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Ashkboos Amini
Agricultural Research, Education and Extension Organization (AREEO)
Ali Akbar Asadi
asadipm@gmail.com

Agricultural Research, Education and Extension Organization (AREEO)
Hamidreza Nikkhah Chamanabad
Agricultural Research, Education and Extension Organization (AREEO)
Mehrdad Chaichi
Agricultural Research, Education and Extension Organization (AREEO)
Alireza Eivazi
Agricultural Research, Education and Extension Organization (AREEO)
Masoud Ezzat -Ahmadi
Agricultural Research, Education and Extension Organization (AREEO)
Marefat Ghasemi Kalkhoran
Agricultural Research, Education and Extension Organization (AREEO)
Adel Ghadiri
Agricultural Research, Education and Extension Organization (AREEO)
Seyyed Karim Hosseini Bay
Agricultural Research, Education and Extension Organization (AREEO)
Parviz Salehi
Agricultural Research, Education and Extension Organization (AREEO)
Nader Mir Fakhraei
Agricultural Research, Education and Extension Organization (AREEO)
Seyyed Mohammad Mehdi Mir Fattah
Agricultural Research, Education and Extension Organization (AREEO)

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Comparison of Results from AMMI and REML/BLUP Methods in Assessing the Adaptability and Stability of Wheat Genotypes Yield

Ashkboos Amini[1], Ali Akbar Asadi (Corresponding Author)[*2], Hamidreza Nikkhah Chamanabad[3], Mehrdad Chaichi[4], Alireza Eivazi[5], Masoud Ezzat -Ahmadi[6], Marefat Ghasemi Kalkhoran[7], Adel Ghadiri[8], Seyyed Karim Hosseini Bay[9], Parviz Salehi[10], Nader Mir Fakhraei[11], Seyyed Mohammad Mehdi Mir Fattah[8],

1-Research Associate Professor, Seed and Plant Improvement Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran,
Ashkboos Amini ORCID ID: 0000-0001-9472-6918
2-Research Assistant Professor, Zanjan Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Zanjan, Iran,
Ali Akbar Asadi ORCID ID: 0000-0002-6096-0850
3-Research Assistant Professor, Razavi Khorasan Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Mashhad, Iran,
Marefat Ghasemi Kalkhoran ORCID ID: 0009-0006-7867-7873
4-Research Assistant Professor, Hamedan Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Hamedan, Iran,
Mehrdad Chaichi ORCID ID: 0000-0002-7757-8762
5-Research Associate Professor, West Azarbaijan Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Urmia, Iran,
Alireza Eivazi ORCID ID: 0000-0002-7167-2774
6-Research Assistant Professor, Razavi Khorasan Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Mashhad, Iran,
Masoud Ezzat -Ahmadi ORCID ID: 0000-0002-1574-7073
7-Research Assistant Professor, Ardabil Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Ardabil, Iran,
Marefat Ghasemi Kalkhoran ORCID ID: 0000-0002-9987-0609
8-Researcher, Markazi Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Arak, Iran,
Adel Ghadiri ORCID ID: 0000-0002-9046-6650
Seyyed Mohammad Mehdi Mir Fattah ORCID ID: 0009-0002-8226-3148
9-Researcher, Gazvin Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Gazvin, Iran,
Seyyed Karim Hosseini Bay ORCID ID: 0000-0002-0839-6543
10-Researcher, Fars Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Shiraz, Iran,
Parviz Salehi ORCID ID: 0000-0001-5941-0280
11-Researcher, East Azarbaijan Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Tabriz, Iran
Nader Mir Fakhraei ORCID ID: 0000-0002-5054-7229
***: Corresponding author Email**: asadipm@gmail.com

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Abstract
Genotype × environment interaction (GEI) is a major source of variation in multi-environment trials (METs) and complicates the identification of wheat genotypes with high yield and stable yield across diverse environments. Although the Additive Main Effects and Multiplicative Interaction (AMMI) model is widely used to investigate GEI, its interpretation may be limited when the first interaction principal components explain only a small proportion of the total interaction variance. This study compared the conventional AMMI model with a Restricted Maximum Likelihood/Best Linear Unbiased Prediction (REML/BLUP) approach for evaluating grain yield stability and adaptability of bread wheat genotypes grown under irrigated conditions in the cold regions of Iran. Twenty bread wheat genotypes were evaluated across nine environments during two growing seasons using a randomized complete block design with three replications. Combined analysis of variance, AMMI analysis, and mixed-model analyses were performed, and stability was assessed using conventional AMMI statistics together with the WAASB and WAASBY indices. Environmental effects, genotype × environment interaction, and genotype effects were all significant ($P < 0.01$), with the environment accounting for 61.0% of the total variation, followed by GEI (18.4%) and genotype (3.0%). The first two AMMI interaction principal components explained 47.18% of the total interaction variance, indicating that a substantial proportion of GEI remained in higher-order components. Consequently, BLUP-based stability statistics, which incorporate all significant interaction principal components, provided a more comprehensive assessment of genotype performance than conventional AMMI biplots. Among the evaluated genotypes G9, G3, G15 and G18 consistently combined high grain yield with specific adaptation and stable yield across environments. Overall, the integration of AMMI and REML/BLUP improved genotype discrimination and provided a more reliable framework for selecting superior wheat genotypes in multi-environment trials.
Keywords: AMMI; BLUP; Genotype × environment interaction; grain yield stability; WAASB; bread wheat

1. Introduction

The identification of crop genotypes that combine high productivity, specific adaptation, and yield stability across diverse environments remains one of the principal objectives of plant breeding programs. Because genotype performance is strongly influenced by environmental conditions, the genotype × environment interaction (GEI) has become a major source of variation in multi-environment trials (METs). A significant GEI complicates cultivar recommendation because genotypes often respond differently to environmental changes, reducing the efficiency of selection based solely on mean grain yield (Finlay & Wilkinson, 1963). Consequently, evaluating both productivity and stability has become an essential component of cultivar development (Lin & Binns, 1988).

Numerous statistical approaches have been developed to investigate GEI, including parametric, non-parametric, and multivariate methods. Among multivariate techniques, the Additive Main Effects and Multiplicative Interaction (AMMI) model and the Genotype plus Genotype × Environment (GGE) biplot are the most widely applied tools for studying genotype adaptation and stability in METs (Gauch & Zobel, 1997; Yan *et al*., 2000). The AMMI model combines analysis of variance with principal component analysis, allowing both the additive effects of genotypes and environments and the multiplicative structure of GEI to be investigated simultaneously. In addition to its statistical efficiency, the graphical interpretation provided by AMMI biplots has made this method one of the most frequently used approaches in crop breeding research (Van Eeuwijk *et al*., 2016). Numerous studies have successfully applied AMMI analysis to evaluate wheat performance under diverse environmental conditions (Mohammadi *et al*., 2015;

Karimizadeh *et al*., 2016, 2017; Esmaeilzadeh Moghaddam *et al*., 2018; Najafi Mirak *et al*., 2018; Amini *et al*., 2023, 2025).

To facilitate genotype ranking, several stability statistics have been derived from the AMMI decomposition, including ASV, ASI, ASTAB, SIPC, EV, DA, DZ, FA, AVAMGE, MASV, and MASI (Annicchiarico, 1997; Purchase *et al*., 2000; Rao & Prabhakaran, 2005; Jambhulkar *et al*., 2014; Ajay *et al*., 2018). These indices summarize different aspects of genotype stability and have been widely used to identify genotypes with either broad or specific adaptation. Nevertheless, most AMMI-based interpretations rely primarily on the first one or two interaction principal components. When these components explain only a limited proportion of the total GEI, a substantial amount of interaction information remains in the higher-order principal components, potentially reducing the reliability of genotype classification.

An alternative strategy for analyzing MET data is provided by linear mixed models using the Restricted Maximum Likelihood (REML) and Best Linear Unbiased Prediction (BLUP) framework. In contrast to fixed-effect approaches, mixed models estimate genetic values while accounting for random environmental effects and genotype × environment interaction, resulting in more accurate prediction of genotype performance (Smith *et al*., 2005; Olivoto *et al*., 2019a). BLUP has therefore become an important tool in modern breeding programs because genotype selection is based on predicted breeding values rather than simple phenotypic means (Quddus *et al*., 2019). In addition, mixed models enable a more flexible partitioning of GEI into its underlying variance components, providing a more realistic representation of genotype performance across environments (Yang, 2002).

To combine the strengths of AMMI and BLUP, Olivoto *et al*. (2019a) proposed the Weighted Average of Absolute Scores from BLUP (WAASB) index. Rather than decomposing the original phenotypic observations, WAASB applies singular value decomposition to the BLUP-derived genotype × environment interaction matrix, thereby incorporating information from all significant interaction principal components into a single stability statistic. Consequently, WAASB is less affected by extreme observations and generally provides a more complete representation of genotype stability than conventional AMMI statistics. Furthermore, the WAASBY index extends this approach by integrating grain yield and WAASB into a single selection criterion, allowing breeders to assign different weights to productivity and stability according to specific breeding objectives.

Although WAASB and WAASBY have recently been applied in several studies on wheat and durum wheat (Karimizadeh *et al*., 2021; Mousavi *et al*., 2023; Amini *et al*., 2025), direct comparisons between conventional AMMI analysis and the integrated AMMI/BLUP approach under irrigated conditions in the cold regions of Iran remain limited. A comprehensive comparison of these analytical frameworks is therefore needed to determine whether incorporating all significant interaction principal components improves the identification of stable and high-yielding genotypes.

