

RESEARCH ARTICLE

Multi-index based analysis of genotype × environment interaction and selection of superior maize (*Zea mays* **L.) hybrids**

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Abstract

Genotype-environment interaction (GEI) plays a critical role in genotype adaptation, making it essential for selecting stable, widely adapted genotypes for cultivation. GEI estimation enables the identification of genotypes that perform consistently across diverse conditions. Models and stability indices derived from fixed-effect and/ or mixed-effect models are frequently utilized for analyzing GEI and selecting genotypes. In this study, thirty hybrids developed through a diallele fashion, along with two checks, were grown across three environments during *kharif* 2023. Analysis of variance revealed significant contributions from the environment and GEI, alongside genotypic effects for eight traits studied, covering flowering, plant architecture and yield. Plot yield (t/ha) was subjected to additive main effects and multiplicative interaction effects (AMMI) analysis to study the stability and genotype interactions with the environment. The first two principal components (PCs) of AMMI analysis explained 69.1% and 30.9% of the total variation, respectively, identifying stable hybrids such as MH-TN-15 and MH-TN-30. The Genotypegenotype×environment (GGE) biplot further highlighted the adaptability and stability of all the genotypes, with the first two PCs explaining 86.11% of the G+GE variation. A multi-trait stability index (MTSI) was employed to select stable and highperforming genotypes across multiple traits. A comprehensive analysis of all the genotypes through various indices showed that hybrids MH-TN-15 and MH-TN-30 were consistently selected as stable and high-yielding genotypes across all indices, demonstrating higher yields than check hybrids and being identified for cultivation. These methods underscore the importance of combining yield and stability metrics for effective genotype selection in varied environments.

Keywords

AMMI; genotype×environment interaction; hybrids; MTSI; stability; WAASBY

Introduction

The importance of a crop in the agricultural landscape can be defined based on parameters such as demand, cultivated area, production quantity and its use. Maize (*Zea mays* L.), also touted as the "Queen of cereals", is one of the most important cereal crops that is majorly cultivated across the globe (1). The golden-colored cereal's vast usefulness as food or an industrial input has led to its increased demand and widespread commercial cultivation worldwide (2). Maize hybrids are cultivated globally to meet the ever-increasing grain demand; hence, hybrid breeding has become the cornerstone of maize breeding programs (3, 4). Maize

production on a global scale totaled 1.16 billion tonnes in 2022, spread across a 203 M ha around the globe, with an increase to 1.22 billion tonnes in 2023 (5, 6). In India, maize production spanned 11.24 M ha, with a total yield of 37.66 M tonnes, underscoring its demand and importance in the agricultural sector during the year 2023-24 (7).

The environment plays an important role in trait expression and growth of a genotype. The interactive effect between the genotype and the growing environment, also known as GEI, dictates the suitability of a genotype to a particular growing environment (8, 9). A genotype is said to be stable when the difference in the quantifiable trait expression is minimal between the test environments. Hence, testing genotypes across different environments is very important for cultivar release (10). This indicates the importance of testing maize hybrids for stability and mean performance across multiple environments to quantify the GEI, which enables the cultivation of these hybrids under a wide range of conditions, thereby increasing production quantity to meet grain demands (11).

Multiple researchers have developed models for quantification of the GEI, which range from regression models to the AMMI model (12-15). Graphical models such as GGE models are also included (16). Recently, the best linear unbiased predictors (BLUPs) based models, such as the weighted average of absolute scores of BLUPs (WAASB), which rely on linear mixed models, have been introduced (17). The AMMI and GGE models are widely used for assessing the stability of the genotypes under study and have formed an integral part of GEI and stability studies (18-20). AMMI, which is based on fixed-effect models, provides more accurate estimates compared to traditional ANOVA-based models (21). However, according to previous research, linear mixed-effect models offer greater accuracy through the estimation of BLUPs (22). Generally, GEI is estimated for a single trait, mostly for grain yield. However, selecting for multiple trait stability and mean performance generally increases the reliability of genotype selection. AMMI and GGE weigh equally between yield and stability and rank the genotypes accordingly. WAASB, on the other hand, enables the breeder to weigh yield and stability according to their goals. MTSI assesses both the mean performance and stability of a genotype over multiple traits, providing a more comprehensive approach to genotype selection through multi-environmental trials (21).

