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Integrated analysis of genotype by yield trait and genotype by environment interactions for selecting superior maize genotypes

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During variety improvement, unfavorable correlations between key traits pose a significant challenge for breeders. Additionally, genotype-environment interaction (GEI), which critically influences plant performance, is recognized as another major challenge. Understanding the nature of this interaction is essential for developing effective breeding programs that enhance crop production. This study investigated the associations between key traits such as kernel yield (KY), number of kernel row (KR), number of kernels in a row (KIR), 1000 kernel weight (KW), kernel moisture (KM), plant height (PH), and ear height (EH), as well as the stability of KY in nine maize genotypes and a control variety. The experiment was conducted at seven locations using a randomized complete block design with three replications over two consecutive years. The results revealed a very high positive correlation between KY and the KR, as well as between KY and PH. A very weak negative correlation was observed between KY and the KIR, and between KY and KM. Genotype 7 was identified as the best genotype for the combinations of KY × KIR and KY × EH. For the combinations of KY × KR, KY × PH, KY × KW, and KY/KM, genotypes 1, 10, and 3 were recognized as the best performers. Based on the multi-trait stability index (MTSI), genotypes 4, 2, and 3 were identified as the most ideal genotypes across all traits. The additive effects analysis of the additive main effects and multiplicative interaction (AMMI) model indicated that the effects of genotype, environment, and GEI were significant for KY. The multiplicative effect analysis of the AMMI, decomposed into principal components (PCs), showed that four significant PCs explained 87.90% of the variation in the GEI. According to the biplot of mean yield versus the weighted average absolute scores (WAAS), genotypes 4 and 3 were identified as stable genotypes with favorable yield. Using the GGE biplot method, genotypes 10 and 3 were identified as the best stable genotypes in the MHD2022, MGN2023, KRJ2022, MGN2022, SYZ2022, and SYZ2023. Genotype 7 was the best in the KRJ2023, KER2022, HDM2022, and IFN2023, while genotype 1 was the best in the KER2023, HDM2023, IFN2022, and MHD2023. The likelihood ratio test (LRT) results showed that the effects of genotype and GEI were significant for KY. Genotype 1 had high mean values of best linear unbiased predictions (BLUP), making it a suitable genotype for KY. Among the experimental genotypes, genotypes 4 and 3 had higher WAASBY compared to others, indicating that they were stable genotypes with high KY. Among the various analytical models, the WAASBY index proved particularly effective for the simultaneous selection of yield and stability. Overall, genotypes 4 and 3 consistently emerged as the most desirable candidates, showcasing a superior combination of high KY and broad stability, making them valuable assets for future maize breeding programs.

Keywords Breeding, Biplot, Component, Correlation, Key traits

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Addressing global food security has been recognized as one of the most significant scientific challenges of our time[1,2]. The current global population is over eight billion and is projected to rise to around 10 billion by 2050, potentially reaching 11 billion by the end of the century[3]. As the population grows, the demand for food is expected to increase substantially[4]. Despite the escalating pressure on food production, advancements in agricultural efficiency have thus far managed to meet this demand[5]. However, food production needs to increase by about 70% by 2050 to sustain a population of over nine billion[6,7]. There are significant concerns that existing crop varieties and farming practices may not be sufficient to meet the growing demand for plant products[5]. Moreover, the degradation of agricultural lands, climate change, and the depletion of natural resources pose significant threats to food security[8,9]. In agricultural sciences, maize (*Zea mays* L.) breeding is a crucial area of study, playing a vital role in global food security. Maize is a staple food for billions worldwide and is essential in animal feed, biofuel production, and various industrial applications[10,11]. Developing new, high-yielding, and resilient varieties is a key strategy to tackle the issue of food security[12,13].

Achieving these breeding goals is complicated by two major challenges. First, negative and unfavorable correlations between key traits are significant hurdles in breeding processes[14]. These associations complicate breeding programs, as improving one trait may inadvertently lead to a decline in another when traits are negatively correlated[15]. Therefore, breeders must balance enhancing multiple traits while managing the constraints imposed by these correlations[2]. The second major challenge is the phenomenon of GEI. Improved maize genotypes often exhibit varying adaptations to different environments, primarily due to yield instability resulting from the differential response of genotypes across a wide range of locations and years[16]. Quantitative traits such as yield, which are economically and agronomically important, can be significantly influenced by GEI, making the selection of broadly adapted and stable genotypes a complex task[17].

To address the challenge of complex trait correlations, several multivariate techniques have been proposed. The genotype by yield × trait (GYT) biplot method was developed based on the principle that yield is the primary trait of interest, and the value of other key traits should be evaluated in the context of high yield, as a genotype with desirable secondary traits is useful only if it also produces high yield[14].Thus, in selecting superior varieties, the combination of yield with other traits is more critical than evaluating varieties based on individual traits. More recently, Olivoto et al.[18] introduced the MTSI, which allows for the simultaneous selection for average yield and stability across multiple traits. For instance, Sellami et al.[19] applied the MTSI to identify lentil genotypes that were not only high-yielding but also stable under rainfed conditions. This index is particularly useful for breeders as it provides a unique and easy-to-interpret selection process that accounts for the correlation structure among traits and allows for selection in a desired direction (increase or decrease) for each trait.

A thorough examination of GEIs necessitates robust statistical methods. Among the multivariate approaches, the AMMI model is particularly significant for its ability to partition the GEI into meaningful PCs, providing a deeper understanding of genotype stability[20,21]. The AMMI model is frequently used alongside the GGE biplot graphical model to identify mega-environments and superior genotypes within each environment[22]. In addition, the BLUP method has been widely used to evaluate multi-environmental trial data by efficiently estimating the average performance of genotypes in mixed models[23]. To leverage the advantages of both AMMI and BLUP, indices such as the weighted average of absolute scores (WAAS) and the WAASB stability index were introduced. These indices, which consider all significant PCs of the GEI, provide a more comprehensive measure of stability and can be combined with yield to create a superiority index like WAASBY for simultaneous selection of high-yielding and stable genotypes[18].

In this study, a collection of new maize genotypes was evaluated across various environments with differing conditions to identify successful genotypes. The main goal was to examine the impact of GEI on the potential for quantitative kernel production and to assess their adaptability. Additionally, the study explored the associations between key traits that influence yield and ultimately selected the superior genotype(s) based on these critical traits.

Materials and methods

Plant materials

A total of 10 maize genotypes were used in this study, including nine new genotypes and one control variety (Table 1). These new genotypes were previously developed at the Seed and Plant Improvement Institute (SPII) with the goal of reducing the growth period (early maturity) while maintaining suitable quantitative and qualitative performances. From this set of developed genotypes, nine superior genotypes were selected based on their quantitative and qualitative traits for further evaluation.

Genotype number	Pedigree	Genotype number	Pedigree
1	KE 79,017/5111 × K 1264/5–1	6	KE83007/2211 × B73
2	KE72012/12 × B73	7	OH43/1–42 × B73
3	KE76009/311 × B73	8	K1263/17 × S61
4	KE77003/3 × B73	9	KE72012/12 × K1263/1
5	KE79015/6222 × B73	10	KSC410 (Check)

Table 1. Selected maize genotypes used in the research.

Environment code	Year	Location of the research station	Altitude (m)	Latitude	Longitude
IFN2022	2022	Isfahan, Isfahan, Iran	1575	32°40'N	51°40'E
IFN2023	2023				
KRJ2022	2022	Karaj, Alborz, Iran	1312	35°55'N	50°54'E
KRJ2023	2023				
MHD2022	2022	Mashhad, Khorasan Razavi, Iran	1316	36°30'N	59°37'E
MHD2023	2023				
MGN2022	2022	Moghan, Ardebil, Iran	66	36°39'N	47°47'E
MGN2023	2023				
SYZ2022	2022	Shiraz, Fars, Iran	1484	29°32'N	52°36'E
SYZ2023	2023				
HDM2022	2022	Hamedan, Hamedan, Iran	1818	34°47'N	48°30'E
HDM2023	2023				
KER2022	2022	Kerman, Kerman, Iran	1764	30°17'N	57°04'E
KER2023	2023				

Table 2. Characteristics of environmental conditions at experimental research stations.

	Average temperature (°C)							Rainfall (mm)						
Month	Isfahan	Karaj	Mashhad	Moghan	Shiraz	Hamedan	Kerman	Isfahan	Karaj	Mashhad	Moghan	Shiraz	Hamedan	Kerman
2022														
May	17.68	19.39	17.90	12.60	18.00	13.19	18.00	8.00	26.00	23.00	8.10	19.00	14.00	17.00
June	24.45	25.55	24.00	17.80	25.26	19.48	24.13	6.00	18.00	2.00	12.40	0.00	24.00	8.00
July	28.13	27.84	24.97	23.90	28.45	24.97	27.39	0.00	0.00	1.00	23.10	0.00	0.00	0.00
August	26.39	29.42	24.48	27.50	26.71	25.13	24.23	0.00	0.00	0.00	4.80	0.00	0.00	0.00
September	24.29	24.77	24.48	27.30	25.74	21.55	24.06	0.00	0.00	3.00	8.60	0.00	0.00	0.00
October	17.20	17.93	16.88	20.30	19.90	14.03	17.70	3.00	26.00	12.00	6.80	1.00	12.00	1.00
2023														
May	15.97	17.77	13.97	18.00	15.39	12.06	15.35	46.00	31.00	68.00	44.60	77.00	67.00	50.00
June	24.35	24.74	21.39	25.80	25.06	19.97	23.55	6.00	14.00	3.00	15.60	0.00	7.00	5.00
July	29.03	27.74	23.94	26.40	28.90	25.06	27.90	0.00	4.00	2.00	41.60	0.00	1.00	0.00
August	30.48	28.42	25.16	28.90	29.10	26.26	28.26	0.00	0.00	0.00	0.00	4.00	0.00	0.00
September	23.77	25.48	18.77	23.40	24.39	21.97	22.13	0.00	2.00	0.00	58.00	0.00	0.00	2.00
October	17.83	18.89	15.83	18.30	19.63	15.10	18.68	1.00	32.00	1.00	56.50	5.00	2.00	3.00

Table 3. Average temperature and rainfall data at research stations for 2022 and 2023.

Research sites and experimental design

Phenotypic evaluations of the experimental genotypes were conducted over two consecutive crop years (2022 and 2023) at seven agricultural research stations located in Isfahan, Karaj, Kerman, Mashhad, Moghan, Shiraz, and Hamedan (Table 2). These sites were selected for their diverse ecological characteristics, including differences in rainfall, and atmospheric temperature (Table 3). At each research station, the experiment was conducted using a randomized complete block design with three replications. Each genotype was planted in four rows, each 3.25 m in length. The distance between the planting rows was 75 cm, and each row contained 17 seedlings spaced 19 cm apart. Across all trial locations, the maize growing season typically spanned from May (sowing) to October (harvest). Fertilizer was applied based on soil test results and regional recommendations, with a standard application of 150 kg N, 80 kg P_2O_5, and 60 kg K_2O per hectare. Weed control was managed through a combination of pre-emergence herbicides and manual weeding as needed. The plots were regularly monitored for common pests, particularly the European corn borer (*Ostrinia nubilalis*). Control measures were implemented following integrated pest management (IPM) principles only when pest populations reached economic threshold levels. Agronomic traits were recorded from 10 plants randomly selected within the two central rows of each plot. At physiological maturity, PH was measured in cm from the soil surface to the base of the tassel, and EH was measured from the soil surface to the upper bearing node. At the time of harvest, KM was determined as a percentage using a portable digital moisture meter. KY was calculated in t ha^{-1}, after adjusting to a standard 15.5% moisture content, from all ears harvested from the central rows. For yield component analysis, the KR and KIR were counted on 10 representative ears from each plot. Finally, KW was measured in g by weighing a random sample of 1000 kernels taken from the bulked grain of each plot.

