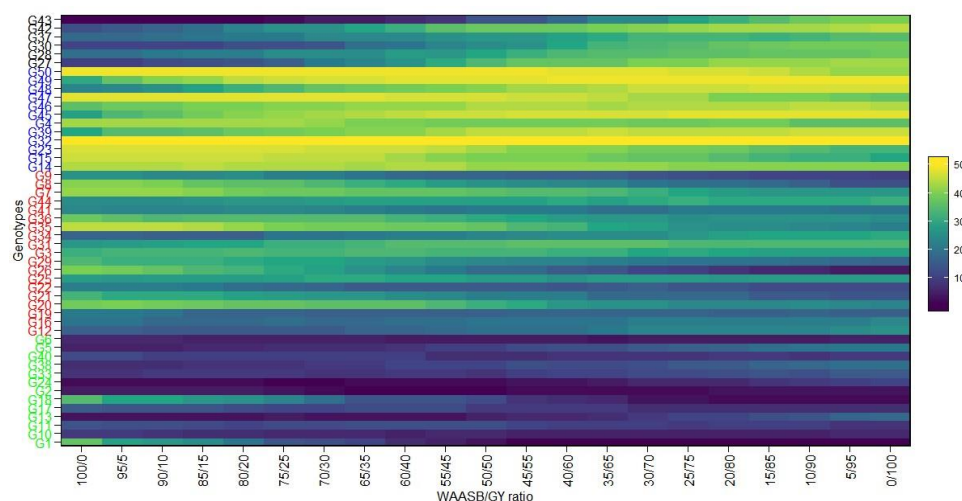
**Figure 6.** Based on the predicted grain yield of the 50 wheat genotypes

To illustrate the ranking of genotypes in terms of stability using WAASB analysis, a heatmap was developed based on the number of IPCAs utilized in the WAASB estimation (Figure 9). Genotypes with identical stability performance were grouped and are indicated by similar-colored letters on the left side of the heatmap. Genotypes highlighted with red color, i.e., G6, G5, G43, G42, G40, G36, G34, G33, had low WAASB values considering all IPCA and therefore were the group of highly stable genotypes (highlighted with red color text). Genotypes highlighted with black color, i.e., G8, G7, G50, G47, G4, G35, G32, G26, G23, and G15, had high WAASB values considering all IPCA and therefore were the group of least stable genotypes (Figure 7).



**Figure 6.** Ranking of genotypes according to significant IPSA used in WAASB

However, Figure 08 shows how the ranking of the genotypes changes with the change of weights assigned to WAASB/GY. The ranks in the left-most side column of the heat map are achieved while stability was the only choice; when we move from left to right, the weight for the grain yield increased by 5% in each scenario, while the weight of the stability decreased. Furthermore, clustering of the genotypes, which are colored differently and depicted on the left side of Figure 08, was used to identify groups of genotypes that performed similarly in terms of stability and productivity. Genotypes, i.e., G6, G5, G40, G33, G38, G24, G2, G18, G17, G13, G11, G10, and G1 within a green color cluster, were identified as productive and well-adapted genotypes. Genotypes with blue color cluster G50, G49, G48, G47, G46, G45, G4, G39, G32, G23, G15 and G14 were poorly productive and unstable. G43, G42, and G27 genotypes, which were included in the black color cluster, were stable but ranked lower in yield. Red color cluster genotypes, such as G9, G8, G7, and G44, are moderately productive and stable genotypes (Figure 8).



**Figure 7.** Clustering of the 50 wheat genotypes according to WAASB/GY ratio.

## DISCUSSION

Multilocation/multi-environment trials are crucial for the approval of a variety for general cultivation in plant breeding programs. Prior to cultivar recommendations for general cultivation, prediction accuracy plays a critical role in successfully identifying genotypes with greater and consistent yields, as well as demarcating mega-environments. The primary obstacle to finding a stable genotype with high yield potential is GEI, which alters the genotype's performance in different environments (Yan & Fréneau-Reid, 2018). AMMI (Verma & Singh, 2021), BLUP (Sood et al., 2020), and WAASB (T. Olivoto et al., 2019) methods have been used for this purpose. All of these techniques have been applied in the current investigation to identify more stable and higher-yielding genotypes.

First, the combined ANOVA revealed the significance of GEI in the current study. According to the estimation of variance components based on REML/BLUP, the  $\sigma^2_{ge}$  is 98.19%, whereas the  $\sigma^2_g$  is only 1.38%. It was also discovered that the broad sense heritability was low due to high  $\sigma^2_{ge}$ .