Given the importance of GEI, it is crucial to use suitable models that accurately quantify the interactive ability of genotypes. The use of multiple statistical models together offers reliable estimates of GEI and aids in the selection of stable genotypes. This study focuses on employing linear fixed models such as AMMI, GGE and mixed models like WAASB and MTSI to identify stable maize hybrids.

Materials and Methods

The current study comprises thirty hybrids that are derived from six different inbreds. The hybrids were generated in a full diallele fashion, comprising both direct and indirect crosses. The hybridization program was carried out in experimental fields at the Department of Millets, Tamil Nadu Agricultural University, Coimbatore, during the late *rabi* season of 2022-23. The details of the hybrids are given in Table 1. The hybrids are grown across

three different locations: Department of Millets, Coimbatore (E_1) (11°01'22.9" N, 76°55'44.2"E), Agricultural Research Station, Bhavanisagar (E_2) (11°28'57.6"N, 77°08'12.3"E) and Maize Research Station, Vagarai (E3) (10°34'10.1"N, 77°33'41.9"E) during *kharif* 2023. The hybrids are planted in a randomized block design with three replications. Planting was done in rows of 3 m size with a spacing of 60×25 cm. A standard agronomic regimen has been followed throughout the cropping period.

Observations recorded comprise flowering traits, such as days to 50% tasseling (DT) and days to 50% silking (DS), as well as plant architectural traits, including plant height (cm), ear altitude (cm) (EA) and ear traits like ear length (cm) (EL), ear diameter (cm) (ED) and the number of kernels per ear (NKE). The yield of hybrids (PY) was calculated on a plot basis and is expressed in tons per hectare (t/ha).

Table 1. Details of the 30 hybrids along with two checks studied across three environments for stability analysis

Code	Hybrid	Pedigree/Source
Н1	MH-TN-01	UMI1252 × UMI 1210
H2	MH-TN-02	UMI1252 × UMI1250
H3	MH-TN-03	UMI1252 \times LM 13
Η4	MH-TN-04	UMI1252 \times LM 14
H5	MH-TN-05	UMI1252 × UMI1268
Η6	MH-TN-06	UMI 1210 × UMI1252
Н7	MH-TN-07	UMI 1210 × UMI1250
Η8	MH-TN-08	UMI $1210 \times LM$ 13
H9	MH-TN-09	UMI $1210 \times LM$ 14
H10	MH-TN-10	UMI 1210 × UMI1268
H11	MH-TN-11	UMI1250 × UMI1252
H12	MH-TN-12	UMI1250 × UMI 1210
H13	MH-TN-13	UMI1250 \times LM 13
H14	MH-TN-14	UMI1250 \times LM 14
H ₁₅	MH-TN-15	UMI1250 × UMI1268
H16	MH-TN-16	LM $13 \times$ UMI1252
H17	MH-TN-17	LM 13 × UMI 1210
H18	MH-TN-18	LM $13 \times$ UMI1250
H19	MH-TN-19	LM $13 \times$ LM 14
H20	MH-TN-20	LM $13 \times$ UMI1268
H21	MH-TN-21	LM $14 \times$ UMI1252
H22	MH-TN-22	LM 14 × UMI 1210
H23	MH-TN-23	LM $14 \times$ UMI1250
H24	MH-TN-24	LM $14 \times$ LM 13
H25	MH-TN-25	LM $14 \times$ UMI1268
H26	MH-TN-26	$UMI1268 \times UMI1252$
H27	MH-TN-27	UMI1268 × UMI 1210
H ₂₈	MH-TN-28	UMI1268 × UMI1250
H29	MH-TN-29	UMI1268 × LM 13
H30	MH-TN-30	UMI1268 × LM 14
C1	S6668	Syngenta Global AG
C2	COH(M)8	Tamil Nadu Agricultural University

Statistical Analysis

Testing for genotype×environment interaction

A joint analysis of variance (ANOVA) was employed to estimate the presence of GEI (23). The ANOVA model equation can be expressed as follows:

$$
Y_{ijk} = \mu + G_i + E_j + G E_{ij} + B_{ij} + \varepsilon_{ijk} \qquad (\text{Eqn. 1})
$$

Where μ = overall mean of the trait in the population; G_i = effect of the ith genotype; E_i = efficacy of the jth environment; GE_{ij} = interaction of the ith genotype with the jth environment; B_{ij} = effect of the kth replication in the jth environment and ϵ_{ik} = random error.