Statistical analysis

Before conducting any analysis on the experimental data, the assumption of normality was verified using the Grubbs test[24]. The Pearson correlation method was employed to assess the association among KY, KR, KIR, KW, PH, EH, and KM, based on the Eq. (1):

$$\mathrm{r} = \frac{\mathrm{n}(\sum \mathrm{xy}) - (\sum \mathrm{x})(\sum \mathrm{y})}{\sqrt{[\mathrm{n}\sum \mathrm{x}^2 - (\sum \mathrm{x})^2][\mathrm{n}\sum \mathrm{y}^2 - (\sum \mathrm{y})^2]}} \tag{1}$$

where, r represents the Pearson correlation coefficient, x and y are the values of each variable, and n is the number of observations. The values of the correlation coefficient range from 1 to −1. A correlation coefficient of 1 indicates a perfect direct association between two traits, meaning that as one variable increases or decreases, the other variable does the same. As the coefficient approaches zero, the direct association weakens, and a coefficient of zero indicates no linear association between the variables. As the coefficient moves towards −1, the inverse association between the variables strengthens, with a coefficient of −1 indicating a perfect inverse association. This means that as one variable increases, the other decreases, and vice versa.

To examine the GYT, the original data were first standardized according to the Eq. (2)[14]:

$$\mathrm{P_{ij}} = \frac{\mathrm{T_{ij}} - \overline{\mathrm{T}}_{\mathrm{j}}}{\mathrm{S_j}} \tag{2}$$

where $\mathrm{P_{ij}}$ is the standardized value of genotype i for trait or yield-trait (YT) combination j in the standardized table, $\mathrm{T_{ij}}$ is the original value of genotype i for trait or YT combination j, $\overline{\mathrm{T}}_{\mathrm{j}}$ is the mean across genotypes for trait or YT combination j, and S_j is the standard deviation for YT combination j. The biplot representing the GYT was constructed using the first and second PCs derived from the singular value decomposition of the standardized data. In this model, the singular value decomposition breaks down the GYT data into genotype eigenvalues, YT combination eigenvalues, and singular values. This process is based on Eq. (3) as proposed by Yan and Frégeau-Reid[14]:

$$\mathrm{P_{ij}} = (d\lambda_1^{\alpha}\zeta_{\mathrm{i1}})\left(\lambda_1^{1-\alpha}\tau_{\mathrm{1j}}/\mathrm{d}\right) + (d\lambda_2^{\alpha}\zeta_{\mathrm{i2}})\left(\lambda_2^{1-\alpha}\tau_{\mathrm{2j}}/\mathrm{d}\right) + \varepsilon_{\mathrm{ij}} \tag{3}$$

where ζ_{i1} and ζ_{i2} are the eigenvalues for PC1 and PC2, respectively, for genotype i; τ_{1j} and τ_{2j} are the eigenvalues for PC1 and PC2, respectively for trait j, and ε_{ij} is the residual from fitting the PC1 and PC2 for genotype i on trait j; λ_1 and λ_1 are the singular values for PC1 and PC2, respectively. α is the singular value partitioning factor. The GYT biplot is created by plotting $d\lambda_1^{\alpha}\zeta_{i1}$ against $d\lambda_2^{\alpha}\zeta_{i2}$ for genotypes, and $\lambda_1^{1-\alpha}\tau_{1j}/d$ against $\lambda_2^{1-\alpha}\tau_{2j}/d$ for YT combinations. This analysis focuses on four main patterns: 1. Exploring the associations between different YT combinations, 2. Identifying the best genotype for each YT combination, 3. Ranking genotypes based on a superiority index and evaluating their strengths and weaknesses, and 4. Ranking genotypes based on an ideal hypothetical genotype.

To identify the most superior maize genotypes, a comprehensive evaluation of key traits such as KY, KR, KIR, KW, PH, EH, and KM is necessary. In order to assess the stability of these traits simultaneously, the MTSI was calculated using Eq. (4)[18]:

$$\mathbf{MTSI_i} = \left[\sum_{\mathbf{j=1}}^{\mathbf{f}}\left(\boldsymbol{\gamma}_{\mathbf{ij}} - \boldsymbol{\gamma}_{\mathbf{j}}\right)^2\right]^{0.5} \tag{4}$$

where $\mathrm{MTSI_i}$ is the multi-trait stability index of the genotype i, γ_{ij} is the score of the genotype i in the factor j, and γ_{j} is the score of the ideal genotype in the factor j. The scores were calculated using factor analysis for genotypes and traits. Finally, stable genotypes were selected based on positive selection differentials for traits intended to increase and negative selection differentials for traits intended to decrease.

The effect of GEI on KY, considered a final indicator for decision-making, was investigated. To ensure the validity of the statistical analyses, the uniformity of variance of experimental errors was first examined using Bartlett's test[25]. Following this, stability analysis of the experimental genotypes was conducted based on the AMMI model using the Eq. (5)[20]:

$$Y_{ge} = \mu + \alpha_g + \beta_e + \sum_n \lambda_n\gamma_{gn}\delta_{en} + \rho_{ge} \tag{5}$$

where $\mathrm{Y_{ge}}$ is the yield of genotype g in environment e; μ is the grand mean; α_g is the genotype deviation from the grand mean; β_e is the environment deviation; λ_n is the singular value for $\mathrm{IPC_n}$ and correspondingly λ_n^2 is its eigenvalue; γ_{gn} is the eigenvector value for genotype g and component n; δ_{en} is the eigenvector value for environment e and component n, with both eigenvectors scaled as unit vectors; and ρ_{ge} is the residual. By performing AMMI analysis of variance using R software (metan package), the IPCs were obtained for each genotype and environment. WAAS were estimated from the IPCs of AMMI analysis of variance using Eq. (6)[26]. The IPC1 in the traditional AMMI1 biplot was replaced by WAAS.

$$\mathrm{WAAS_i} = \frac{\sum_{\mathrm{k=1}}^{\mathrm{P}} |\mathrm{IPCA_{ik}} \times \mathrm{EP_k}|}{\sum_{\mathrm{k=1}}^{\mathrm{P}} \mathrm{EP_k}} \tag{6}$$

where $\mathrm{WAAS_i}$ is the weighted average of absolute scores of the $\mathrm{i^{th}}$ genotype or environment; $\mathrm{IPCA_{ik}}$ is the absolute score of the $\mathrm{i^{th}}$ genotype or environment in the $\mathrm{k^{th}}$ IPC; and $\mathrm{EP_k}$ is the magnitude of the variance explained by the $\mathrm{k^{th}}$ IPC. Graphic analysis of GGE biplot was done based on singular value decomposition according to Eq. (7):

$$\mathrm{Y_{ij}} - \mu - \beta_{\mathrm{j}} = \lambda_1 \xi_{\mathrm{i1}} \eta_{\mathrm{j1}} + \lambda_2 \xi_{\mathrm{i2}} \eta_{\mathrm{j2}} + \varepsilon_{\mathrm{ij}} \tag{7}$$

where, $\mathrm{Y_{ij}}$ is the mean of $\mathrm{i^{th}}$ genotype in $\mathrm{j^{th}}$ environment, μ is the mean of all genotypes, β_{j} is the main effect of $\mathrm{j^{th}}$ environment, λ_1 and λ_2 are the special quantities for the first and second components, respectively, ξ_{i1} and ξ_{i2} are the special vectors of genotypes, and η_{j1} and η_{j2} are the environmental vectors of first and second components, respectively, and $\varepsilon_{\mathrm{ij}}$ is the remaining quantity for the $\mathrm{i^{th}}$ genotype in $\mathrm{j^{th}}$ environment.

To evaluate the genotypes stability across the environments, a linear mixed model (LMM) was used. The variance components were estimated by restricted LRT. The estimations were performed assuming genotype and GEI as random effects. The significance of random effects was tested by the LRT. The BLUP of $\mathrm{i^{th}}$ genotype was predicted as the sum of the general mean overall environments and genotypic effect. WASSB were estimated based on singular value decomposition of the GEI effects from the matrix of the BLUP. The equation of WAASB Eq. (8) is similar to WAAS[26]. The WAASBY was calculated allowing weighting between its mean performance and stability based on Eq. (9)[26]:

$$\mathrm{WAAS}B_{\mathrm{i}} = \frac{\sum_{\mathrm{k=1}}^{\mathrm{P}} |\mathrm{IPCA_{ik}} \times \mathrm{EP_k}|}{\sum_{\mathrm{k=1}}^{\mathrm{P}} \mathrm{EP_k}} \tag{8}$$

$$\mathrm{WAASBY_i} = \frac{(\mathrm{rG_g} \times \theta_{\mathrm{Y}}) + (\mathrm{rW_g} \times \theta_{\mathrm{S}})}{\theta_{\mathrm{Y}} + \theta_{\mathrm{S}}} \tag{9}$$

where (Eq. 8) $\mathrm{WAASB_i}$ is the weighted average of absolute scores of the $\mathrm{i^{th}}$ genotype or environment; $\mathrm{IPCA_{ik}}$ is the absolute score of the $\mathrm{i^{th}}$ genotype or environment in the $\mathrm{k^{th}}$ IPC; and $\mathrm{EP_k}$ is the magnitude of the variance explained by the $\mathrm{k^{th}}$ IPC. In Eq. (9), $\mathrm{WAASBY_i}$ is the superiority index with different weights between yield and stability for the $\mathrm{g^{th}}$ genotype; θ_{Y} and θ_{S} are the weights for yield and stability, respectively; $\mathrm{rG_g}$ and $\mathrm{rW_g}$ are the rescaled values of the $\mathrm{g^{th}}$ genotype for yield and WAASB, respectively.

Results and discussion

Correlation analysis

The Pearson correlation analysis of various traits in the experimental maize genotypes, including KY, KR, KIR, KW, PH, EH, and KM, is presented in Fig. 1. The highest positive and significant correlation at the 1% probability level was observed between PH and EH, with a correlation coefficient (r) of 0.77. This strong correlation indicates that as PH increases, EH also tends to increase. Moderate to weak positive and significant correlations were found between several other traits at the 1% and 5% probability levels. KY was most directly and positively influenced by KW (r = 0.54), with smaller contributions from KIR (r = 0.30) and KR (r = 0.26). Interestingly, plant architecture played a significant role, as both PH and EH were positively correlated not only with each other but also with yield components such as KR, KIR, and KW, suggesting that larger plant stature in these genotypes was associated with improved yield-related traits. The highest negative correlation was observed between the KIR and the KR (r = -–0.14), suggesting that an increase in the KIR is associated with a decrease in the KR. Additionally, a weak negative correlation was found between KW and KM (r = -–0.13). Similar associations between KY and yield-related components have been reported in previous studies[27–29]. These complex associations between key traits introduce challenges in the breeding process. Breeders must carefully manage these correlations to optimize the selection of desirable traits. In this context, GYT biplot graphical method is comprehensive and efficient tool for visualizing and interpreting these associations[2,14].