Accordingly, the objective of the present study was to compare AMMI and REML/BLUP-based AMMI approaches for evaluating genotype adaptability and stability in wheat. Specifically, conventional AMMI stability statistics were compared with the WAASB and WAASBY indices to determine whether the combined mixed-model approach provides a more reliable assessment of genotype × environment interaction and to identify wheat genotypes with superior grain yield, specific adaptation, and stable performance under irrigated conditions in the cold regions of Iran.

2. Materials and Methods

The study was conducted using 20 bread wheat genotypes, comprising 18 advanced breeding lines selected from the National Advanced Yield Trial for winter and facultative growth habits, together with the commercial cultivars Zarrineh and Heyran, which served as check cultivars. Field experiments were established during the 2021–2022 growing season at nine research stations located in Karaj, Mashhad, Miandoab, Qazvin, Eghlid, Jolgeh Rokh, Hamedan, Ardabil, and Zanjan**,** representing diverse agro-climatic conditions under irrigated environments (Table 1). At each experimental site, the trials were arranged in a randomized complete block design (RCBD) with three replications. Standard agronomic practices recommended for each location were consistently implemented throughout the growing season to minimize management-related variation among environments. Land preparation involved primary tillage followed by

disc harrowing, two perpendicular passes with a roller, fertilizer incorporation, and the formation of irrigation furrows spaced 60 cm apart. Basal fertilization consisted of potassium sulfate and ammonium phosphate, while urea was applied in two split applications, at sowing and again during the tillering stage. Each genotype was planted in experimental plots of 6 m^2, with the seeding rate adjusted according to the thousand-kernel weight to achieve a target plant density of 450 seeds m^{-2}**.**

Before sowing, seeds were treated with the fungicide Carboxin–Thiram at a concentration of 2 gkg^{-1} seed to reduce the risk of seed-borne diseases, particularly common bunt. Weed management was performed during the vegetative growth period using a tank mixture of Puma Super (1 Lha^{-1}) and Granstar (20 gha^{-1}), following the recommended application rates for effective control of grass and broadleaf weeds. Following physiological maturity, all experimental plots were harvested individually, and grain yield was determined for each genotype in every environment. The resulting dataset was subsequently used for statistical analyses of genotype performance, adaptability, and stability across test environments.

Grain yield data obtained from each experimental environment were initially subjected to separate analyses of variance to evaluate the performance of the tested genotypes within individual locations and growing seasons. Before combining the datasets, the homogeneity of residual variances across environments was examined using Bartlett's and Hartley's Fmax tests. As the assumption of variance homogeneity was satisfied, a combined analysis of variance was subsequently conducted using pooled data from all experimental environments.

The significance of the different sources of variation was determined using the appropriate F-tests, where genotype effects were considered fixed, whereas environmental effects (locations, years, and their combinations) were treated as random. This analytical framework enabled the partitioning of phenotypic variation into its major contributing components and provided the basis for evaluating genotype × environment interaction (GEI).

To investigate the adaptability and stability of the wheat genotypes across environments, the Additive Main Effects and Multiplicative Interaction (AMMI) model was employed. This approach combines analysis of variance for the additive effects of genotypes and environments with principal component analysis (PCA) of the interaction matrix, thereby allowing a more detailed interpretation of GEI patterns. Scores of the interaction principal component axes (IPCAs) were calculated for both genotypes and environments and subsequently used to construct AMMI biplots for graphical assessment of adaptation patterns and specific genotype–environment associations (Rodriguez *et al*., 2007).

A comprehensive set of AMMI-derived stability statistics was then estimated to characterize genotype stability from different analytical perspectives. These included the AMMI Stability Value (ASV) (Purchase *et al*., 2000), Sum of the Interaction Principal Component Scores (SIPC) (Sneller *et al*., 1997), Eigenvalue Stability Parameter (EV) (Zobel *et al*., 1988), AMMI Stability Parameter (ASTB) (Rao & Prabhakaran, 2005), AMMI Stability Index (ASI) (Jambhulkar *et al*., 2014), AMMI-Based Stability Measure (FA) (Raju, 2002), Zhang's Stability Parameter (DZ) (Zhang *et al*., 1998), Annicchiarico's Stability Parameter (DA) (Annicchiarico, 1997), Modified AMMI Stability Index (MASI) (Ajay *et al*., 2018), the absolute contribution of interaction principal component scores (ZA), the Modified AMMI Stability Value (MASV), and the Average of the Absolute Values of the Modeled GEI Effects (AVAMGE) (Zali *et al*., 2012). Because individual stability statistics often emphasize different aspects of GEI, the Selection Index of Ideal Genotype (SIIG) was additionally applied to integrate the information obtained from these complementary measures and facilitate the identification of genotypes exhibiting both high stability and desirable agronomic performance (Zali *et al*., 2015)

To complement the AMMI analysis, genotype performance was further evaluated using a linear mixed model (LMM) based on the restricted maximum likelihood/best linear unbiased prediction (REML/BLUP) framework. Within this approach, genotype × environment interaction effects were predicted and subsequently summarized through singular value decomposition (SVD), allowing the interaction pattern to be partitioned into orthogonal multiplicative components suitable for stability assessment.

The significance of random effects included in the mixed model was examined using the Likelihood Ratio Test (LRT). Estimates of the variance components were obtained by the REML procedure, and the relative

contribution of each random source of variation was visualized using scree plots to facilitate interpretation of the interaction structure.

To quantify genotype stability across environments, the Weighted Average of Absolute Scores from the BLUP Matrix (WAASB) was calculated according to Equation 1 (Olivoto *et al*., 2019a). This statistic summarizes the weighted contribution of all significant interaction principal components derived from the BLUP-based decomposition, providing a comprehensive measure of genotype stability. Genotypes exhibiting smaller WAASB values were considered to have greater stability because they contributed less to the genotype × environment interaction.

Equation 1

$$WAASB_i = \frac{\sum_{k=1}^{p} |IPCA_{ik} \times EP_k|}{\sum_{k=1}^{p} E\,P_k}$$

where $WAASB_i$represents the weighted average of the absolute interaction scores for the *i*th genotype, $IPCA_{ik}$is the score of the *i*th genotype on the *k*th interaction principal component axis, and EP_kdenotes the proportion of interaction variance explained by that principal component.

Because high yield alone does not necessarily indicate specific adaptation, genotype selection was also performed using the Weighted Average of Absolute Scores and Yield (WAASBY) index, which simultaneously considers grain yield and stability. This integrated index provides a balanced ranking of genotypes by combining productivity with consistency of performance across environments.

Equation 2

$$WAASBY_i = \frac{\left[W_Y \left(\frac{GY_i}{GY_{max}} \times 100\right)\right] + \left[W_S \left(100 - \frac{WAASB_i}{WAASB_{min}}\right)\right]}{W_Y + W_S}$$

where $WAASBY_i$denotes the combined yield–stability index for the *i*th genotype; $GY_i$is the mean grain yield of the genotype; $GY_{max}$is the highest mean grain yield observed among all genotypes; $W_Y$and $W_S$are the relative weights assigned to grain yield and stability, respectively; $WAASB_i$is the stability value of the genotype; and $WAASB_{min}$corresponds to the minimum WAASB value, representing the most stable genotype in the experiment.

To identify superior genotypes from the mixed-model perspective, three additional adaptability and stability statistics were estimated: the Harmonic Mean of Genotypic Values (HMGV), the Relative Performance of Genotypic Values (RPGV), and the Harmonic Mean of the Relative Performance of Genotypic Values (HMRPGV) (Resende, 2004). These statistics simultaneously account for productivity and stability, with larger values indicating genotypes that combine superior mean performance with greater adaptability across test environments.

All analyses related to the combined analysis of variance and tests for homogeneity of residual variances were conducted using SAS software. The AMMI and REML/BLUP analyses, together with the calculation of stability statistics and graphical outputs, were performed in RStudio using the metan package for multi-environment trial analysis (Olivoto & Lúcio, 2020).

Table 1. Bread wheat genotypes evaluated in this study and their pedigrees

Genotype no.	Pedigree
G1	Zarrineh(check1)
G2	Heyran (check2)
G3	EVWYT2/Azd//Rsh*2/10120/3/ Yan7578.128//Chil/2*Star
G4	Mihan/3/CMH79A.955/Bow//Pastor
G5	Mihan/3/CMH79A.955/Bow//Pastor
G6	Weizen/Mihan//Gaspard
G7	Weizen/Mihan//Gaspard
G8	Khalij/4/Ghk"s"/Bow"s"//90Zhong87/3/Shiroodi/5/Khalij/3/Yan7578.128//Chil/2*Star
G9	Catbird/Titona//Oroum

G10	Mo/4/Nd/WW15//Lee/Fn/3/N.5/Nac/5/2*Yan7578.128//Chil/2*Star
G11	Khalij/MV17
G12	Babaga/Fiocco
G13	DORADE-5/3/PROGRESS//PYN/BAU/4/TX71A983.4/TX69D4812//PYN/3/VPM/MOS83.11.4.8//PEW
G14	KINGBIRD/BILINMIYEN96.7
G15	BABAX/LR42//BABAX*2/3/KURUKU/4/TX96V2427/5/KAUZ//ALTAR 84/AOS/3/F10S-1
G16	MV22-2000/9/KLEIBER/2*FL80//DONSK.POLUK./8/KS82W409/STEPHENS/7/ NAI /HN7// BUC /5/ F59-71~
G17	PFAU/SERI.1B//AMAD/3/KRONSTAD F2004/4/SHARK-6/5/SHARK-6
G18	AP04T8531/KS04WKS-19//KS980554-12~9
G19	DORADE-5/3/SHI#4414/CROW//GK SAGVARI/CA8055
G20	EVEREST/CEDAR

3. Results and Discussion

3.1. Homogeneity of Error Variances

Prior to conducting the combined analysis across environments, separate analyses of variance were performed for grain yield in each individual environment to evaluate the homogeneity of experimental error variances (Table 2). The assumption of homogeneous residual variances was subsequently examined using both Bartlett's and Hartley's Fmax tests. Because different statistical procedures may provide inconsistent conclusions regarding variance homogeneity, it has been recommended that more than one test be considered before making a final decision (Valizadeh & Moghaddam, 2010).