Stability indices Additive main effects and multiplicative interaction analysis (AMMI)

The data were subjected to AMMI analysis for plot yield (t/ha) for estimate the GEI. The model explains additive variance through ANOVA and non-additive variance through principal component analysis (PCA) (15). The AMMI1 biplot was plotted between the mean yield and PC₁, while the AMMI2 biplot was plotted between PC₁ and PC₂. The AMMI stability value (ASV) is calculated for genotype stability based on a fixed effects model (24). The yield stability index (YSI) is expressed as the cumulative rank obtained by adding the yield ranking and ASV rank of a genotype (25).

Genotype-genotype×environment (GGE) biplot

The GGE biplots were developed using site regression (SREG) analysis (26, 27). These biplot tools were employed to identify genotypes best suited to specific environments through the "What-Won-Where" graph, to highlight high-yielding, highly adaptable genotypes using the "Mean vs Stability" plot and to visualize the relationships between genotypes and environments through the "Representativeness vs Discriminativeness" graph (28).

Best linear unbiased predictor (BLUPs) based stability indices

The WAASBY index for yield was calculated by assigning weightings to both yield and stability, with a higher emphasis on yield over stability in a 60:40 ratio (17). The MTSI is determined by converting factor analysis scores into a genotype-ideotype distance index (21). A selection intensity of 15% was used to identify the hybrids with the lowest MTSI scores. All analysis were performed using the "metan" package in R studio, running on R version 2024.09.0 (29, 30).

Results and Discussion

Analysis of variance (ANOVA) for eight traits studied across three environments

The joint analysis of variance revealed significant differences (p<0.001) for all eight studied biometrical traits. The genotypes, environment and the crossover interaction (GEI) showed significant differences, while the replications across environments were non-significant (Table 2). The sum of squares explained by the three components (G, E and GEI) was partitioned to explain the contribution of each component to the variation shown by a particular trait under study. The environment explained the highest variation among the three components for DT (49.95%), DS (49.08%), PH (41.91%) and EA (45.64%). Genotypes contributed the highest to the variation recorded for EL (41.94%) and PY (42.46%), while the interaction between the two (GEI) significantly influenced the variation explained by ED (52.66%) and NKE (43.52%). The results show that the environment is highly influential in creating variation in flowering periods and plant architectural traits, while yield is more influenced by genotypes. Studies have shown that genotypes contribute to around 50% of the total variation recorded for yield (31). A similar delineation of total variation into percent contributions by the environment, genotype and GEI was conducted in previous studies (32-34).

Additive main effects and multiplicative interaction (AMMI) analysis of plot yield (t/ha)

The AMMI model is one of the powerful statistical model that have been widely utilized for GEI analysis (35). The AMMI analysis for plot yield (t/ha) exhibited significant differences attributed to environment, genotype and the genotype-environment interaction (Table 2). The multiplicative part of the AMMI analysis has dissected the GEI sum of squares into two principal components, $PC₁$ and $PC₂$, which explained 69.1% and 30.9% of the total variation explained, respectively (Table 3).

The AMMI1 biplot was generated by plotting $PC₁$ against the mean yield (Fig. 1). Fifteen genotypes exhibited yields higher than the mean yield. The biplot provides information about the performance and stability of genotypes across different environments (36). Genotypes that lie closer to the ordinate are

ENV: Environment; GEN: Genotype; GEN:ENV/GEI: Genotype-environment interaction; REP(ENV): Replication across environments; DF: Degrees of freedom; MSS: Mean sum of squares; DT: Days to 50% tasselling; DF: Days to 50% silking; PH: Plant height (cm); EA: Ear altitude (cm); EL: Ear length (cm); ED: Ear diameter (cm); NKE: Number of kernels per ear; PY: Plot yield (t/ha)

Table 3. AMMI ANOVA for plot yield (t/ha)