Genotype by Yield × Trait (GYT)

To elucidate the genetic factors influencing crop productivity, a GYT graphical analysis method was employed. The results indicated that the first and second PCs accounted for 58.91% and 24.81% of the total variation in the KY data, respectively, cumulatively explaining 83.72% of the total variance. The ability of the first two PCs to explain more than 70% of the variance in the data underscores the high validity of the biplots generated in this study for interpreting GYT variations[30]. It is noteworthy that the proportion of variance explained by the initial PCs is context-dependent and influenced by factors such as the number of genotypes and environments included in the analysis. Therefore, while the value of 83.72% is high and suggests a reliable biplot, it should be interpreted within the specific genetic and environmental scope of this study. When the sum of the PC1 and PC2 fails to account for the majority of the variation in the data, it often indicates the data's inherent complexity[31]. However, this does not necessarily invalidate the biplot[32]. Shojaei et al.[33] demonstrated that approximately 50% of the GYT variation was explained by the first two PCs. Faheem et al.[34] estimated that the PC1 and PC2 collectively explained nearly 85% of the total variation in their experiment, with the PC1 contributing about 74% and the PC2 close to 11%. Hassani et al.[2] reported that the PC1 and PC2 explained 50.53% and 34.96%, respectively, totaling 85.49% of the variation in yield data. Similarly, Hosseini et al.[28] found that the PC1 and PC2 accounted for 64.54% and 14.38%, respectively, summing to 78.92% of the total variation in KY data. These

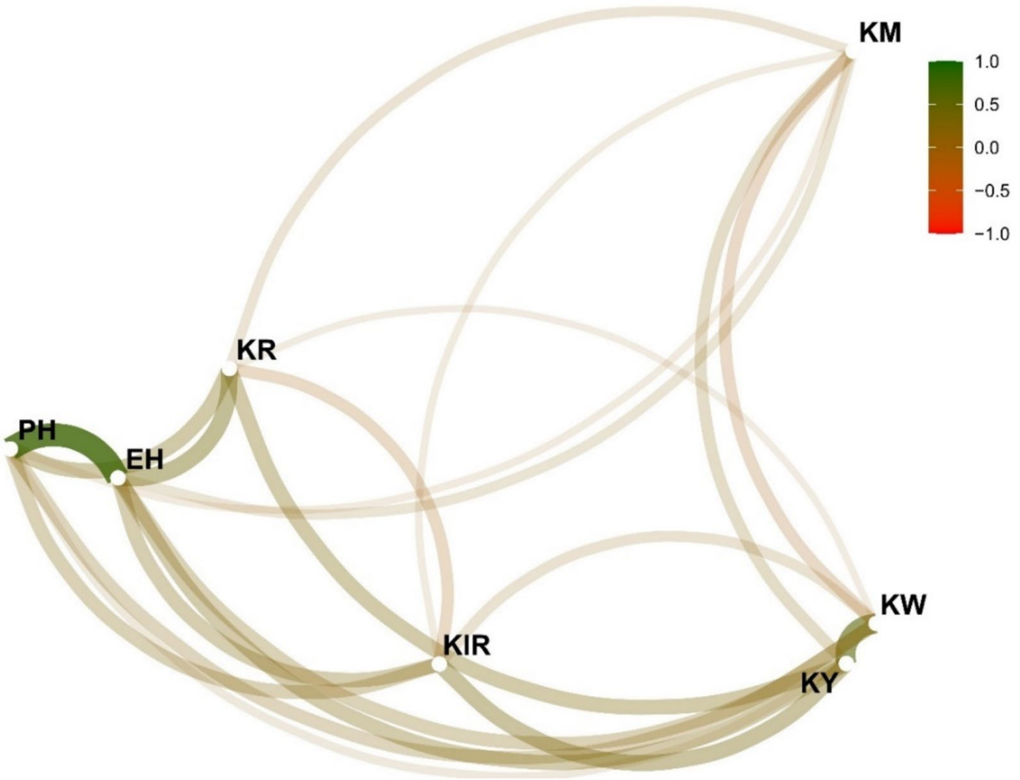


Fig. 1. Pearson correlation analysis among key traits in maize genotypes. KY, Kernel yield; KR, Kernel row; KIR, Kernel in row; KW, 1000 kernel weights; KM, Kernel moisture; PH, Plant height; EH, Ear height.

results collectively highlight the efficacy of PCA in capturing the majority of the variation in GYT data, thereby validating the use of biplots for interpreting complex genetic interactions and their impact on crop productivity.

The study of correlations between YT combinations is crucial for understanding the associations between traits and can be instrumental in future experiments aimed at developing new genotypes. In the biplot (Fig. 2a), the acute angles between the majority of vectors indicate positive correlations between YT combinations. This tendency for positive correlation is primarily due to the inclusion of yield as a PC in these combinations[14]. The high correlation between YT combinations suggests a strong correlation between the ranks of genotypes based on these combinations. Specifically, there is a very high positive correlation between KY × KR and KY × PH. However, no correlation was observed between the KY × KIR and the KY × KW. Interestingly, KY × KIR showed a weak positive correlation with the KY × PH, KY × KR, and KY × EH. No correlation was found between KY × EH, and KY/KM. However, KY × EH are positively correlated with KY × KW, KY × PH, and KY × KR. KY × KIR exhibited a very weak negative correlation with KY/KM. The positive correlations between KY × PH, and KY × KR with other combinations suggest that increasing PH and the KR in a genotype may lead to an increase in the KIR, EH, and KW, while simultaneously decreasing KM to some extent. The presence of KY/KM in these combinations complicates the improvement process for certain traits, necessitating precise breeding programs. Similar associations among different trait combinations and KY have been reported by Hosseini et al.[28], aligning with the findings of this study.

In biplot of Fig. 2b, a polygon is formed by connecting genotypes 5, 8, 9, 1, and 7, which are located at the maximum distance from the coordinate origin. Perpendicular lines were drawn from the coordinate origin to the sides of this polygon to determine the grouping of GYT. The sections where YT combinations are located, with genotypes at the vertices, indicate that these genotypes have high performance for those combinations, meaning they are the best genotypes for the relevant YT. In the KY × KIR and KY × EH, genotype 7 was identified as the best. For the KY × KR, KY × PH, KY × KW, and KY/KM, genotypes 1, 10, and 3 were identified as the best. Genotypes that were not located in any of the desirable combinations, i.e., those not within the polygon, were considered weak genotypes. Based on the polygonal biplot, the YTs were divided into two groups. The first group includes KY × KIR and KY × EH. The second group includes other combinations: KY × KR, KY × PH, KY × KW, and KY/KM. Hosseini et al.[28] reported similar results, showing that KY × KIR was placed in one group, while KY/ear wood weight, KY/KM, KY × PH, KY × KR, KY × EH, and KY × KW were placed in another group. These findings highlight the utility of polygonal biplots in identifying superior genotypes for specific YTs and in grouping YTs for more targeted breeding efforts.

To evaluate the ranking of genotypes based on YTs, the average tester coordinate biplot (Fig. 2c) and the superiority index (Table 4) were utilized. In the average tester coordinate biplot, the axis with an arrow passing through the origin of the coordinates represents the average of all YTs. The axis perpendicular to the mean

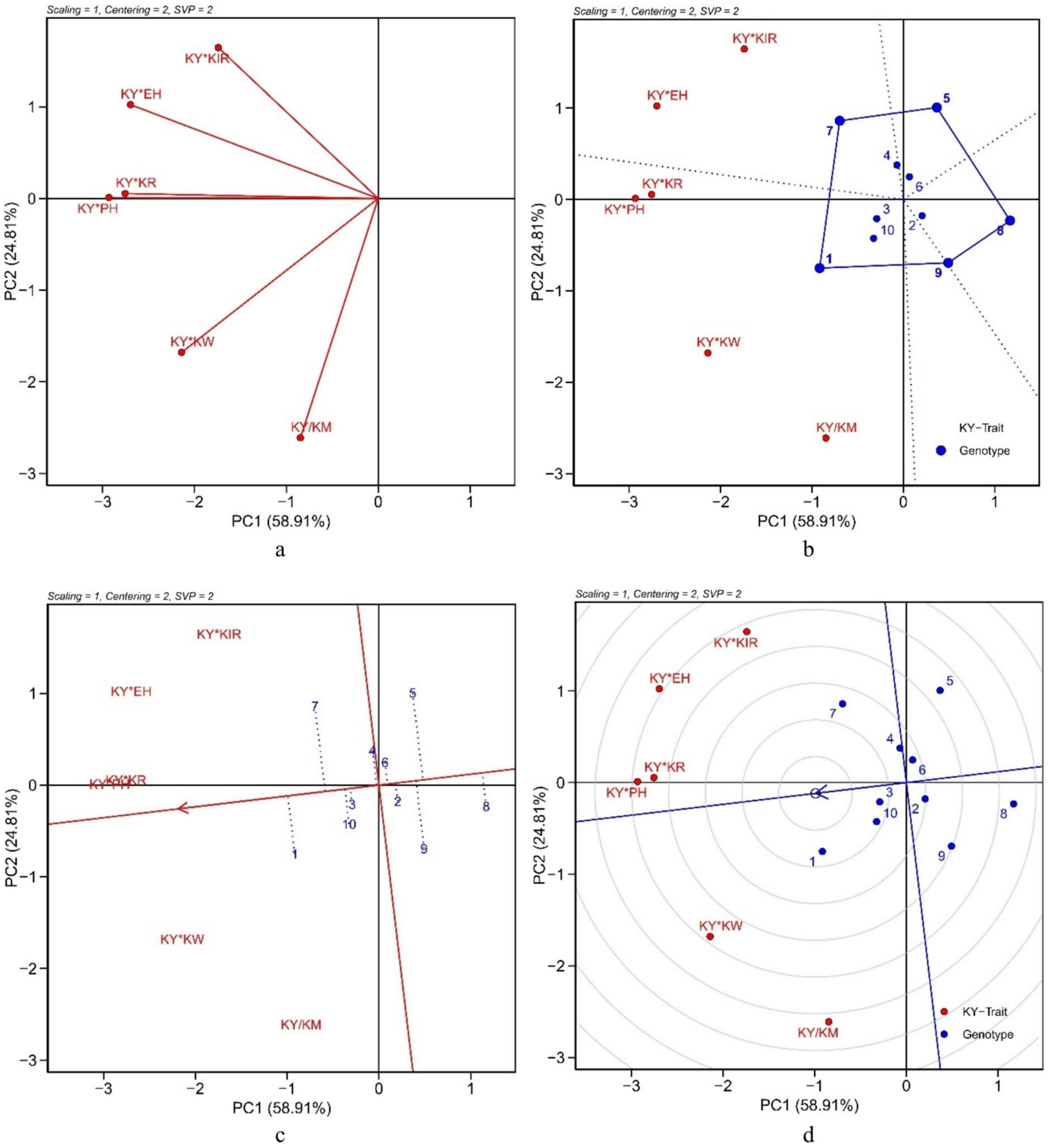


Fig. 2. Tester vector view of the genotype by kernel yield-trait biplot for associations among the yield-trait combinations (**a**), Which-won-where view of genotype by kernel yield-trait biplot highlighting genotypes with outstanding profiles (**b**), Average Tester Coordination view of genotype by kernel yield-trait biplot ranking genotypes based on overall superiority and strengths and weaknesses (**c**), Genotype ranking view of genotype by kernel yield-trait biplot ranking genotypes based on ideal genotype (**d**). KY, Kernel yield; KR, Kernel row; KIR, Kernel in row; KW, 1000 kernel weights; KM, Kernel moisture; PH, Plant height; EH, Ear height.

axis indicates the balance of genotypes in terms of these combinations. As indicated, genotype 1, followed by genotypes 7, 10, and 3, exhibited the highest average KY and were identified as the best genotypes. Conversely, genotype 8 had the lowest average KY. The results of the average tester coordinate biplot were corroborated by the superiority index. Numerous studies[2,14,28,33,34] and the findings of this study demonstrate that the biplot of the average tester coordinates in the GYT biplot method is a valuable tool that provides comprehensive information about genotypes.