In the present study, Hartley's Fmax test was not significant, whereas Bartlett's test indicated significance at the 1% probability level. Considering the overall evidence from both tests, the residual variances among environments were regarded as sufficiently homogeneous to justify a combined analysis of variance. Nevertheless, to obtain a more reliable assessment of genotype performance and stability, the data were further analyzed using both the AMMI approach and the REML/BLUP mixed-model methodology.

Table 2. Experimental error variances across environments and homogeneity of variance tests.

Year	df	Karaj	Qazvin	Eqlid	Hamedan	Ardabil	Miandoab	Mashhad	Jolge-Rokh	Zanjan
Year1	38	0.3499	0.5701	0.5509	0.3428	05053	0.4786	0.4127	0.6067	0.4534
Year2	38	0.5528	0.4719	0.6254	0.5094	0.6272	0.4041	0.5245	0.612	0.4025
Hartley's F_{max} = 1.815 ^{ns}										
Bartlet's statistic = 46.64**										

**: Significant at 1% probability levels,

3.2. Combined analysis of variance

The combined analysis of variance revealed significant differences among the evaluated sources of variation (Table 3). The main effects of year, location, genotype, and the genotype × location interaction were significant at the 5% probability level, whereas the interactions year × location and genotype × year × location were highly significant ($P < 0.01$). The significance of the three-way interaction demonstrates that genotype performance was not consistent across years and locations, indicating that the response of individual genotypes depended on the specific environmental conditions in which they were evaluated. Similar findings have been reported by Amini *et al*. (2023), who emphasized that substantial genotype × environment interaction (GEI) complicates the identification of specific adapted genotypes.

Variation in genotype yield across environments is primarily attributable to differences in genetic constitution and the differential expression of genes under contrasting climatic and geographical conditions. Environmental factors may alter the magnitude of gene expression, resulting in changes in the relative performance of genotypes from one environment to another (Jafari & Farshadfar, 2018). Partitioning of the total sum of squares indicated that environmental effects accounted for the largest proportion of the observed variation (61%), followed by the genotype × environment interaction (18.4%), whereas the genotypic effect contributed only 3% of the total variation (Table 3). The predominance of environmental

variation reflects the substantial diversity among the test environments and agrees with previous reports in wheat multi-environment trials (Ehyaei *et al*., 2022; Amini *et al*., 2023).

The relatively small proportion of variation attributed to genotype may indicate that the advanced breeding lines evaluated in this study had already undergone intensive selection in earlier breeding stages, thereby reducing genetic variability for grain yield (Golkari *et al*., 2016). Although environmental effects represented the major source of phenotypic variation, the comparatively smaller contribution of the GEI component suggests that several genotypes maintained relatively stable performance across the evaluated environments (Ehyaei *et al*., 2022).

Based solely on mean grain yield, G11, G2, G3, G9, G1, and G8 were identified as the highest-yielding genotypes, whereas G14, G13, G5, and G4 produced the lowest grain yields. However, because the genotype × environment interaction was statistically significant, ranking genotypes exclusively according to their average yield could lead to misleading conclusions regarding their agronomic superiority. Consequently, stability and adaptability analyses are required to distinguish genotypes that combine high productivity with consistent yield across diverse environments. This conclusion is further supported by the distribution of genotype mean yields across environments (Fig. 1), where noticeable changes in genotype ranking were observed from one environment to another. Such crossover responses demonstrate that genotype evaluation based solely on average grain yield is insufficient for cultivar recommendation. Instead, reliable selection requires the simultaneous assessment of productivity, adaptability, and stability over multiple years and locations, as also suggested by Olivoto *et al*. (2019).

Table 3. Combined analysis of variance for grain yield in bread wheat genotypes

Source of variation	Digree of freedom	Sum of square	Mean square	Percentage of total sum of square
Year	1	175.28	175.28*	8.3
Location	8	943.17	117.89*	44.3
Year×Location	8	177.22	22.152**	8.4
Block (Year×Location)	36	26.01	0.722	1.2
Genotype	19	61.234	3.223*	2.9
Year×Genotype	19	17.468	0.919	0. 8
Location×Genotype	152	215.331	1.417*	10. 2
Year×Location×Genotype	152	157.214	1.034**	7.4
Error	684	342.39	0.50058	16.2
CV(%)			9.41	

* and **: Significant at 5% and 1% probability levels, respectively.

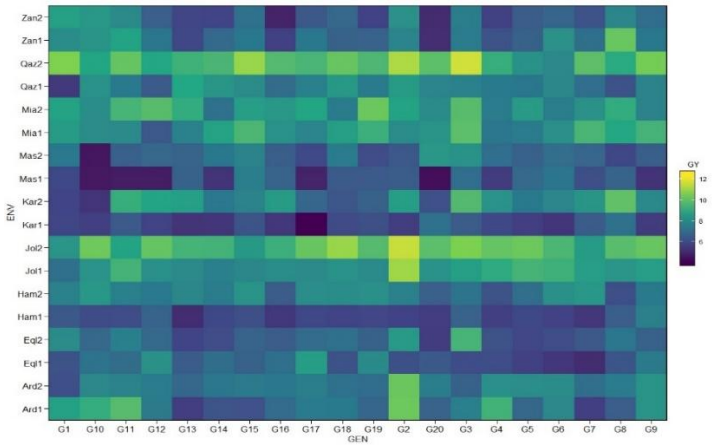


Fig. 1. Heatmap showing the grain yield of bread wheat genotypes across environments.

3.3. AMMI Analysis

The AMMI analysis of variance revealed that the first seven interaction principal component axes (IPCAs) were highly significant ($P < 0.01$), indicating that a substantial proportion of the genotype × environment interaction (GEI) was captured by these multiplicative components (Table 4). Collectively, the significant IPCAs accounted for approximately 85.7% of the total GEI variation, demonstrating that the AMMI model effectively described the interaction pattern observed across the experimental environments. Among the extracted interaction axes, IPCA1 and IPCA2 explained the largest proportion of the interaction sum of squares, jointly accounting for 47.4% of the total GEI variation. Because these two components retained nearly half of the interaction information, they provide a reliable basis for graphical interpretation of genotype responses and for evaluating genotype adaptation and stability across environments.

The first two IPCAs have frequently been used as the foundation for AMMI-based stability assessment because they summarize the most important interaction patterns while reducing the complexity of multi-environment data. Consequently, numerous stability statistics and AMMI biplots are derived primarily from these components to facilitate the identification of specific adapted and specifically adapted genotypes (Purchase *et al*., 2000; Grausgruber *et al*., 2000; Taherian *et al*., 2019; Amini *et al*., 2023).

The proportion of GEI variance explained by each interaction principal component reflects its relative contribution to describing the interaction structure. Components explaining larger percentages of the interaction variation generally provide greater discriminatory power and improve the interpretation of genotype adaptation across environments. Nevertheless, the proportion of GEI captured by the first two IPCAs is not constant and varies depending on the crop species, the diversity of testing environments, and the genetic constitution of the evaluated materials. Although IPCA1 and IPCA2 represented the largest share of the interaction variance, together they explained less than half of the total GEI. Similar observations have been reported in previous wheat studies, where a considerable proportion of the interaction was distributed among subsequent IPCAs, reflecting the complexity of genotype responses under diverse environmental conditions (Karimizadeh *et al*., 2021; Aghaee Sarbezeh *et al*., 2012; Amini *et al*., 2023)

Table 4. AMMI analysis of variance for grain yield of promising bread wheat genotypes.