From the AMMI biplots, G2 was identified as a highly stable genotype. Similarly, WAASB also identifies G2 as a stable one. However, highly contradictory results were obtained when BLUP-based stability indexes were used for identifying stable genotypes. HMGV, RPGY, and HMPRGY found G1 as the most stable. These paradoxes highlight a crucial idea about "stability." The term "high stability" is valid only with high mean performance. Considering this concept, G43 was identified as highly "stable", but their performance is just near the mean, so they are not considered well. This means that they were not suitable for cultivation in all environments; however, this indicates that the performance of these genotypes was relatively stable. However, the genotypes G2 and G1, along with stability, had high mean performance. So, they are suitable genotypes for general cultivation. These results should also make it clear that identifying and choosing



only stable genotypes may be deceptive. “Stable” genotypes are required only when they have greater average performance along with stability.

The G1 genotype had the highest BLUP-based predicted grain yield and was identified as a desirable genotype through BLUP.  $Y \times WAAS$  also grouped the G1 in highly productive and broadly adapted genotypes in the quarter. However, regarding WAASBY, G1 is in second position after G2. In addition, WAASB was found to be a more effective model than AMMI and BLUP for grouping genotypes based on mean and stability performance. WAASB considers all the IPCA when quantifying stability, whereas AMMI considers only the first two IPCA, which contribute 51.1% to GEI, and ignores IPCA 3, which contributes 15.8% to GEI. (Hussain et al., 2021) Also found WAASB helpful in identifying high-yielding and stable chickpea genotypes for drought-susceptible regions.

### **Conclusion**

We concluded that while AMMI, BLUP, and WAASB are three multivariate methods that can be used to select stable genotypes, using just one method could be misleading because stable genotypes can change when stability methods are changed. This conclusion was based on the evaluation of 50 wheat genotypes in ten environments. Breeders should focus on selecting stable genotypes with good mean performance because only stable genotypes may be low yielders and unsuitable for widespread cultivation.  $Y \times WAAS$  and WASSBY graphs were found to be more helpful for this objective since they categorized the genotypes into groups based on their stability and productivity performance. Consequently, out of the 50 genotypes, genotypes G1 and G2 were chosen as the most stable and highly productive genotypes. Before releasing high-yielding stable genotypes for general cultivation, we advise breeders to use various multivariate methods to find them. This will help to improve the crop's yield per unit area by minimizing GEI.

### **Competing Interests**

The authors declare that the research was conducted without any commercial or financial relationships that could be perceived as a potential conflict of interest.

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### **References**

- Aarthi, S., Suresh, J., Leela, N. K., & Prasath, D. (2020). Multi-environment testing reveals genotype-environment interaction for curcuminoids in turmeric (*Curcuma longa* L.). *Industrial Crops and Products*, 145, 1–8. <https://doi.org/10.1016/j.indcrop.2020.112090>
- Ahakpaz, F., Abdi, H., Neyestani, E., Hesami, A., Mohammadi, B., Mahmoudi, K. N., . . . Alipour, H. (2021). Genotype-by-environment interaction analysis for grain yield of barley genotypes under dryland conditions and the role of monthly