The ideal genotype is conceptually defined as one that combines high mean performance across all yield-trait combinations with high stability. Any genotype that is closest to this hypothetical genotype is considered superior, while the genotype furthest from it is deemed the most undesirable. Based on this diagram, genotypes 1, 10, and 3 were identified as the best genotypes due to their proximity to the hypothetical ideal genotype. In

Genotype	KY × KR	KY × KIR	KY × KW	KY × PH	KY × EH	KY/KM	Mean superiority index
1	1.61	−0.30	1.44	1.74	1.00	1.43	1.15
2	−0.70	−0.42	0.48	−0.13	−0.50	−0.29	−0.26
3	0.55	−0.07	0.97	0.28	0.28	0.05	0.34
4	−0.46	1.66	−0.21	0.21	−0.22	0.02	0.17
5	−0.08	0.33	−1.46	−0.72	−0.03	−1.71	−0.61
6	−0.53	−0.68	0.27	0.04	0.62	−1.07	−0.22
7	1.15	1.47	−0.35	1.14	1.62	−0.61	0.74
8	−1.34	−1.73	−1.77	−1.75	−1.77	−0.03	−1.40
9	−1.09	−0.45	0.17	−0.96	−1.20	1.06	−0.41
10	0.89	0.20	0.45	0.14	0.21	1.14	0.50
Mean	0.00	0.00	0.00	0.00	0.00	0.00	–
Standard deviation	1.00	1.00	1.00	1.00	1.00	1.00	–

Table 4. Standardized genotype by kernel yield-trait data and superiority index for maize genotypes. KY, Kernel yield; KR, Kernel row; KIR, Kernel in row; KW, 1000 kernel weights; KM, Kernel moisture; PH, Plant height; EH, Ear height.

Variable	FA1	FA2	FA3	Communality	Uniqueness's	Goal	h^2	SD (%)	SG (%)
KY	0.89	−0.27	−0.11	0.88	0.12	increase	0.69	2.23	1.53
PH	0.88	−0.17	0.23	0.85	0.15	increase	0.95	−0.69	−0.65
EH	0.88	0.15	0.04	0.80	0.20	increase	0.96	−3.69	−3.53
KW	0.36	−0.82	−0.14	0.83	0.17	increase	0.89	1.51	1.35
KM	−0.06	−0.94	0.03	0.89	0.11	decrease	0.90	3.20	2.89
KR	0.24	0.35	0.81	0.84	0.16	increase	0.96	−4.41	−4.22
KIR	0.07	0.14	−0.91	0.85	0.15	increase	0.87	0.47	0.41
Eig	2.72	1.90	1.30	–	–	–	–	–	–
Proportion (%)	38.87	27.27	18.53	–	–	–	–	–	–
Accumulated (%)	38.87	66.14	84.67	–	–	–	–	–	–

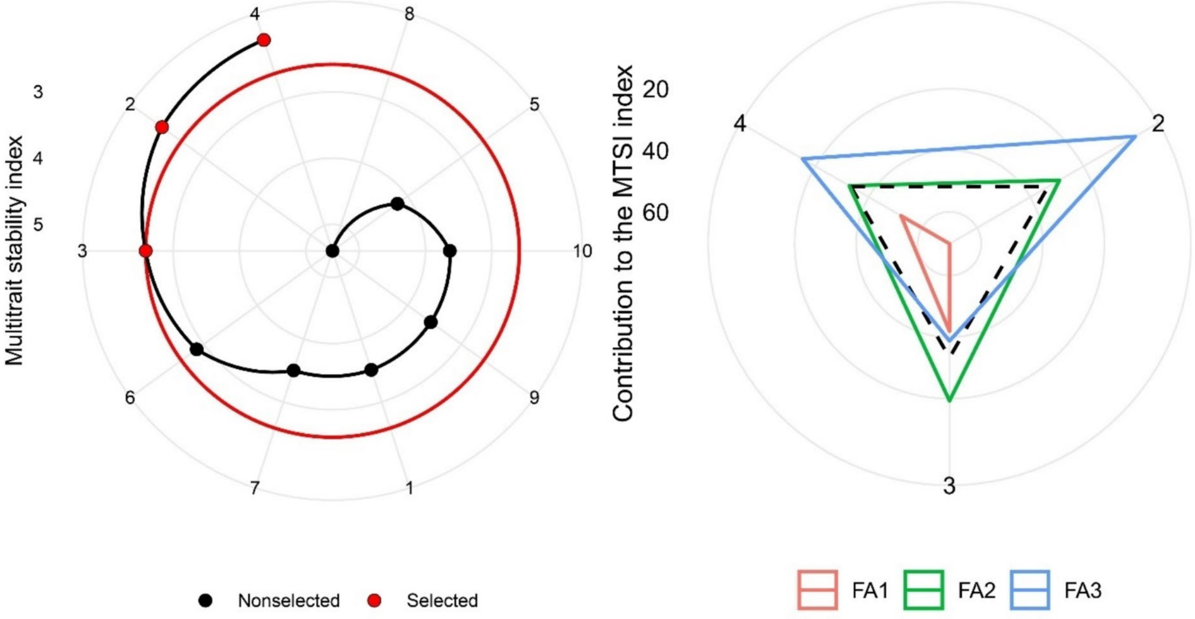
Table 5. Prediction of selection differential for studied traits based on multi-trait stability insex (MTSI). KY, Kernel yield; KR, Kernel row; KIR, Kernel in row; KW, 1000 kernel weights; KM, Kernel moisture; PH, Plant height; EH, Ear height, Eig, Eigen value; FA, Factor; h^2, Heritability; SD, Selection differential; SG, Selection gain.

contrast, genotypes 8, 5, and 9 were classified as undesirable genotypes because they were the furthest from the hypothetical ideal genotype (Fig. 2d). Hassani et al.[2] and Hosseini et al.[28] employed the GYT graphical method to analyze trait associations and select superior genotypes, yielding useful results. This method further supports the efficacy of graphical analysis in identifying and ranking superior genotypes for breeding programs.

Multi-trait stability index (MTSI)

Factor analysis was conducted using PCA, and the results were interpreted following Varimax rotation. Table 5 displays the outcomes of the factor analysis. Factors with eigenvalues exceeding one were selected, and the variance of each factor was expressed as a percentage to indicate its importance in interpreting the overall data variations. In this analysis, three independent factors collectively accounted for 84.67% of the data variations. The first factor, with an eigenvalue of 2.72, explained 38.87% of the total data variance. This factor exhibited large positive factor coefficients for KY, PH, and EH. The second factor, with an eigenvalue of 1.90, explained 27.27% of the variations and had large negative factor coefficients for KW and KM. The third factor, with an eigenvalue of 1.30, accounted for 18.53% of the total data variance. This factor showed a large positive factor coefficient for the KR and a large negative factor coefficient for the KIR. These findings are largely consistent with those of Hosseini et al.[28], who also identified a primary factor in maize that grouped KY with plant architecture traits (PH and EH). This consistency across studies reinforces that a major axis of genetic variation in maize is linked to overall plant stature and its direct impact on yield. While the grouping of secondary traits showed minor differences, likely due to variations in the germplasm and environments tested, the fundamental structure of the main factors was analogous, thus validating the results of this analysis.

The MTSI for the studied genotypes was calculated based on the factor scores of the three factors. According to this index, a lower value indicates a smaller distance from the ideal genotype, suggesting greater similarity with the ideal stable genotype. Conversely, a higher value of the MTSI indicates a greater distance from the ideal stable genotype and suggests that the genotype should not be selected. In Fig. 3a, the experimental genotypes are ranked from the highest to the lowest value of the MTSI. The genotype with the highest value of the index is



a

b

Fig. 3. Ranking of genotypes in ascending order based on multi-trait stability index (MTSI) (**a**), and Strengths and weaknesses of selected genotypes as the ratio of each factor in the calculated multi-trait stability index (MTSI) (**b**).

positioned in the center, while the genotype with the lowest value is located in the outermost orbit. By applying a selection pressure of 25%, genotype 4 ranked first, and genotypes 2 and 3 ranked second as the most ideal stable genotypes across all studied traits.

A comparison of trait values in selected genotypes based on the MTSI with those in all experimental genotypes revealed that the average values of KY, KW, KM, and KIR increased in the selected genotypes. However, the average values of PH, EH, and KR decreased. While the increase in KY, KW, and KIR aligned with the breeding goals, the decrease in PH, EH, and KR was not a desired outcome in maize breeding programs. The goal for KM is to reduce its value, which was not achieved as the selected genotypes showed an increase in this trait. Overall, the selected genotypes exhibited a selection differential and favorable selection gains in some traits, including KY, KW, and KIR (Table 5). In the selected genotypes, all traits except KY had high heritability.

Figure 3b illustrates the strengths and weaknesses of the selected genotypes based on the contribution of each factor to the MTSI. The lowest contribution explained by a factor (close to the outer edge) indicates that the traits within that factor are close to the stable ideal. The dashed line represents the theoretical value if all factors contribute equally. Since each genotype is closer to the ideal genotype in terms of traits within a factor that has a lower contribution, genotypes 3 and 4, which had the lowest value in the first factor, are close to the ideal genotype for the traits of KY, PH, and EH, which had the highest factor coefficients in this factor. The ideal genotype is defined based on the traits included in each factor and the goals set for improving those traits. Genotype 3 had the lowest contribution to the second factor, indicating that these genotypes were very close to the ideal genotype for the KW and KM. The highest contribution of the third factor was made by genotypes 2 and 4, which were identified as very close to the ideal genotype for the KR and KIR. Using the MTSI, Hosseini et al.[28] identified seven genotypes resulting from diallel crossing as ideal genotypes. Based on the MTSI, Rajabi et al.[35] introduced five genotypes as stable genotypes under disease-infected conditions. Taleghani et al.[36] identified four ideal genotypes simultaneously for different traits using the MTSI. These findings highlight the effectiveness of the MTSI in selecting genotypes that exhibit desirable traits and stability[37], thereby aiding in the optimization of breeding programs.