Source	DF	Sum of square	Mean square	Percentage of sum of square	Accumulative percentage of sum of square
ENV	17	1295.709	76.218**		
REP(ENV)	36	26.012	0.723		
GEN	19	61.239	3.223**		
GEN:ENV	323	390.011	1.207**		
PC1	35	120.304	3.437**	30.8	30.8
PC2	33	64.371	1.951**	16.5	47.4
PC3	31	42.628	1.375**	10.9	58.3
PC4	29	30.727	1.060**	7.9	66.2
PC5	27	30.514	1.130**	7.8	74
PC6	25	23.976	0.959**	6.1	80.1
PC7	23	21.656	0.942**	5.6	85.7
Noise	120	55.835	0.465		
Residuals	684	342.390	0.501		
Total	1402	2505.372	1.787		

** and *: significant at the level of 1 and 5%, respectively

3.4. AMMI1 and AMMI2 Biplot Interpretation

Genotypes with IPCA1 scores close to zero are generally considered less responsive to environmental variation because they contribute minimally to the genotype × environment interaction (GEI). In the present study, G8, G6, G9, G3, G18, and G15 exhibited IPCA1 values near zero, indicating relatively high stability across the test environments. Among these, G8, G9, G3, G18, and G15 also produced grain yields above the overall mean, suggesting that they combined superior productivity with specific adaptation and could therefore be regarded as desirable genotypes for cultivation across a wide range of environments (Table 5; Fig. 1).

Conversely, genotypes G2, G7, and G10 displayed comparatively large positive or negative IPCA1 scores, indicating stronger interactions with environmental conditions and, consequently, greater variation in performance across testing sites. Regarding the environments, Ard2, Eql2, Jol1, Ham1, and Mia2 had

IPCA1 scores close to zero, implying lower interaction effects and greater environmental stability than the remaining test locations. Among these environments, Ard1, Jol1, and Mia2 also recorded mean grain yields above the overall average, indicating favorable conditions for evaluating genotype performance (Table 5). Although IPCA1 accounted for the largest proportion of the interaction variation (30.8%), it did not fully describe the complexity of GEI. Therefore, IPCA2, which explained an additional 16.5% of the interaction sum of squares, was also considered in the stability assessment. Based on this component, G14, G15, G17, G2, and G4 showed the smallest absolute IPCA2 scores. When these values were interpreted together with grain yield performance, G15, and to a lesser extent G9, emerged as the most stable genotypes across the evaluated environments (Fig. 2). Similarly, the environments Mia2 and Jol1 exhibited IPCA2 scores close to zero, indicating comparatively stable environmental conditions.

The AMMI2 biplot provides additional insight into genotype adaptation patterns by simultaneously displaying the first two interaction principal components. Genotypes located near the origin contribute little to the GEI and are generally considered to possess specific adaptation, whereas those positioned close to a particular environment tend to exhibit specific adaptation to that environment. Based on this interpretation, G14 and G15 were located relatively close to the origin and therefore can be regarded as specificly adapted genotypes with relatively stable performance across environments. In contrast, genotypes positioned far from the center of the biplot—particularly those located at the vertices of the polygon are characterized by stronger interaction effects, lower overall stability, and greater environmental specificity (Gauch & Zobel, 1997). According to the AMMI2 biplot, G19 and G20 showed specific adaptation to the environments Mas2, Qaz1, Mas1, and Mia1, whereas G1, G2, and G10 were more closely associated with Ard1. Likewise, G11 and G12 were specifically adapted to the Zan1 environment, reflecting their superior performance under those particular environmental conditions.

Table 5. Scores of the first seven interaction principal component axes (IPCA1–IPCA7) for bread wheat genotypes.

Genotype	Yield	IPCA1	IPCA2	IPCA3	IPCA4	IPCA5	IPCA6	IPCA7
G1	7.703	-0.728	0.533	-0.529	-0.787	-0.061	0.606	0.109
G2	7.928	-1.070	0.138	0.246	0.119	-0.424	0.294	0.045
G3	7.902	0.267	-0.591	-0.022	0.325	0.364	0.339	0.511
G4	7.292	-0.424	0.132	-0.136	0.336	-0.551	-0.140	0.603
G5	7.282	0.373	0.777	0.618	-0.202	0.381	0.230	0.438
G6	7.428	0.170	0.514	0.666	-0.060	-0.170	-0.171	0.485
G7	7.296	0.993	-0.372	0.213	-0.323	-0.746	0.018	-0.144
G8	7.636	-0.135	-1.014	0.316	-0.828	0.344	-0.620	0.231
G9	7.850	-0.202	0.173	-0.398	-0.175	-0.090	-0.916	-0.224
G10	7.512	-0.865	0.222	0.446	0.445	0.470	-0.370	-0.206
G11	7.947	-0.426	-0.640	-0.165	-0.460	-0.060	0.452	-0.610
G12	7.446	-0.528	-0.623	-0.455	0.176	0.389	0.230	0.406
G13	7.203	0.729	-0.462	0.459	0.334	0.407	0.472	-0.131
G14	7.197	0.315	0.021	-0.538	0.338	0.120	-0.285	-0.047
G15	7.562	0.268	0.034	0.005	0.455	-0.303	0.353	-0.539
G16	7.329	0.325	-0.231	-0.258	0.241	-0.875	-0.130	0.265
G17	7.545	-0.525	0.016	0.150	0.578	0.088	-0.165	-0.513
G18	7.568	0.207	0.704	0.086	-0.440	0.093	0.099	-0.350
G19	7.400	0.437	0.156	0.358	-0.140	0.104	-0.229	-0.386
G20	7.297	0.819	0.514	-1.061	0.068	0.520	-0.068	0.056
Ard1	7.608	-1.421	0.703	-0.239	0.366	-0.850	0.078	0.006
Ard2	7.603	0.004	0.215	0.113	0.081	-0.116	-0.167	0.518
Eql1	6.630	-0.417	-0.095	-0.191	0.834	0.192	-0.491	-0.212
Eql2	6.501	0.009	-0.359	0.363	0.016	0.139	-0.036	-0.033
Ham1	6.364	-0.061	0.255	-0.230	-0.325	0.312	-0.332	-0.055
Ham2	7.537	0.181	0.161	0.134	0.194	-0.327	0.255	-0.182
Jol1	8.538	-0.068	0.327	0.274	-0.354	0.048	0.349	0.335
Jol2	9.699	-0.116	0.345	-0.190	0.227	0.878	-0.276	0.728
Kar1	6.003	0.309	-0.255	-0.190	-0.746	0.232	-0.078	0.012
Kar2	8.134	0.167	-1.533	-0.301	0.381	-0.374	-0.305	0.505
Mas1	5.941	0.845	0.369	0.885	0.118	-0.567	0.273	0.532
Mas2	6.702	0.652	0.564	-1.233	-0.318	-0.108	0.225	0.173
Mia1	8.200	0.836	0.175	0.093	-0.356	-0.583	-0.934	-0.639
Mia2	8.736	0.024	-0.526	0.370	-0.203	0.118	0.647	-0.131

Qaz1	7.668	0.730	0.413	0.350	0.787	0.638	0.132	-0.577
Qaz2	9.548	0.103	-0.438	-0.657	0.194	-0.053	0.796	-0.509
Zan1	7.068	-0.865	-0.315	0.391	-0.455	0.068	-0.095	-0.243
Zan2	6.811	-0.911	-0.004	0.260	-0.442	0.353	-0.041	-0.226

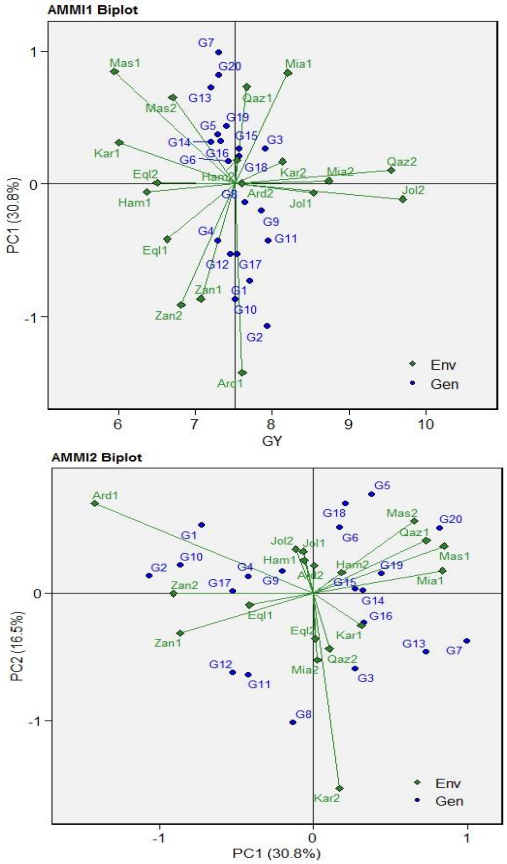


Fig. 2. AMMI biplots of bread wheat genotypes and environments: (A) mean grain yield versus IPCA1 (AMMI1) and (B) IPCA1 versus IPCA2 (AMMI2).

3.5. AMMI stability parameters

A wide range of AMMI-derived stability statistics has been proposed to improve the identification of stable genotypes across contrasting environments, and their usefulness has been demonstrated in several previous studies (Chimdesa *et al*., 2019; Pouresmael *et al*., 2018; Danyali *et al*., 2012). Unlike the graphical interpretation of AMMI biplots, these indices incorporate information from multiple interaction principal component axes, thereby providing a more comprehensive evaluation of genotype stability.

The stability rankings obtained from the different AMMI statistics were generally consistent, although minor differences were observed among individual indices (Table 6). According to the ASV statistic, G9, G15, G14, and G6 were identified as the most stable genotypes. Similar rankings were obtained using the ASI and MASI indices, which also recognized G9, G15, G14, and G6 as the most stable entries. The ASTAB statistic ranked G19, G14, G15, and G18 among the most stable genotypes, whereas the EV and SIPC indices consistently highlighted G14, G19, G15, and G18. Likewise, DZ, FA, ZA, MASV, and AVAMGE produced highly comparable rankings, with G14, G15, G18, and G19 repeatedly appearing among the most stable genotypes regardless of the specific statistic employed.