- rainfall. *Agricultural Water Management*, 245, 1–9.  
<https://doi.org/10.1016/j.agwat.2020.106665>
- De Leon, N., Jannink, J. L., Edwards, J. W., & Kaeppler, S. M. (2016). Introduction to a special issue on genotype by environment interaction. *Crop Science*, 56(5), 2081–2089. <https://doi.org/10.2135/cropsci2016.07.0002in>
- Dempster, A. P., Laird, N. M., & Rubin, D. B. (1977). Maximum Likelihood from Incomplete Data Via the EM Algorithm. *Journal of the Royal Statistical Society: Series B (Methodological)*, 39(1), 1–22.  
<https://doi.org/https://doi.org/10.1111/j.2517-6161.1977.tb01600.x>
- Filho, J. M. C., Marcos Deon Vilela de Resende, Orlando Peixoto de Moraes, Adriano Pereira de Castro, Élcio Perpétuo Guimarães, José Almeida Pereira, . . . Breseghello, F. (2013). Upland rice breeding in Brazil: a simultaneous genotypic evaluation of stability, adaptability and grain yield. *Euphytica*, 192(1), 117–129.  
<https://doi.org/10.1007/s10681-013-0922-2>
- Fox, P. N., J., C., & I., R. (1997). Multi-environment testing and genotype  $\times$  environment interaction. In *Statistical Methods for Plant Variety Evaluation*. (pp. 117–138).
- Hussain, T., Akram, Z., Shabbir, G., Manaf, A., & Ahmed, M. (2021). Identification of drought tolerant Chickpea genotypes through multi trait stability index. *Saudi Journal of Biological Sciences*, 28(12), 6818–6828.  
<https://doi.org/10.1016/j.sjbs.2021.07.056>
- Kindie, Y., Tesso, B., & Amsalu, B. (2022). AMMI and GGE biplot analysis of genotype by environment interaction and yield stability in early maturing cowpea [*Vigna unguiculata* (L) Walp] landraces in Ethiopia. *Plant-Environment Interactions*, 3(1), 1–9. <https://doi.org/10.1002/pei3.10068>
- Malosetti, M., Ribaut, J. M., & van Eeuwijk, F. A. (2013). The statistical analysis of multi-environment data: Modeling genotype-by-environment interaction and its genetic basis. *Frontiers in Physiology*, 4, 1–17.  
<https://doi.org/10.3389/fphys.2013.00044>
- Olivoto, Lúcio, A. D. C., da Silva, J. A. G., Marchioro, V. S., de Souza, V. Q., & Jost, E. (2019). Mean performance and stability in multi-environment trials I: Combining features of AMMI and BLUP techniques. *Agronomy Journal*, 111(6), 1–12.  
<https://doi.org/10.2134/agronj2019.03.0220>
- Olivoto, T., & Lúcio, A. D. C. (2020). metan: An R package for multi-environment trial analysis. *Methods in Ecology and Evolution*, 11(6), 783–789.  
<https://doi.org/https://doi.org/10.1111/2041-210X.13384>
- Olivoto, T., Lúcio, A. D. C., da Silva, J. A. G., Sari, B. G., & Diel, M. I. (2019). Mean performance and stability in multi-environment trials II: Selection based on multiple traits. *Agronomy Journal*, 111(6), 2961–2969.  
<https://doi.org/10.2134/agronj2019.03.0221>
- Pour-Aboughadareh, A., Khalili, M., Pocza, P., & Olivoto, T. (2022). Stability Indices to Deciphering the Genotype-by-Environment Interaction (GEI) Effect: An Applicable Review for Use in Plant Breeding Programs. In *Plants* (Vol. 11, pp. 1–24).

- Santos, F., & Marza, y. F. (2020). Selection of forage oat genotypes through GGE Biplot and BLUP. *bioRxiv*, 1–9. <https://doi.org/https://doi.org/10.1101/2020.03.10.986422>
- Sitairesmi, T., Suwarno, W. B., Gunarsih, C., Nafisah, Nugraha, Y., Sasmita, P., & Daradjat, A. A. (2019). Comprehensive stability analysis of rice genotypes through multi-location yield trials using PBSTAT-GE. *SABRAO Journal of Breeding and Genetics*, 51(4), 355–372.
- Sood, S., Bhardwaj, V., Kumar, V., & Gupta, V. K. (2020). BLUP and stability analysis of multi-environment trials of potato varieties in sub-tropical Indian conditions. *Heliyon*, 6(11), 1–10. <https://doi.org/10.1016/j.heliyon.2020.e05525>
- Verma, A., & Singh, G. P. (2021). Stability, Adaptability Analysis of Wheat Genotypes by AMMI with BLUP for Restricted Irrigated Multi Location Trials in Peninsular Zone of India. *Agricultural Sciences*, 12(03), 198–212. <https://doi.org/10.4236/as.2021.123013>
- Yaghotipoor, A., Farshadfar, E., & Saeidi, M. (2017). Evaluation of phenotypic stability in bread wheat accessions using parametric and non-parametric methods. *The Journal of Animal and Plant Science*, 27(4), 1269–1275.
- Yan, W., & Frégeau-Reid, J. (2018). Genotype by Yield\*Trait (GYT) Biplot: A Novel Approach for Genotype Selection based on Multiple Traits. *Scientific Reports*, 8(1), 1-10. <https://doi.org/10.1038/s41598-018-26688-8>
- Yan, W., Hunt, L. A., Sheng, Q., & Szlavics, Z. (2000). Cultivar evaluation and mega-environment investigation based on the GGE biplot. *Crop Science*, 40(3), 597–605. <https://doi.org/10.2135/cropsci2000.403597x>
- Yan, W., & Tinker, N. A. (2006). Biplot analysis of multi-environment trial data: Principles and applications. *Canadian Journal of Plant Science*, 86, 623–645. <https://doi.org/10.4141/P05-169>
- Yue, H., Gauch, H. G., Wei, J., Xie, J., Chen, S., Peng, H., . . . Jiang, X. (2022). Genotype by Environment Interaction Analysis for Grain Yield and Yield Components of Summer Maize Hybrids across the Huanghuaihai Region in China. *Agriculture (Switzerland)*, 12(5), 1-17. <https://doi.org/10.3390/agriculture12050602>
- Zobel, R. W., Wright, M. J., & Gauch Jr., H. G. (1988). Statistical Analysis of a Yield Trial. *Agronomy Journal*, 80(3), 388–393. <https://doi.org/https://doi.org/10.2134/agronj1988.00021962008000030002x>

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