Additive main effects and multiplicative interaction (AMMI)

The combined analysis of variance for KY, based on the AMMI model, is presented in Table 6. The additive effects of both environment and genotype were significant at the 1% probability level. The significant main effect of the environment confirms that variation among locations and, critically, between the two years of the study was a major determinant of KY. This underscores the importance of the year effect, driven by annual fluctuations in weather and other conditions, as a substantial component of the total phenotypic variation. Additionally, the GEI significantly influenced KY at the 1% probability level. The significant GEI underscores the importance of considering environmental factors when selecting stable and high-yielding genotypes. These results are consistent with previous studies that have emphasized the inevitability of GEIs in agricultural research[38,39]. Previous studies have documented significant variations across experimental years, locations, and genotypes, attributing these differences to changes in environmental conditions and plant genetic structure[2,40]. The findings of this study

Source of variation	Df	Sum of square	Mean of square	Proportion (%)	Accumulated (%)
Environment	13	3870.09	297.70**	–	–
Error 1	28	247.18	8.83	–	–
Genotype	9	145.33	16.15**	–	–
Environment-genotype interaction	117	589.54	5.04**	–	–
Principal component 1	21	299.73	14.27**	50.80	50.80
Principal component 2	19	90.31	4.75**	15.30	66.20
Principal component 3	17	71.46	4.20**	12.10	78.30
Principal component 4	15	56.82	3.79*	9.60	87.90
Principal component 5	13	36.43	2.80^{ns}	6.20	94.10
Principal component 6	11	20.94	1.90^{ns}	3.60	97.70
Principal component 7	9	8.85	0.98^{ns}	1.50	99.20
Principal component 8	7	3.03	0.43^{ns}	0.50	99.70
Principal component 9	5	1.96	0.39^{ns}	0.30	100.00
Residuals	252	513.69	2.04	–	–
Total	536	5955.36	11.11	–	–
Coefficient of variation (%)			12.84		

Table 6. Combined analysis of variance for kernel yield of maize genotypes based on additive main effects and multiplicative interaction (AMMI) model. ns, *, **: non-significant and significant at 5% and 1% probability levels, respectively.

support these observations, demonstrating that the environment and its interaction with the genetic structure of different genotypes play a crucial role in the phenotypic expression of key traits. The study by Sadeghzadeh Hemayati et al.[41] showed that genotypes respond differently to different locations, emphasizing the need to evaluate genotypes in diverse environments. Similarly, Saremirad and Taleghani[40] demonstrated that GEIs affect both quantitative and qualitative traits. Therefore, understanding the significant effects of these interactions on key traits is essential for selecting the most suitable genotypes for specific environmental conditions. This knowledge is crucial for optimizing breeding strategies and minimizing yield losses due to environmental variability.

While GEIs are often seen as a challenge, they also present opportunities to identify genotypes that perform exceptionally well under specific environmental conditions. This can lead to the development of specialized varieties suited to particular climates or regions, thereby maximizing yield and quality. In this regard, multivariate statistical methods have been instrumental in revealing and better understanding these interactions[2]. Therefore, agricultural researchers should consider these methods to achieve desirable results in advancing agricultural research. By leveraging the insights gained from multivariate statistical analyses, breeders can make more informed decisions, leading to the development of stable and high-yielding genotypes that are well-adapted to diverse environmental conditions. This approach not only enhances the efficiency of breeding programs but also contributes to the sustainability and productivity of agricultural systems.

Given the significant effect of GEI on KY, a multiplicative effects analysis was performed to identify stable genotypes based on the AMMI. The AMMI, which includes two PCs of significant GEI, is considered the best predictive model[42,43]. Based on the results presented in Table 5, the first three PCs were significant at the 1% probability level, and the fourth PC was significant at the 5% probability level. Each of these PCs accounted for 50.80%, 15.30%, 12.10%, and 9.60% of the GEI variation, respectively, totaling 87.90%. The remaining PCs of the AMMI were not significant and had the lowest mean squares, indicating a high degree of accuracy in this model[44]. Omrani et al.[45] found that the first four PCs explained a total of 83% of the variation in the GEI. Fathi et al.[46] estimated that the PC1 and PC2 of the GEI contributed 49.10% and 22.50%, respectively, accounting for a total of 71.60% of the variation. In a study using the AMMI, the PC1 was significant and explained about 63% of the variation in the data[47]. Rajabi et al.[48] reported that the first six PCs of the GEI had a significant effect and explained a total of 98.80% of the total variation. These findings collectively highlight the effectiveness of the AMMI in capturing the complex interactions between genotypes and environments. By identifying the significant PCs of the GEI, researchers can better understand the stability and yield of different genotypes across various environmental conditions.

The mean yield versus WAAS biplot, referred to as the WAAS biplot for simplicity (Fig. 4), differs from the AMMI by considering all PCs of the GEI rather than just the PC1. This allows WAAS to account for the entire variance of the GEI when identifying stable genotypes. In this biplot, the vertical line in the middle represents the total KY average across the 14 experimental environments. Genotypes and environments to the right of this line have yield values higher than the total average, while those to the left have lower values. The horizontal axis in the middle of the biplot represents the mean area of the WAAS. From the intersection of this axis with the vertical axis (mean KY), the biplot is divided into four quadrants. Genotypes in different quadrants can be classified based on their suitability to different environments. Genotypes 8 and 9 in the first quadrant had high WAAS and KY lower than the overall average, indicating high fluctuation and instability, and are not recommended for cultivation. Genotypes 7, 10, and 1 in the second quadrant had high WAAS and KY higher than the overall average, showing good discrimination ability under favorable conditions and are recommended for cultivation

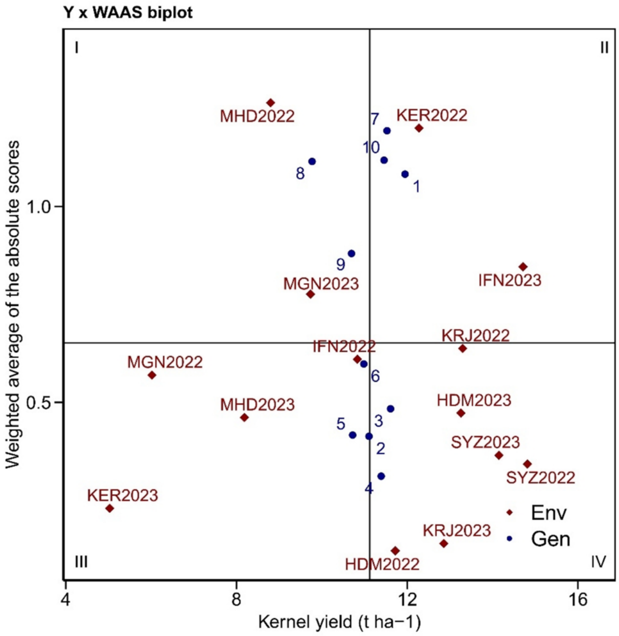


Fig. 4. Mean vs. weighted average absolute scores (WAAS) biplot from additive main effects and multiplicative interaction (AMMI) model for kernel yield of maize genotypes. IFN2022: Isfahan in 2022, IFN2023: Isfahan in 2023, SYZ2022: Shiraz in 2022, SYZ2023: Shiraz in 2023, KRJ2022: Karaj in 2022, KRJ2023: Karaj in 2023, KER2022: Kerman in 2022, KER2023: Kerman in 2023, MHD2022: Mashhad in 2022, MHD2023: Mashhad in 2023, MGN2022: Moghan in 2022, MGN2023: Moghan in 2023, HDM2022: Hamedan in 2022, HDM2023: Hamedan in 2023.

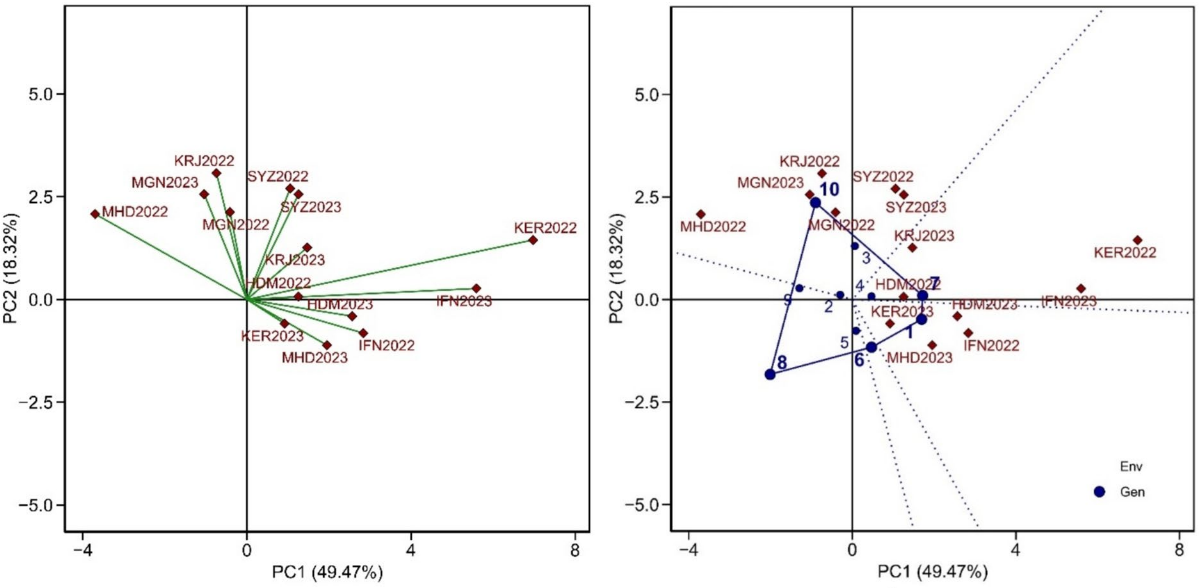
in ideal conditions for maize growth. Genotypes 5, 2, and 6 in the third quadrant had lower WAAS, indicating stability or insensitivity to environmental conditions but lower KY. Genotypes 4 and 3 in the fourth quadrant had lower WAAS values and higher KY than the total average, making them stable genotypes with favorable yields due to their low sensitivity to environmental conditions and good yield.

In general, the WAAS biplot can be interpreted more simply as genotypes with zero or close to zero WAAS are considered the most stable genotypes. Therefore, genotypes 4, 2, 5, and 3 have low GEI and high stability. However, ideal genotypes are those that, in addition to having zero or close to zero WAAS, also have higher KY than the total average. Genotypes 4 and 3, in addition to stability, had KY higher than the total average, so they were selected as stable genotypes with desirable yield values. The advantage of using the WAAS, which is calculated based on AMMI, is that unlike the AMMI that only considers the PC1 of GEI, the WAAS expresses stability based on all scores of the PCs of GEI. Therefore, WAAS considers the entire variance of GEI in identifying stable genotypes. Since the PC1 related to yield accounts for nearly 51% of the variation in the GEI, it cannot be successful alone in identifying genotypes with stable yield. Therefore, in situations where the number of significant GEI PCs is high and the PC1 cannot explain a large part of the GEI, it is suggested that the WAAS be used, as this index can fully apply the GEI variance in identifying stable genotypes. So far, these stability indexes have been used to identify genotypes with favorable and stable yields in various plants such as wheat[49], lentils[19], soybeans[50], rice[51], sugar beet[36,41], and maize[52], and useful results have been presented.

GGE biplot

The GGE biplot graphical analysis method was employed to identify the variation between genotypes, environments, and to study the GEI. The results of the GGE biplot method revealed that the PC1 and PC2 explained 49.47% and 18.32% of the total variation in the KY data, respectively, accounting for a total of 67.79%. Since the first two PCs accounted for a significant share of the data variance, it can be confirmed that the biplots obtained from this study are relatively reliable in explaining genotype and GEI variations. It has been stated that when the biplots explain at least 60% of the variance in the data, they can be used to identify MEs[53]. Saremirad et al.[54] estimated the sum of the PC1 and PC2 to be close to 74%, with the PC1 accounting for about 46% and the PC2 for close to 27% of the total data variation. By explaining a significant portion of the variance, the GGE biplot provides valuable insights into the yield and stability of genotypes across different environmental conditions, aiding in the identification of MEs and the selection of superior genotypes for breeding programs.