The high degree of agreement among these stability measures indicates that most of the AMMI-derived statistics captured similar patterns of genotype × environment interaction. Consequently, the simultaneous use of a large number of highly correlated stability indices may provide redundant

information, suggesting that a smaller subset of representative statistics could adequately describe genotype stability, as previously reported by Amini *et al*. (2023).

Although stability statistics are valuable for identifying genotypes with consistent performance, they do not account for mean grain yield. Consequently, selecting genotypes solely on the basis of stability may result in the recommendation of genotypes with excellent stability but limited agronomic value. Therefore, stability should always be interpreted together with productivity to ensure that selected genotypes combine both high yield potential and wide adaptation. Among the genotypes consistently classified as stable by the AMMI statistics, only G9, G15, and G18 produced grain yields above the overall mean, making them more suitable candidates for cultivar recommendation.

To overcome the limitations of individual stability indices, the Selection Index of Ideal Genotype (SIIG) was also employed. This index integrates grain yield with multiple AMMI-derived stability statistics, enabling simultaneous evaluation of productivity and stability. Based on SIIG values, G17, G16, G3, G4, G9, G6, G18, G19, G14, and G15 were ranked closest to the ideal genotype. Among these, G17, G3, G9, G18, and G15 exhibited grain yields exceeding the overall mean and were therefore considered the most promising genotypes for specific adaptation across the evaluated environments.

The reliability of AMMI biplots largely depends on the proportion of genotype × environment interaction explained by the first two interaction principal component axes. Crossa (1990) suggested that AMMI biplots provide the most informative graphical interpretation when the first two IPCAs explain more than 50% of the total interaction variation. In the present study, however, IPCA1 and IPCA2 together accounted for less than half of the GEI sum of squares. Consequently, interpretation based exclusively on these two axes should be approached with caution because an appreciable proportion of the interaction remained unexplained.

The significance of the third through seventh IPCAs further demonstrates that the interaction structure was distributed across multiple multiplicative components rather than being concentrated in the first two axes. Under such circumstances, restricting stability assessment to IPCA1 and IPCA2 may oversimplify the interaction pattern and potentially lead to inaccurate conclusions. Similar observations have been reported by Olivoto *et al*. (2019a), who emphasized that when GEI is highly complex, meaningful interaction information may be retained in higher-order IPCAs. Therefore, a comprehensive assessment of genotype stability should incorporate all statistically significant interaction principal components rather than relying solely on the first two axes.

Table 6. AMMI-based stability indices for the evaluated bread wheat genotypes.

GEN	Y	ASTAB	ASI	ASV	AVAMGE	DA	DZ	EV	FA	MASI	MASV	SIPC	ZA	SIIG
G1	7.7	2.1	0.241	1.46	10.3	2.96	0.74	0.078	8.79	0.258	2.03	3.35	0.223	0.247
G2	7.93	1.51	0.33	2	8.85	2.9	0.541	0.042	8.44	0.334	2.14	2.34	0.191	0.333
G3	7.9	1.03	0.128	0.774	6.14	1.96	0.547	0.043	3.86	0.138	1.33	2.42	0.139	0.753
G4	7.29	1.02	0.132	0.803	5.93	1.91	0.557	0.044	3.65	0.147	1.3	2.32	0.134	0.753
G5	7.28	1.56	0.172	1.04	7.83	2.53	0.634	0.058	6.38	0.19	1.77	3.02	0.189	0.512
G6	7.43	1.03	0.0996	0.604	6.02	1.97	0.536	0.041	3.89	0.127	1.37	2.23	0.13	0.793
G7	7.3	1.85	0.312	1.89	10.2	3.04	0.642	0.059	9.22	0.319	2.21	2.81	0.214	0.233
G8	7.64	2.39	0.172	1.04	10.7	3.01	0.811	0.094	9.06	0.194	2	3.49	0.194	0.333
G9	7.85	1.16	0.0684	0.415	6.65	1.9	0.618	0.055	3.62	0.1	1.28	2.18	0.113	0.766
G10	7.51	1.59	0.269	1.63	9.78	2.75	0.614	0.054	7.55	0.279	1.97	3.02	0.209	0.340
G11	7.95	1.41	0.168	1.02	8.19	2.33	0.634	0.058	5.42	0.179	1.57	2.81	0.171	0.562
G12	7.45	1.27	0.192	1.17	7.34	2.35	0.565	0.046	5.51	0.203	1.66	2.81	0.185	0.561
G13	7.2	1.47	0.237	1.44	8.36	2.59	0.598	0.051	6.71	0.248	1.85	2.99	0.204	0.426
G14	7.2	0.601	0.0972	0.59	5.63	1.54	0.403	0.023	2.37	0.118	1.07	1.66	0.102	0.932
G15	7.56	0.787	0.0826	0.501	5.66	1.6	0.507	0.037	2.55	0.1	1.03	1.96	0.1	0.890
G16	7.33	1.14	0.107	0.649	7.1	2.01	0.58	0.048	4.03	0.132	1.42	2.32	0.135	0.740
G17	7.55	0.93	0.162	0.98	6.43	1.92	0.514	0.038	3.7	0.172	1.29	2.03	0.127	0.736
G18	7.57	0.88	0.132	0.803	5.6	1.9	0.477	0.033	3.6	0.139	1.28	1.98	0.123	0.810
G19	7.4	0.575	0.137	0.831	4.85	1.56	0.391	0.022	2.45	0.145	1.1	1.81	0.118	0.854
G20	7.3	2.34	0.266	1.61	10.7	3.26	0.742	0.079	10.6	0.293	2.36	3.11	0.229	0.163

3.6. BLUP-based AMMI analysis

The scree plot of the interaction principal components (Fig. 3) indicated that the genotype × environment interaction could not be adequately represented by only the first two principal component axes. Although IPCA1 and IPCA2 explained 30.94% and 16.24% of the interaction sum of squares, respectively, a considerable proportion of the interaction variance remained distributed among the subsequent principal components. This finding suggests that the interaction pattern was relatively complex and that higher-order IPCAs also contributed substantially to describing genotype responses across environments.

The significance of the random effects was evaluated using the Likelihood Ratio Test (LRT). The analysis revealed highly significant differences among genotypes for grain yield ($P < 0.01$) and also confirmed a significant genotype × year interaction ($P < 0.01$) (Table 7). These results indicate that the relative performance of the evaluated genotypes was not consistent across years, reflecting differences in their phenotypic responses under varying environmental conditions. Because the genotype × year interaction was statistically significant, the interaction effects were predicted using the Best Linear Unbiased Prediction (BLUP) procedure within the REML framework. The resulting BLUP estimates were subsequently subjected to singular value decomposition (SVD) in order to quantify genotype stability while accounting for the interaction structure. This combined analytical strategy provides a more robust assessment of genotype performance by separating predictable genetic effects from environmental noise.

Prediction accuracy is a critical consideration in multi-environment trials because reliable genotype recommendation depends on the ability of statistical models to estimate genetic values as closely as possible to their true values (Olivoto *et al*., 2019a). In mixed-model analyses, prediction accuracy can be enhanced through improvements in experimental design, the use of an adequate number of replications, and the application of statistical models capable of efficiently partitioning genetic and environmental sources of variation (Gauch & Zobel, 1977). The integration of AMMI with the REML/BLUP framework has therefore become an effective approach for increasing the precision of genotype evaluation while simultaneously improving the interpretation of genotype × environment interaction.

Comparison of the BLUP-predicted grain yield values demonstrated clear differences among the evaluated genotypes (Fig. 4). G11, G2, G3, G9, G1, G8, G18, G15, and G17 exhibited predicted grain yields above the overall mean, indicating superior genetic potential across the tested environments. In contrast, the remaining genotypes produced BLUP estimates below the grand mean, suggesting comparatively lower productivity under the experimental conditions.

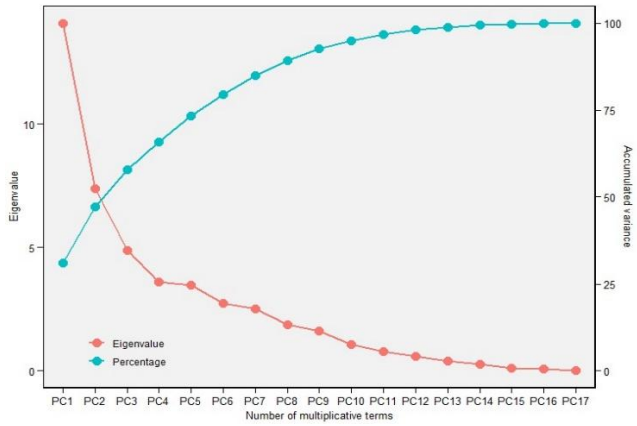


Fig. 3. Scree plot of the eigenvalues of the BLUP-based genotype × environment interaction (BLUP-GEI) matrix for grain yield.

Table 7. Significance of model effects for grain yield based on the likelihood ratio test (LRT).