Studying the correlation between the environments under study can determine the associations between the environments and be useful in future experiments in terms of time and cost. In this regard, the smaller the angle between the vectors of the environments, the greater the correlation between the environments. High correlation between the environments means that there is a high correlation between the ranks of the genotypes in those environments. As shown in Fig. 5a, a complete positive correlation was observed between the environments KRJ2022 and MGN2022, HDM2022 and IFN2023, and also between KER2023 and MHD2023, which indicates



a

b

Fig. 5. Biplot examine association between environments based on kernel yield (**a**), and polygon of GGE biplot method for determine the appropriate genotypes in each environment based on kernel yield (**b**). IFN2022, Isfahan in 2022; IFN2023, Isfahan in 2023; SYZ2022: Shiraz in 2022; SYZ2023, Shiraz in 2023; KRJ2022, Karaj in 2022; KRJ2023, Karaj in 2023; KER2022, Kerman in 2022; KER2023, Kerman in 2023; MHD2022, Mashhad in 2022; MHD2023, Mashhad in 2023; MGN2022, Moghan in 2022; MGN2023, Moghan in 2023; HDM2022, Hamedan in 2022; HDM2023, Hamedan in 2023.

a similar response of the genotypes in these locations. The environments MHD2022, MGN2023, KRJ2022, MGN2022, SYZ2022, and SYZ2023 showed a range of strong positive correlations to zero with each other. Similarly, the environments MGN2023, KRJ2022, MGN2022, SYZ2022, SYZ2023, KRJ2023, and KER2022 had such associations with each other, as well as the environments SYZ2022, SYZ2023, KRJ2023, KER2022, HDM2022, IFN2023, HDM2023, IFN2022, MHD2023, and KER2023. In contrast, the environment MHD2022 had a range of negative to zero correlations with the environments KER2023, MHD2023, IFN2022, HDM2023, IFN2023, HDM2022, KER2022, and KRJ2023. Such associations were observed between environments MGN2023, KRJ2022, and MGN2022 with environments KER2023, MHD2023, IFN2022, HDM2023, IFN2023, and HDM2022. A particularly critical finding from this analysis was the negative or near-zero correlation observed between the two experimental years within the same location, for instance between MHD2022 and MHD2023, or KER2022 and KER2023 (Fig. 5a). This indicates a strong crossover genotype × year interaction, meaning that the ranking of genotypes changed drastically from one year to the next in these locations. Such a strong temporal interaction presents a significant challenge for breeders, as it complicates the selection of genotypes with stable yield. It underscores the absolute necessity of conducting multi-year trials to identify varieties that possess true stability over time. Furthermore, from a breeding program perspective, locations exhibiting such high year-to-year variability may not be ideal as representative testing sites for selecting specifically adapted genotypes. However, they are highly valuable as discriminating environments for identifying broadly adapted genotypes that can buffer against unpredictable annual climatic fluctuations.

The vector length of environments is an approximation of the standard deviation within each environment and also serves as an indicator for the differentiation of environments, such that environments with greater vector length have greater standard deviation and therefore have greater differentiation[39]. Therefore, one of the important characteristics of each environment is its differentiation, such that environments without differentiation cannot provide useful information about the genotypes used in the experiment[39]. The study of the environment vectors indicated that the majority of environments had long vector lengths, indicating high discrimination of these environments, and only the Hamedan had lower discrimination during the two years of the experiment (HDM2022 and HDM2023) due to its shorter vector length. Finally, the biplot study of the environment correlations showed that most of the tested environments had high discrimination and could provide appropriate discrimination between maize genotypes in experiments studying GEIs. By identifying environments with high discrimination and strong positive correlations, researchers can more effectively evaluate genotype yield and stability across diverse conditions, ultimately enhancing the efficiency and success of breeding programs.

Figure 5b is a polygon biplot drawn to identify MEs and superior genotypes in different environments. In this biplot, a polygon is formed by connecting the genotypes that have the maximum distance from the origin of the coordinates. Genotypes 10, 7, 1, 6, and 8 were located at the farthest distance and formed the polygon. Perpendicular lines were then drawn from the origin of the coordinates to the sides of this polygon to identify the MEs[22]. In the sections where environments are located and genotypes are at the vertices, it indicates that these genotypes have good KY in those environments; in other words, they are the best genotypes for cultivation in these environments. Accordingly, in the MHD2022, MGN2023, KRJ2022, MGN2022, SYZ2022, and SYZ2023, genotypes 10 and 3 were the best in terms of KY. In the KRJ2023, KER2022, HDM2022, and IFN2023, genotype 7 was superior, and in the KER2023, HDM2023, IFN2022, and MHD2023, genotype 1 was the best. Genotypes that were placed in sections where there is no environment are not favorable for cultivation in any of the studied areas and are among the weak genotypes in most of the tested areas. Based on the polygon biplot, the experimental environments were placed in three different MEs: the first ME includes MHD2022, MGN2023, KRJ2022, MGN2022, SYZ2022, and SYZ2023; the second ME includes KRJ2023, KER2022, HDM2022, and IFN2023; and the third ME includes KER2023, HDM2023, IFN2022, and MHD2023. Previous studies have also utilized similar methods to identify stable genotypes and classify environments. For instance, Mostafavi and Saremirad[16] identified stable genotypes in different environments by evaluating various maize genotypes. Saremirad et al.[54] evaluated genotypes in different regions for stability and found that GEI significantly affects the quantitative and qualitative characteristics of yield, emphasizing the importance of considering this interaction when breeding new genotypes. Estimating GEI allows for decisions regarding breeding for general or specific adaptation, which depends on the stability and sustainability of yield in a limited or wide range of environmental conditions. This approach is crucial for developing varieties that are stable and adaptable to target environments, ultimately improving agricultural productivity and sustainability.

Best linear unbiased prediction (BLUP)

The results of Table 7 showed that, based on the LRT, the effect of genotype and environment was significant at the 1% probability level. The GEI was also significant at the 1% probability level. The significant effect of GEI indicates that the genetic structure of the genotype, the environmental conditions prevailing in the field, and the interaction between them determine the phenotypic expression of KY. Due to the polygenic nature of the mentioned trait, it is continuously influenced by the regions controlling quantitative traits, genomic regions with associated genes, and environmental conditions[55]. As a result, genes that affect yield and its components are highly sensitive to environmental conditions and show the interaction of quantitative trait loci. This interaction between the quantitative trait loci and the environment can facilitate or limit the response to selection[56]. Therefore, breeding programs should take these effects seriously and manage them properly[57], for which purpose the use of BLUP to estimate genetic variance components will provide better and more reliable results[18]. Therefore, the values of genetic variance components were estimated using the REML/BLUP method.

The results indicated that environmental variance accounted for the largest amount of phenotypic variance (61.71%), while genotypic variance contributed the smallest share (8.01%) (Table 7). This finding is of critical importance for breeders. A low ratio of genotypic variance to total phenotypic variance indicates that selection based purely on observed phenotypes would be inefficient. The reason is that the large environmental and GEI effects can mask the true underlying genetic potential of the genotypes, making it difficult to distinguish genuinely superior lines from those that performed well simply due to favorable local conditions (Falconer and Mackay, 1996). This situation powerfully underscores the value of the BLUP methodology, which is specifically designed to dissect these variance components and provide more accurate estimates of genotypic breeding values by effectively filtering out the environmental noise. The results of the study by Taleghani et al.[58] confirmed that the environment had the greatest effect on yield, followed by genotype, which accounted for the most explained variance. The GEI accounted for the least changes in the data. In the study by Basafa and Taherian[59], the explained variance by the GEI was 84.7%. In the study by Mostafavi and Saremirad[47], genotype caused the most change in yield, indicating the high diversity of genotypes. Hassani et al.[2] showed that environmental variance accounted for the largest amount, followed by GEI variance in second place, and genotypic variance in third place, which was consistent with the results obtained for the present study. These findings highlight the significant role of environmental factors in influencing KY and the relatively lower impact of genetic factors. This underscores the importance of considering environmental conditions in breeding programs to optimize selection and improve crop yield across diverse environments.

Heritability plays an important role in breeding programs by helping plant breeders select plants with desirable traits and develop effective breeding strategies[18,60]. By understanding the nature of heritability, breeders can make informed decisions[61,62]. In fact, heritability is a statistical concept that estimates the degree

Source of variation	Variance	Proportion variance	Accumulation variance
Environment	2.03**	61.71	61.71
Genotype	0.26**	8.01	69.72
Genotype-environment interaction	1.00**	30.28	100.00
Genetic variance component			
δ^2_{P}	3.30	-	–
$\mathrm{h}^2_{\mathrm{mg}}$	0.69	-	-
R^2 Genotype-environment interaction	0.30	-	-
Accuracy	0.83	-	-

Table 7. Estimation of variance components from linear mixed model (LMM) for kernel yield in maize genotypes. **, Significant at 1% probability level.

of correspondence between the phenotypic and genotypic values of a trait. If the heritability of a trait is less than 0.30, it indicates low heritability; if it is between 0.30 and 0.60, it has moderate heritability; and if it is more than 0.60, it has high heritability[63]. The results obtained showed that KY had high heritability (Table 7). High heritability means that, to some extent, the phenotype is a good indicator of the genotype, and selection based on the phenotype will be effective. The genotypic correlation between environments was low. Low correlation indicates difficulties in selecting superior stable genotypes because this correlation suggests that the genetic effects of KY were low in different environments. In this regard, accurate information and details are needed to select superior genotypes[37]. A critical measure of the predictive quality of mixed model is selection accuracy, which represents the correlation between the BLUPs and the true (but unknown) genotypic values. In this study, the selection accuracy for KY was 0.83 (Table 7), a value considered high in plant breeding trials[64]. Such a high accuracy indicates that the experimental design and statistical model were robust, allowing for a reliable differentiation among genotypes. This provides strong confidence that the selections based on BLUP estimates are likely to reflect true genetic superiority, thereby increasing the probability of success in future breeding cycles. High heritability and selection accuracy provide confidence that the phenotypic traits observed are good indicators of the underlying genotype, making the selection process more effective and reliable. This, in turn, enhances the potential for developing superior genotypes that can perform well across different environmental conditions.

The predicted mean values of the genotypes in terms of KY are presented in Fig. 6. Blue circles indicate predicted means that were higher than the overall mean, while red circles indicate predicted means that were lower than the overall mean. Out of the 10 genotypes tested, five genotypes had a predicted mean value higher than the overall mean, with genotype 1 having the highest value (Fig. 6). In METs, the WAASBY has emerged as a useful and valuable simultaneous selection index based on stability and yield, and its results have high validity. This index allows breeders to select genotypes with different ratios of stability and yield, depending on the specific goals of their breeding programs. In Fig. 7, it was possible to rank and select genotypes based on KY and stability values simultaneously. Blue circles indicate a higher-than-average WAASBY, and red circles indicate a lower-than-average WAASBY. Five genotypes had a higher-than-average WAASBY, among which genotypes 4 and 3 had relatively higher WAASBY compared to other genotypes. Accordingly, these two genotypes were identified as stable genotypes with high KY. Given that maize is a staple food for billions of people worldwide[11,65,66] and a critical component in animal feed, biofuel production, and industrial uses[10], and given concerns about climate change and the need to transition from fossil fuels to renewable fuels[67], achieving optimal and sustainable quantitative and qualitative performance under different environmental conditions is considered an important strategy[36,41,48]. In this context, the WAASBY, as a quantitative measure of stability by weighting average yield and stability, provides an opportunity to select genotypes with high yield and yield stability[36,68,69]. This approach not only enhances the efficiency of breeding programs but also contributes to the sustainability and productivity of corn production in the face of global challenges.

A key observation emerging from this study is the apparent lack of complete congruence among the different analytical models in identifying ideal genotypes. For instance, the GYT analysis highlighted genotype 1 for specific yield-trait combinations, the MTSI pointed to genotypes 4, 2, and 3 for overall multi-trait stability,

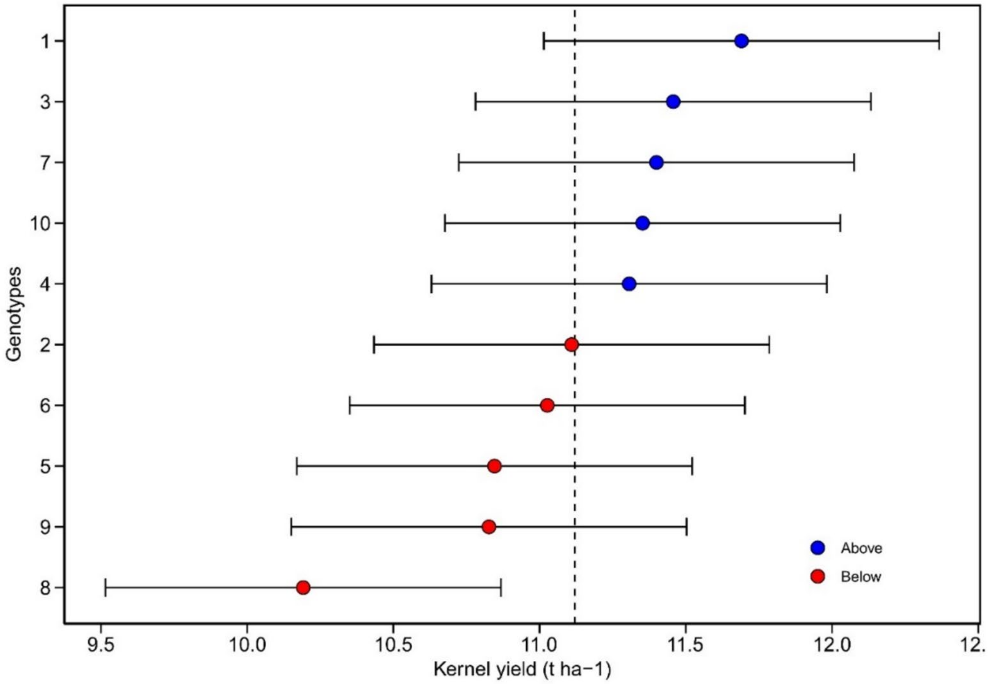


Fig. 6. Best linear unbiased prediction (BLUP) mean values for kernel yield in maize genotypes.

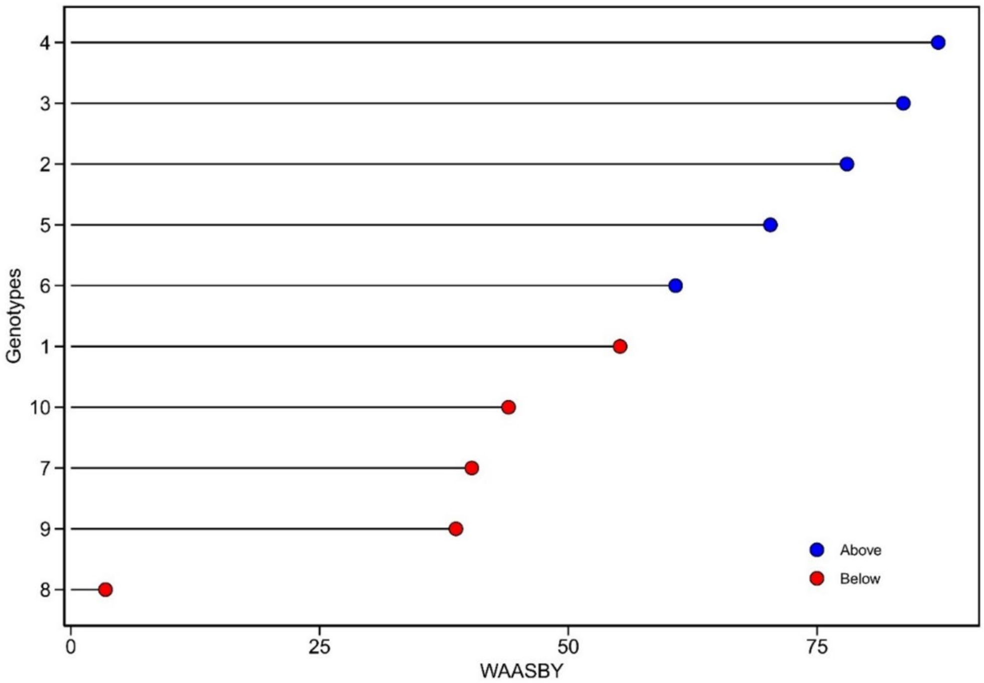


Fig. 7. Estimated weighted average absolute scores by yield (WAASBY) considering weights of 50 for kernel yield and 50 for WAASB in maize genotypes.

and the GGE biplot identified genotypes 10, 3, and 7 as top performers in specific mega-environments. This divergence is not a contradiction but rather a reflection of the distinct objectives each model is designed to address. The GYT biplot excels at selecting for specific trait associations, while the MTSI provides a balanced index for simultaneous improvement across all traits, regardless of their correlations. Similarly, AMMI and GGE biplots are powerful for dissecting GEI patterns and identifying genotypes with either broad or specific adaptation.

For breeders, the choice of the most reliable model is therefore context-dependent and hinges on the specific goals of their program. If the objective is to develop a variety for a particular mega-environment, the GGE biplot is most informative. If the goal is to improve a complex set of traits simultaneously, the MTSI is invaluable. However, for the overarching goal of identifying broadly adapted genotypes that combine high mean yield with high stability across diverse environments—a common objective in many breeding programs—the WAASBY index provides the most practical and decisive framework. By integrating both BLUP-predicted yield and a comprehensive stability score (WAASB) into a single, weighted index, WAASBY offers a robust and reliable criterion for final selection. In this study, its identification of genotypes 4 and 3 as superior candidates represents the most balanced and actionable conclusion for advancing elite germplasm.

A potential limitation of this study is the relatively small number of genotypes evaluated. It is important to note that these 10 genotypes were pre-selected as elite lines from a broader breeding program at SPII, based on their promising performance in preliminary trials. However, future studies incorporating a larger and more diverse set of maize germplasm would be valuable to validate these findings and to explore the genetic basis of the observed trait associations more comprehensively.

Conclusion

This study underscores the complexity of breeding programs aimed at improving maize varieties, particularly in the presence of GEIs and unfavorable trait correlations. The comprehensive analysis of nine maize genotypes and a control variety across multiple environments revealed crucial insights into the associations between key traits and their influence on KY. The high positive correlations between KY and traits such as the KR and PH highlight potential pathways for targeted breeding interventions. Conversely, the weak negative correlations between KY and traits like the KIR and KM suggest areas for cautious optimization. The identification of genotypes 4, 2, and 3 as most ideal across all traits, based on the MTSI, provides valuable candidates for breeding programs targeting broad adaptability and stability. The AMMI further validated the significance of genotype, environment, and their interaction on KY, with four PCs explaining a substantial portion of the variation. The GGE biplot identified genotypes 10, 3, and 7 as stable and high-yielding across specific environments, offering a robust framework for environmental-specific recommendations. Genotype 1 emerged as particularly promising due to its high BLUP, suggesting its stability for consistent KY in diverse conditions. The WAASBY further confirmed genotypes 4 and 3 as stable and high-yielding, reinforcing their potential as elite breeding lines. These findings provide a strategic foundation for developing more resilient maize varieties capable of thriving in diverse and challenging environments, ultimately contributing to enhanced crop production and food security.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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References