Likelihood test		Statistics
Genotype*Environment	Genotype	
91.8**	11.67**	Chi-square X2

9.54×10^{-22}	6.33×10^{-4}	p-Value

** and *: significant at the level of 1 and 5%, respectively

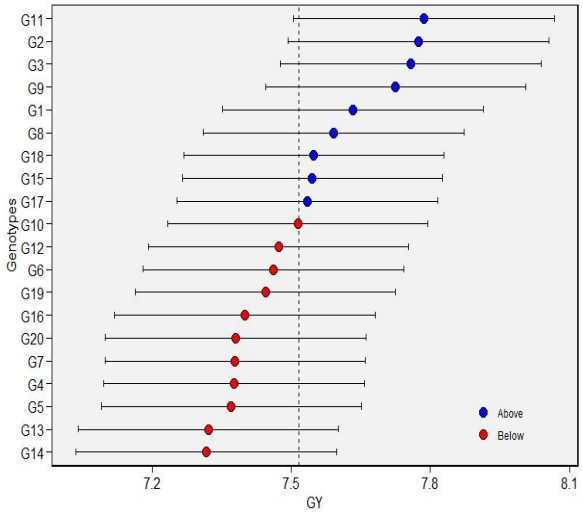


Fig. 4. BLUP-predicted grain yield of the 20 bread wheat genotypes.

3.7. BLUP-Based Deviation Plot

The AMMI1 and AMMI2 biplots generated using the AMMI/BLUP approach were largely consistent with those obtained from the conventional AMMI model, showing nearly identical patterns of genotype grouping in both biplots. Under the AMMI/BLUP analysis, the first and second principal components (PC1 and PC2) accounted for 30.94% and 16.24% of the total interaction sum of squares, respectively. Genotypes G8, G6, G9, G3, G18, and G15 exhibited PC1 scores close to zero, indicating relatively low interaction with the test environments. Among these genotypes, G8, G9, G3, G18, and G6 produced grain yields above the overall mean and therefore can be regarded as stable, high-yielding genotypes with specific adaptability across environments (Fig. 5). Likewise, genotypes G14, G15, G17, G2, and G4 showed the lowest PC2 scores. Based on their yield performance and interaction patterns, G14 and G15, and to a lesser extent G9, may be considered relatively stable genotypes across the tested environments (Fig. 5). The biplot also indicated specific associations between genotypes and environments. In particular, G19 and G20 showed greater affinity with Mas2, Qaz1, Mas1, and Mia1, whereas G1, G2, and G10 were more closely associated with Ard1. Similarly, G11 and G12 showed a stronger association with the Zan1 environment.

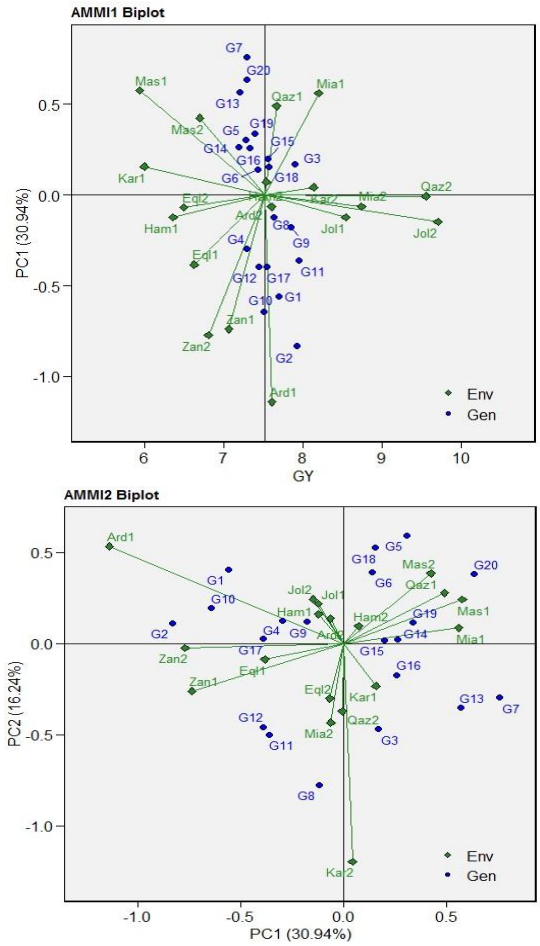


Fig. 5. AMMI/BLUP biplots of bread wheat genotypes and environments: (A) BLUP-predicted grain yield versus IPCA1 (AMMI1) and (B) IPCA1 versus IPCA2 (AMMI2)

Grain yield stability was further investigated through the BLUP-based deviation plot presented in Fig. 6. Rather than relying solely on numerical stability statistics, this graphical approach allows the consistency of genotype performance across the test environments to be visualized. Each genotype is represented by an individual line, and the magnitude of its slope reflects the extent to which its observed yield departs from the corresponding predicted values. Thus, genotypes associated with flatter trajectories can be interpreted as being less affected by environmental changes, whereas steeper trajectories indicate a stronger response to environmental variation and, consequently, lower yield stability.

The environmental positions along the horizontal axis also provide useful information about genotype-specific responses and make it possible to recognize situations in which particular genotypes perform comparatively better in certain environments. From this perspective, G2, G1, G10, G4, G17, G11, G9, G12, G7, and G13 displayed comparatively pronounced slopes, suggesting a greater involvement in the genotype × environment interaction. In contrast, the trajectories of G16, G18, G15, G6, G3, G19, G8, G14, G5, and G20 were relatively flat, indicating weaker sensitivity to environmental changes.

When both productivity and stability are considered simultaneously, genotypes G16, G18, G6, and G15 appear particularly desirable. These genotypes combined relatively high grain yield with small deviations from their predicted performance, suggesting a more consistent expression of yield across the environments investigated. Such genotypes may therefore be considered promising candidates for specific adaptation under the conditions represented in the present trial.

Nevertheless, the graphical interpretation should not be considered independently of the other stability analyses. The horizontal axis represents PC1, and the information provided by the remaining principal

components is not directly incorporated into this display. Consequently, conclusions regarding stability based solely on this plot should be interpreted cautiously (Sharifi *et al*., 2021). The use of graphical approaches based on genotype performance across environments has been reported in previous studies as an effective means of identifying specific genotype–environment associations and distinguishing genotypes with particular adaptation patterns (Gurmu *et al*., 2012; Farshadfar, 2013; Yan *et al*., 2000).

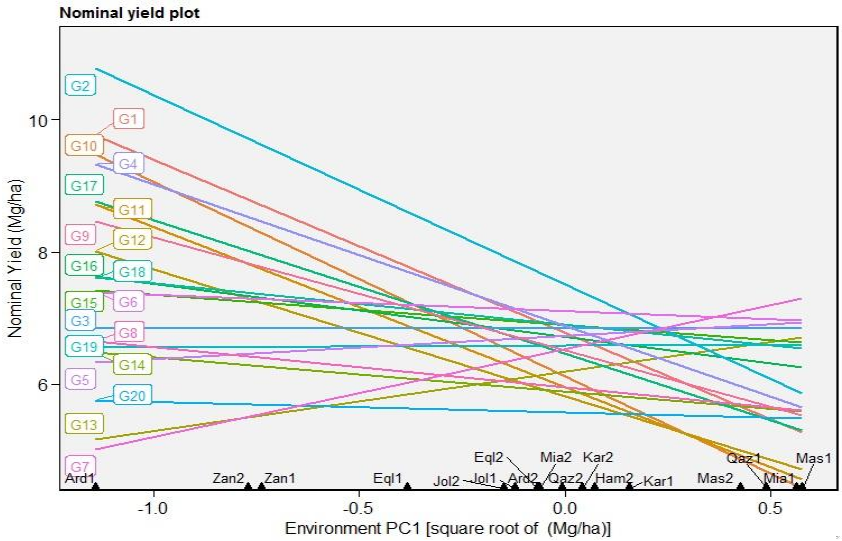


Fig. 6. Evaluation of 20 bread wheat genotypes across 18 environments based on observed grain yield and IPCA1 scores.

3.8. WAASB × Grain Yield Biplot

The combined evaluation of grain yield and stability was performed using the WAASB × grain yield biplot (Fig. 7), which simultaneously classifies genotypes according to their productivity and stability. In this graphical approach, lower WAASB values indicate greater stability because they reflect a smaller contribution of the genotype to the genotype × environment interaction.

The upper-left quadrant contained genotypes with below-average grain yield and high WAASB values, representing materials with both poor productivity and low stability. G7, G20, G13, G5, and G8 were positioned in this group and therefore exhibited limited agronomic value under the evaluated environments. The upper-right quadrant included genotypes with above-average grain yield but relatively high WAASB values, indicating that superior productivity was accompanied by considerable environmental sensitivity. G10, G1, G2, G11, and G12 were classified in this category. Although G1 and G2 (the check cultivars) produced the highest grain yields, their comparatively large WAASB values revealed substantial genotype × environment interaction, indicating that their performance was less stable across the testing environments. Genotypes located in the lower-left quadrant exhibited below-average grain yield but low WAASB values, reflecting high stability despite limited productivity. This group consisted of G6, G19, G4, G16, and G14, suggesting that these genotypes were consistently low yielding across environments. The lower-right quadrant represented the most desirable region of the biplot because it combined above-average grain yield with low WAASB values, thereby identifying genotypes with both high productivity and specific adaptation. G17, G3, G9, G18, and G15 occupied this quadrant and therefore emerged as the most promising genotypes. Among them, G3 and G9 produced grain yields comparable to those of the check cultivars while maintaining substantially greater stability, making them particularly attractive candidates for cultivar recommendation.