1. Cassman, K. G. What do we need to know about global food security?. *Glob. Food Sec.* **2**, 81–82 (2012).
2. Hassani, M., Mahmoudi, S. B., Saremirad, A. & Taleghani, D. Genotype by environment and genotype by yield*trait interactions in sugar beet: analyzing yield stability and determining key traits association. *Sci. Rep.* **13**, 23111. https://doi.org/10.1038/s41598-023-51061-9 (2024).
3. United Nations, Department of Economic and Social Affairs, Population Division (2024). World Population Prospects 2024: Summary of Results. https://population.un.org/dataportal/home?df=77e137b6-0549-487f-ab64-5290523f6dbe
4. Saremirad, A. & Mostafavi, K. Genetic diversity study of sunflower (*Helianthus annus* L.) genotypes for agro-morphological traits under normal and drought stress conditions. *Plant Prod.* **43**, 227–240. https://doi.org/10.22055/ppd.2020.27588.1671 (2020).
5. Voss-Fels, K. P., Stahl, A. & Hickey, L. T. Q&A: Modern crop breeding for future food security. *BMC Biol.* **17**, 1–7 (2019).
6. FAO. in *How to feed the world 2050: High-level expert forum.* (Food and Agriculture Organization of the United Nations, Rome).
7. United Nations. *World Population Prospects: the 2017 Revision*. (United Nations. Department of International Economic, 2017).
8. Asseng, S. et al. Wheat yield potential in controlled-environment vertical farms. *Proc. Natl. Acad. Sci.* **117**, 19131–19135 (2020).
9. FAO. (Food and Agriculture Organization of the United Nation, Rome, Italy, 2023).
10. Foley, J. Its time to rethink americas corn system. *Scientific American* (2013).
11. Jans, W. W. et al. Carbon exchange of a maize (*Zea mays* L.) crop: Influence of phenology. *Agric. Ecosyst. Environ.* **139**, 316–324 (2010).
12. Reynolds, M. et al. Addressing research bottlenecks to crop productivity. *Trends Plant Sci.* **26**, 607–630 (2021).
13. Taleghani, D., Rajabi, A., Saremirad, A. & Darabi, S. Estimation of gene action and genetic parameters of some quantitative and qualitative characteristics of sugar beet (*Beta vulgaris* L.) by line × tester analysis. *Crop Breed.* **15**, 201–212 (2024).
14. Yan, W. & Frégeau-Reid, J. Genotype by Yield∗ Trait (GYT) Biplot: A novel approach for genotype selection based on multiple traits. *Sci. Rep.* **8**, 1–10 (2018).
15. Neyhart, J. L., Lorenz, A. J. & Smith, K. P. Multi-trait improvement by predicting genetic correlations in breeding crosses. *G3 (Bethesda)* **9**, 3153–3165. https://doi.org/10.1534/g3.119.400406 (2019).
16. Mostafavi, K. & Saremirad, A. Genotype-environment interaction study in corn genotypes using additive main effects and multiplicative interaction method and GGE-biplot method. *J. Crop Prod.* **14**, 1–12. https://doi.org/10.22069/ejcp.2022.17527.2293 (2021).
17. Signor, C. E. L. et al. Interpretation of genotype× environment interactions for early maize hybrids over 12 years. *Crop Sci.* **41**, 663–669 (2001).
18. Olivoto, T., Lúcio, A. D., da Silva, J. A., Sari, B. G. & Diel, M. I. Mean performance and stability in multi-environment trials II: Selection based on multiple traits. *Agron. J.* **111**, 2961–2969 (2019).
19. Sellami, M. H., Pulvento, C. & Lavini, A. Selection of suitable genotypes of lentil (*Lens culinaris* Medik.) under rainfed conditions in south Italy using multi-trait stability index (MTSI). *Agronomy* **11**, 1807 (2021).
20. Gauch, H. *Statistical analysis of regional yield trials: AMMI analysis of factorial designs*. (Elsevier Science Publishers, 1992).
21. Gauch, H. G. & Zobel, R. W. Identifying mega-environments and targeting genotypes. *Crop Sci.* **37**, 311–326 (1997).
22. Yan, W., Kang, M. S., Ma, B., Woods, S. & Cornelius, P. L. GGE biplot vs. AMMI analysis of genotype-by-environment data. *Crop Sci.* **47**, 643–653 (2007).
23. Smith, A., Cullis, B. R. & Thompson, R. The analysis of crop cultivar breeding and evaluation trials: An overview of current mixed model approaches. *J. Agric. Sci.* **143**, 449–462 (2005).
24. Grubbs, F. E. Procedures for detecting outlying observations in samples. *Technometrics* **11**, 1–21 (1969).
25. Bartlett, M. S. Properties of sufficiency and statistical tests. *Proc. R. Soc. London. Series A-Mathematical and Physical Sciences* **160**, 268–282 (1937).
26. Olivoto, T. et al. Mean performance and stability in multi-environment trials I: Combining features of AMMI and BLUP techniques. *Agron. J.* **111**, 2949–2960 (2019).
27. Akbari, A., Saremirad, A., Shojaei, H. & Ghasemi, S. H. Genetic diversity Study of corn (*Zea mays* L.) genotypes for morpho-physiological traits under drought stress. *Agronomy Plant Breed.* **14**, 9–23 (2018).
28. Hosseini, S. M. S., Shiri, M., Mostafavi, K., Mohammadi, A. & Miri, S. M. Genetic analysis and association detection of agronomic traits in maize genotypes. *Sci. Rep.* **15**, 399 (2025).
29. Nazeran, H., Saremirad, A. & Sadrabadi Haghighi, R. Study of efficiency of organic and chemical nitrogen on yield, yield components and agronomic characteristics of maize (*Zea mayze* L.). *Agronomy and Plant Breeding* **13**, 49–63 (2018).
30. Cruz, C., Regazzi, A. & Carneiro, P. Modelos biométricos aplicados ao melhoramento. *UFV, Viçosa* (2012).
31. Yan, W. & Tinker, N. A. An integrated biplot analysis system for displaying, interpreting, and exploring genotype× environment interaction. *Crop Sci.* **45**, 1004–1016 (2005).
32. Yan, W. et al. Development and evaluation of a core subset of the USDA rice germplasm collection. *Crop Sci.* **47**, 869–876 (2007).
33. Shojaei, S. h. *et al.* Comparison of genotype× trait and genotype× yield-trait biplots in Sunflower cultivars. *International Journal of Agriculture Environment and Food Sciences* **7**, 136–147 (2023).
34. Faheem, M., Arain, S. M., Sial, M. A., Laghari, K. A. & Qayyum, A. Genotype by yield*trait (GYT) biplot analysis: a novel approach for evaluating advance lines of durum wheat. *Cereal Research Communications* **51**, 447–456. https://doi.org/10.1007/s42976-022-00298-7 (2023).
35. Rajabi, A., Ahmadi, M., Bazrafshan, M., Hassani, M. & Saremirad, A. Evaluation of resistance and determination of stability of different sugar beet (*Beta vulgaris* L.) genotypes in rhizomania-infected conditions. *Food Science & Nutrition* **11**, 1403–1414 (2023). https://doi.org/10.1002/fsn3.3180
36. Taleghani, D., Rajabi, A., Saremirad, A. & Fasahat, P. Stability analysis and selection of sugar beet (*Beta vulgaris* L.) genotypes using AMMI, BLUP, GGE biplot and MTSI. *Scientific Reports* **13**, 10019 (2023). https://doi.org/10.1038/s41598-023-37217-7
37. Koundinya, A. et al. Genetic parameters, stability and selection of cassava genotypes between rainy and water stress conditions using AMMI, WAAS. *BLUP and MTSI. Scientia Horticulturae* **281**, 109949 (2021).
38. Sabaghnia, N., Dehghani, H., Alizadeh, B. & Mohghaddam, M. Genetic analysis of oil yield, seed yield, and yield components in rapeseed using additive main effects and multiplicative interaction biplots. *Agron. J.* **102**, 1361–1368 (2010).
39. Yan, W. & Kang, M. S. *GGE biplot analysis: A graphical tool for breeders, geneticists, and agronomists*. (CRC press, 2002).
40. Saremirad, A. & Taleghani, D. Utilization of univariate parametric and non-parametric methods in the stability analysis of sugar yield in sugar beet (*Beta vulgaris* L.) hybrids. *Journal of Crop Breeding* **14**, 49–63 (2022).
41. Sadeghzadeh Hemayati, S. *et al.* Evaluation of White Sugar Yield Stability of Some Commercially Released Sugar Beet Cultivars in Iran from 2011–2020. *Seed and Plant Journal* **38**, 339–364 (2022). https://doi.org/10.22092/spj.2023.362024.1305

42. Annicchiarico, P., Russi, L., Piano, E. & Veronesi, F. Cultivar adaptation across Italian locations in four turfgrass species. *Crop Sci.* **46**, 264–272 (2006).
43. Zobel, R. W., Wright, M. J. & Gauch, H. G. Jr. Statistical analysis of a yield trial. *Agron. J.* **80**, 388–393 (1988).
44. Anandan, A. & Eswaran, R. in *the Proceeding of 2nd Interaction Rice Congress.*
45. Omrani, S. et al. Application of Additive Main Effects and Multiplicative Interaction and Biplot Graphical Analysis Multivariate Methods to Study of Genotype-Environment Interaction on Safflower Genotypes Grain Yield. *Journal of crop Breeding* **11**, 153–163 (2019).
46. Fathi, M., Ranjbar, G., Zangi, M., Tabar, S. & Zarini, H. N. Analysis of stability and adaptation of cotton genotypes using GGE Biplot method. *Trakia Journal of Sciences* **16**, 51 (2018).
47. Mostafavi, K. & Saremirad, A. Genotype - Environment Interaction Study in Corn Genotypes Using additive main effects and multiplicative interaction method and GGE- biplot Method. *Journal of Crop Production* **14**, 1–12 (2021). https://doi.org/10.22069/ejcp.2022.17527.2293
48. Rajabi, A., Ahmadi, M., Bazrafshan, M., Hassani, M. & Saremirad, A. Evaluation of resistance and determination of stability of different sugar beet (*Beta vulgaris* L.) genotypes in rhizomania-infected conditions. *Food Science & Nutrition* **11**, 1403–1414 (2022). https://doi.org/10.1002/fsn3.3180
49. Verma, A. & Singh, G. Stability index based on weighted average of absolute scores of AMMI and yield of wheat genotypes evaluated under restricted irrigated conditions for peninsular zone. *International Journal of Agriculture, Environment and Biotechnology* **13**, 371–381 (2020).
50. Abdelghany, A. M. et al. Exploring the phenotypic stability of soybean seed compositions using multi-trait stability index approach. *Agronomy* **11**, 2200 (2021).
51. Sharifi, P., Erfani, A., Abbasian, A. & Mohaddesi, A. Stability of some of rice genotypes based on WAASB and MTSI indices. *Iranian Journal of Genetics & Plant Breeding (IJGPB)* **9** (2020).
52. Yue, H. et al. Genotype by environment interaction analysis for grain yield and yield components of summer maize hybrids across the huanghuaihai region in China. *Agriculture* **12**, 602 (2022).
53. Yang, R. C., Crossa, J., Cornelius, P. L. & Burgueño, J. Biplot analysis of genotype× environment interaction: Proceed with caution. *Crop Sci.* **49**, 1564–1576 (2009).
54. Saremirad, A., Mostafavi, K. & Mohammadi, A. Genotype- Environment Interaction Study Based on GGE biplot Method for Kernel Yield in Sunflower (*Helianthus annuus* L.) Cultivars. *Journal of Crop Breeding* **12**, 43–53 (2020). https://doi.org/10.29252/jcb.12.34.43
55. Said, A. A. et al. Genome-wide association mapping of genotype-environment interactions affecting yield-related traits of spring wheat grown in three watering regimes. *Environ. Exp. Bot.* **194**, 104740 (2022).
56. Falconer, D. S. The problem of environment and selection. *Am. Nat.* **86**, 293–298 (1952).
57. El-Soda, M. & Sarhan, M. S. From gene mapping to gene editing, a guide from the Arabidopsis research. *Annu. Plant Rev. Online* **4**, 1–32 (2021).
58. Taleghani, D. *et al.* Genotype × Environment Interaction Effect on White Sugar Yield of Winter-Sown Short-Season Sugar Beet (*Beta vulgaris* L.) Cultivars. *Seed and Plant Journal* **38**, 53–69 (2022). https://doi.org/10.22092/spj.2022.360021.1275
59. Basafa, M. & Taherian, M. Analysis of stability and adaptability of forage yield among silage corn hybrids. *Journal of Crop Breeding* **8**, 185–191 (2016).
60. Benakanahalli, N. K. *et al.* A Framework for Identification of Stable Genotypes Basedon MTSI and MGDII Indexes: An Example in Guar (*Cymopsis tetragonoloba* L.). *Agronomy* **11**, 1221 (2021).
61. Majhi, P. K. *Heritability and Its Genetic Worth for Plant Breeding* 69–75 (Advances in Genetics and Plant Breeding. AkiNik Publications, 2019).
62. Schmidt, U., Thöni, H. & Kaupenjohann, M. Using a boundary line approach to analyze N2O flux data from agricultural soils. *Nutr. Cycl. Agroecosyst.* **57**, 119–129 (2000).
63. Comstock, R. E., Robinson, H. & Harvey, P. H. A breeding procedure designed to make maximum use of both general and specific combining ability. *Agronomy Journal* **41**, 360–367 (1949). https://doi.org/10.2134/agronj1949.00021962004100080006x
64. de Resende, M. D. V. & Duarte, J. B. Precision and quality control in variety trials. *Pesquisa Agropecuária Tropical* **37**, 182–194 (2007).
65. Liu, Y., Li, S., Chen, F., Yang, S. & Chen, X. Soil water dynamics and water use efficiency in spring maize (Zea mays L.) fields subjected to different water management practices on the Loess Plateau, China. *Agricultural Water Management* **97**, 769–775 (2010).
66. Panda, R., Behera, S. & Kashyap, P. Effective management of irrigation water for maize under stressed conditions. *Agric. Water Manag.* **66**, 181–203 (2004).
67. Salazar-Ordóñez, M., Pérez-Hernández, P. P. & Martín-Lozano, J. M. Sugar beet for bioethanol production: An approach based on environmental agricultural outputs. *Energy Policy* **55**, 662–668 (2013).
68. Lee, S. Y. et al. Multi-Environment Trials and Stability Analysis for Yield-Related Traits of Commercial Rice Cultivars. *Agriculture* **13**, 256 (2023).
69. Nataraj, V. et al. WAASB-based stability analysis and simultaneous selection for grain yield and early maturity in soybean. *Agron. J.* **113**, 3089–3099 (2021).

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Author contributions

ASh, AA and SM: Performed the trial, analyzed the datasets, and wrote the manuscript; MSh & AE: Assisted in Performing the trial. All authors have read and approved the final manuscript.

Declarations

Competing interests

The authors have stated that they do not have any conflicting interests.

Ethical approval

Not applicable.

Additional information

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