Compared with the conventional AMMI1 biplot, the WAASB × grain yield biplot provides a more comprehensive representation of genotype performance because the WAASB statistic is calculated using information from all significant interaction principal components rather than relying solely on the first interaction axis. Consequently, this approach captures a larger proportion of the genotype × environment interaction and provides a more reliable assessment of genotype stability.

A similar advantage is observed when comparing the WAASB biplot with the traditional which-won-where graphical approach. Whereas the latter is primarily based on the first interaction principal component, the WAASB index integrates the contribution of all significant IPCAs into a single stability measure. As a result, the interaction structure is represented more completely, leading to a more robust interpretation of genotype adaptation and stability across environments (Olivoto *et al*., 2019a)

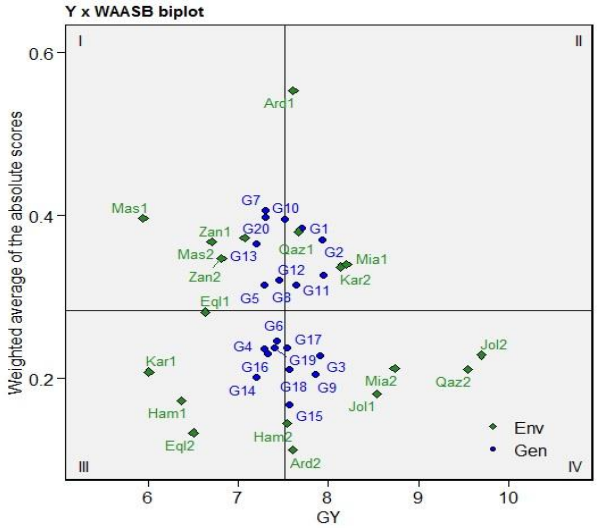


Fig. 7. WAASB biplot showing grain yield versus the weighted average of absolute scores from the BLUP-based genotype × environment interaction matrix.

3.9. WAASBY Index for Simultaneous Evaluation of Yield and Stability

To simultaneously evaluate grain yield and stability, the WAASBY index was calculated by integrating mean grain yield with the WAASB stability statistic into a single selection criterion. In the present study, identical weights were assigned to grain yield and stability, reflecting the assumption that both attributes contribute equally to the identification of superior genotypes. Based on the WAASBY ranking, G9, G3, G15, G11, G18, G17, G2, G6, G19, G8, and G16 achieved the highest index values (Fig. 8), indicating a favorable combination of productivity and stability across the evaluated environments. Unlike stability statistics that consider only the consistency of genotype performance, the WAASBY index provides a balanced assessment by simultaneously rewarding high grain yield and low genotype × environment interaction.

Comparison of the WAASBY rankings with the grain yield × WAASB biplot further clarified the performance of the evaluated genotypes. Some high-ranking genotypes, including G2 and G11, were positioned in the high-yield but relatively unstable region of the biplot, whereas G6 and G16 exhibited high stability despite producing grain yields below the overall mean. In contrast, G3, G9, G15, G17, and G18 were located within the quadrant representing both above-average grain yield and high stability, confirming their superior overall performance across environments.

These results demonstrate that the WAASBY index provides a more comprehensive basis for genotype selection than yield or stability alone. Because its values depend on the relative importance assigned to productivity and stability, different weighting schemes may alter genotype rankings according to breeding objectives. Consequently, simultaneous interpretation of the WAASBY index together with the grain yield × WAASB biplot offers a more robust and biologically meaningful framework for identifying specificly adapted, high-yielding, and stable wheat genotypes.

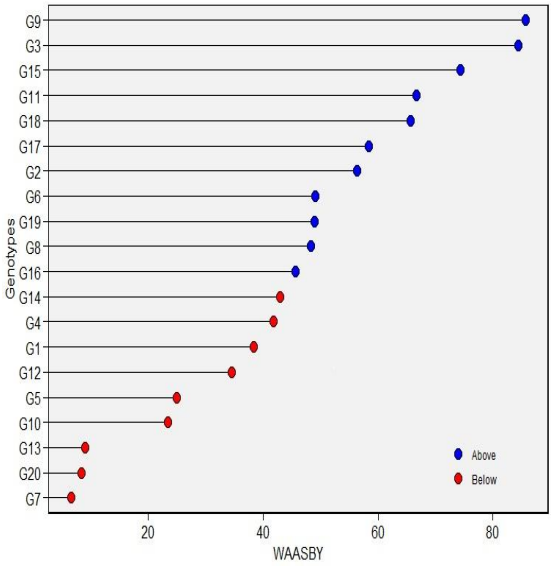


Fig. 8. WAASBY index values for the 20 evaluated bread wheat genotypes using equal weights (50:50) for grain yield and stability.

The consistency of genotype rankings obtained from the WAASB index was further evaluated by examining changes in genotype rank as the number of interaction principal components included in the calculation increased (Fig. 9). This analysis provides an assessment of the robustness of genotype classification when progressively larger proportions of the genotype × environment interaction are incorporated into the stability measure. To facilitate the interpretation of ranking patterns, a hierarchical cluster analysis based on Euclidean distance was performed using the genotype ranks generated under the different WAASB models. The resulting dendrogram grouped genotypes according to the similarity of their ranking profiles, allowing the identification of genotypes that maintained stable positions regardless of the number of principal components considered.

The clustering pattern demonstrated that G9, G3, G18, G15, and G14 consistently remained among the highest-ranked genotypes across all WAASB calculations. The persistence of these genotypes within the same cluster indicates that their superiority was not influenced by the number of interaction principal components retained in the analysis. This consistency suggests that these genotypes possess both high grain yield and stable performance across the evaluated environments, making them reliable candidates for specific adaptation and potential cultivar recommendation.

Overall, the stability of genotype rankings across successive WAASB models demonstrates the robustness of the REML/BLUP-based stability assessment. Genotypes that maintain similar rankings despite the inclusion of additional interaction principal components can be considered more reliable selections because their classification is less sensitive to model complexity and more likely to reflect their true biological performance under diverse environmental conditions

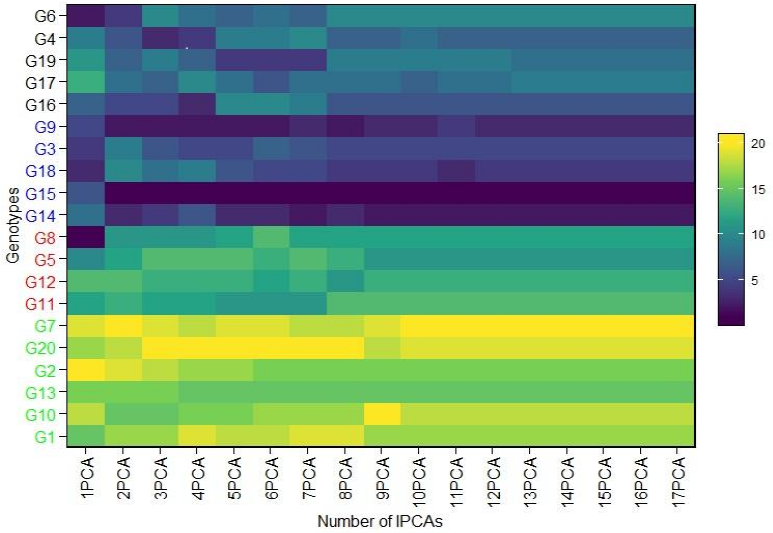


Fig. 9. Hierarchical clustering of the 20 bread wheat genotypes based on the WAASB index using all significant principal components.

The flexibility of the WAASBY index was further explored by assigning different relative weights to grain yield and genotype stability (Fig. 10). Unlike conventional stability statistics, WAASBY allows breeders to adjust the contribution of productivity and stability according to the objectives of a breeding program, thereby providing a more versatile criterion for genotype selection under different production scenarios. As expected, genotype rankings varied substantially as the relative importance assigned to grain yield and stability changed. When 100% of the selection emphasis was placed on stability (WAASB) and grain yield was excluded from the calculation, G15, G9, G3, G18, and G14 achieved the highest rankings, reflecting their superior stability across environments. Conversely, progressively increasing the contribution of grain yield while reducing the weight assigned to stability resulted in noticeable changes in genotype ranking. Under the scenario in which grain yield alone determined the ranking, G11, G2, G9, and G3 emerged as the highest-performing genotypes.

When equal importance was assigned to productivity and stability (50:50 weighting), G9, G3, G15, and G18 consistently occupied the highest positions, indicating that these genotypes successfully combined high grain yield with stable performance across environments. Their consistent ranking under balanced weighting highlights their potential as specificly adapted genotypes suitable for cultivation over a wide range of environmental conditions.

The observed changes in genotype ranking demonstrate that selection decisions are strongly influenced by the relative importance assigned to yield and stability. Consequently, the weighting strategy should be chosen according to the breeding objective. Programs focused on maximizing grain production under favorable conditions may prioritize productivity, whereas breeding for variable or stress-prone environments may require greater emphasis on stability. The WAASBY index provides sufficient flexibility to accommodate both selection strategies within a unified analytical framework.

An important limitation of the conventional AMMI1 biplot is that its interpretation depends primarily on the first interaction principal component. When IPCA1 explains only a limited proportion of the genotype × environment interaction, a substantial amount of biologically relevant information remains in the higher-order interaction components, reducing the reliability of conclusions drawn from the first axis alone. Under these circumstances, graphical interpretation based exclusively on AMMI1 should be approached with caution.

The grain yield × WAASB biplot overcomes this limitation because the WAASB statistic integrates information from all statistically significant interaction principal components, thereby providing a more complete description of genotype × environment interaction. By combining the predictive strength of the REML/BLUP methodology with the interaction-partitioning capability of AMMI, this approach improves

both the accuracy of genotype prediction and the biological interpretation of multi-environment trial data (Ajay & Singh, 2021).

The importance of incorporating multiple interaction principal components has been emphasized in previous studies. Olivoto *et al*. (2019a) demonstrated that genotype rankings based on only the first two IPCAs may differ markedly from rankings obtained after considering all significant interaction components; in their study, a genotype initially classified as the most stable declined to sixth position once the complete interaction structure was incorporated. Similar findings have been reported in wheat and other crops, where IPCA1 accounted for only a relatively small proportion of the total interaction variance (Veenstra *et al*., 2019; Tigabu *et al*., 2017; Amini *et al*., 2025). These studies collectively indicate that restricting stability assessment to one or two interaction axes may oversimplify the complexity of genotype × environment interaction.

The results of the present study support this conclusion. IPCA1 explained approximately 31% of the total genotype × environment interaction, indicating that a considerable proportion of the interaction remained distributed among subsequent principal components. Therefore, incorporating a larger number of significant interaction axes is expected to improve both the precision of genotype ranking and the reliability of biological interpretation. This interpretation is consistent with the findings of Sharifi *et al*. (2021), who reported that AMMI-based rankings progressively converge toward those obtained with the WAASBY index as additional interaction principal components are incorporated, highlighting the greater robustness of WAASBY for simultaneous evaluation of grain yield and stability.

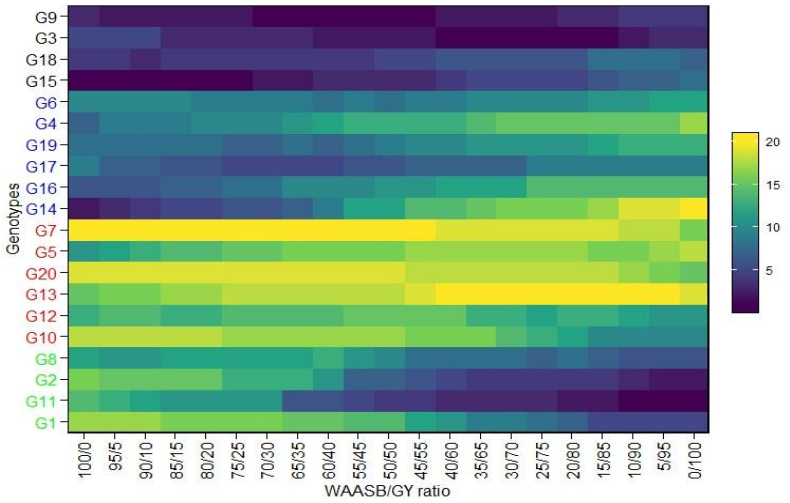


Fig. 10. Ranking of the 20 bread wheat genotypes based on the WAASBY index using different weighting schemes for grain yield and stability.

3.10. Genotype Ranking Based on REML/BLUP Stability Statistics

To further identify superior genotypes, grain yield and stability were jointly evaluated using the REML/BLUP mixed-model approach. Unlike conventional methods that rely primarily on phenotypic means, the BLUP framework estimates genotypic values while accounting for random environmental effects and genotype × environment interaction, thereby providing more accurate predictions for genotype selection (Resende, 2004). Consequently, BLUP-based statistics have become widely used in plant breeding programs for simultaneously assessing productivity, adaptability, and stability.

Three complementary BLUP-derived indices, the Harmonic Mean of Genotypic Values (HMGV), the Relative Performance of Genotypic Values (RPGV), and the Harmonic Mean of the Relative Performance of Genotypic Values (HMRPGV) were calculated to rank the evaluated genotypes (Colombari Filho *et al*., 2013). These statistics integrate information on yield performance and stability, with higher index values indicating genotypes that combine superior productivity with greater consistency across environments.

The three indices produced highly consistent rankings (Table 8), demonstrating strong agreement in the identification of superior and inferior genotypes. According to HMGV, RPGV, and HMRPGV, genotypes G1, G2, G3, G9, and G11 consistently achieved the highest values, indicating outstanding performance in terms of both grain yield and stability. Their agreement across all three statistics suggests that these genotypes possess specific adaptation and represent promising candidates for cultivar recommendation. Conversely, G13, G14, G20, G4, and G5 consistently ranked among the lowest-performing genotypes based on all three BLUP-derived indices. Their low HMGV, RPGV, and HMRPGV values indicate limited productivity together with reduced stability across environments, making them less suitable for recommendation under the conditions evaluated in this study.

The close agreement among the three BLUP-based statistics further demonstrates the robustness of the mixed-model approach for genotype evaluation. Because these indices simultaneously account for predicted genetic values and environmental variability, they provide a more reliable basis for selecting superior genotypes than approaches based solely on observed mean performance. Their combined use therefore strengthens breeding decisions by identifying genotypes that exhibit both high yield potential and stable performance over diverse testing environments.

Table 8. REML/BLUP-based yield stability and adaptability statistics (HMGV, RPGV, and HMRPGV) for the evaluated bread wheat genotypes.

GEN	Yield	HMGV	HMGV_R	RPGV	RPGV_R	HMRPGV	HMRPGV_R
G1	7.7	7.51	5	1.02	5	1.02	5
G2	7.93	7.69	1	1.05	2	1.04	2
G3	7.9	7.68	2	1.04	3	1.04	3
G4	7.29	7.15	17	0.974	18	0.972	15
G5	7.28	7.16	16	0.974	17	0.971	17
G6	7.43	7.29	11	0.991	11	0.989	11
G7	7.3	7.16	15	0.975	15	0.972	16
G8	7.64	7.46	6	1.02	6	1.01	6
G9	7.85	7.64	4	1.04	4	1.04	4
G10	7.51	7.3	10	0.998	10	0.994	10
G11	7.95	7.68	3	1.05	1	1.04	1
G12	7.45	7.27	13	0.991	12	0.989	12
G13	7.2	7.06	20	0.963	20	0.96	20
G14	7.2	7.07	19	0.963	19	0.962	19
G15	7.56	7.39	8	1	8	1	8
G16	7.33	7.19	14	0.979	14	0.977	14
G17	7.55	7.39	9	1	9	1	9
G18	7.57	7.43	7	1.01	7	1.01	7
G19	7.4	7.28	12	0.988	13	0.987	13
G20	7.3	7.13	18	0.974	16	0.97	18

Note: GY - Grain yield (kg/ha), R – Rank of the given parameter, HMGV - Harmonic mean of genotypic values, RPGV - Relative performance of the genotypic values, HMRPGV - Harmonic mean of the relative performance of genotypic values.

4. Conclusion

The present study demonstrated that genotype × environment interaction had a significant influence on grain yield, emphasizing the importance of evaluating both productivity and stability when identifying superior wheat genotypes. Although the conventional AMMI analysis successfully characterized the interaction pattern, the first two interaction principal components explained less than half of the total genotype × environment variation, indicating that a considerable proportion of the interaction remained distributed among higher-order components. Consequently, genotype evaluation based solely on the first two IPCAs may not adequately represent the complexity of multi-environment trial data.

The REML/BLUP-based AMMI approach provided a more comprehensive description of genotype performance by incorporating all significant interaction principal components into the stability assessment. As a result, indices such as WAASB and WAASBY offered a more reliable framework for simultaneously

evaluating grain yield and stability than conventional AMMI biplots. The close agreement observed among the WAASB, WAASBY, and BLUP-derived indices further confirmed the robustness of the mixed-model approach for identifying specificly adapted genotypes across diverse testing environments.

Among the evaluated materials, G9, G3, G15, and G18 consistently ranked among the best genotypes across most stability criteria. These genotypes combined high grain yield with stable performance and exhibited only small yield differences relative to the check cultivars, suggesting specific adaptation to irrigated environments. In contrast, the BLUP-derived statistics (HMGV, RPGV, and HMRPGV) consistently identified G1, G2, G3, G9, and G11 as the highest-ranking genotypes, highlighting the effectiveness of mixed-model predictions for genotype selection.

Overall, the findings indicate that when the first two interaction principal components account for only a limited proportion of the genotype × environment interaction, reliance on conventional AMMI biplots alone may lead to incomplete or potentially misleading conclusions. Under such circumstances, stability measures that integrate all significant interaction principal components, particularly WAASB and WAASBY, provide a more robust basis for genotype evaluation and selection.

From a practical breeding perspective, G9, G3, G15, and G18 emerged as the most promising candidates because they consistently combined high productivity with specificfigure adaptation and yield stability across environments. These genotypes therefore represent valuable materials for advanced multi-location testing and may be considered potential candidates for the development and release of new irrigated wheat cultivars adapted to the cold regions of Iran, subject to confirmation through additional agronomic, quality, and registration evaluations

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