Source	Df	MSS	
ENV	\mathcal{P}	83.343**	
REP(ENV)	6	0.195^{ns}	
GEN	31	$6.506**$	
GEN:ENV	62	$2.151**$	
Residuals	186	0.166	Proportion
PC1	32	2.853	68.5
PC ₂	30	1.402	31.5
Cumulative variance	100		

ENV: Environment; GEN: Genotype; GEN:ENV/GEI: Genotype-environment interaction; REP(ENV): Replication across environments; DF: Degrees of freedom; MSS: Mean sum of squares; ns: Non-significant

Fig. 1. AMM1 biplot of plot yield (t/ha) for the thirty hybrids under study.

less interactive, indicating greater stability in expression. The stable genotypes with yields above the mean include H15, H30, C1, C2, H16, H24 and H1. The vector length of the environments reflects their interactive ability; environments E_1 (Coimbatore) and E3 (Vagarai) showed longer vector projections from the origin, indicating that these environments are highly interactive. In contrast, environment E_2 (Bhavanisagar) has a shorter projection from the origin, suggesting that this environment is less interactive.

The polygon view of the AMMI2 biplot constructed using $PC₁$ and $PC₂$ shows that genotypes specifically adapted to a particular environment are represented as the vertices of the polygon, through which the environment vectors are projected perpendicularly (Fig. 2). Genotypes H28 and H21 performed well in E_1 (Coimbatore), while H3 performed well in E_2 (Bhavanisagar) and H7 and H29 performed well in E_3 (Vagarai), indicating their suitability to a specific environment. Genotypes lying closer to the origin are said to be stable and perform similarly across all environments (37). The sufficiency of the model with two significant PCs was validated in previous studies (38).

The ASV is a stability index based on AMMI values, which provides a metric for the stability of genotypes and ranks them accordingly (24). The lower the ASV value, the higher the stability of the genotype. According to the ASV ranking, the top five stable genotypes are H16, H18, H12, C1 and H1 (Table 4). However, ranking based on stability alone does not aid in the selection of hybrids for further testing. Hence, the YSI is calculated; this value is derived by summing the ASV ranks and yield ranks of the

Fig. 2. Polygon view of AMMI2 biplot for plot yield (t/ha) constructed using PC1 and PC2 scores.

genotypes (39). This index helps in selecting high-yielding genotypes with stable expressions. The hybrids H15 (YSI=8), C1 (YSI=9), H16 (YSI=9), H30 (YSI=12) and H24 (YSI=17) have been found to be high-yielding and stable genotypes.

GGE biplot analysis for plot yield (t/ha)

The GGE biplot method has been reported to be a suitable method for studying GEI (40). A unique feature of GGE biplots is their ability to evaluate potential genotypes that are suitable for specific environments (23). The GGE biplots are constructed using the PC₁ and PC₂, with scaling based on standard deviations (scaling=1), environment-centered data (G+GE) (centering=2) and symmetrical singular value partitioning to accommodate both environments and genotypes (SVP=3). This methodology provides a clear biplot view for interpretation, particularly due to the longer vector lengths of the environments. The first two PC axes accounted for 86.11% of the total variation explained by the G+GE component.

Mean vs. stability biplot

The graphical analysis of the mean vs. stability biplot can be utilized for visualizing stable and high-yielding genotypes. The direction of the arrow on the average environmental coordinate (AEC) points towards higher yield, while the projection of the genotypes onto the AEC indicates their stability. The shorter the projection, the more stable the genotypes are; conversely, longer projections indicate less stability (41). The closer the genotypes are to the AEC arrow, the higher their yield. Based on the biplot (Fig. 3), genotypes H15, H30, C1, C2, H2, H14 and H16 have shorter projections and are relatively closer to the AEC arrow in that order, indicating their stability and performance. The genotype H29, though it lies nearer to the arrow, has a longer projection from the AEC, indicating its unstable nature.

What-won-where biplot

The what-won-where biplot shows the suitable genotypes in each environment. A polygon was drawn by connecting the outer genotypes (16). The biplot consists of a polygon view with the outerlying genotypes acting as the vertices of the polygon.

GEN: Genotype; PY: Plot yield (t/ha); Y_R: Yield based ranking of genotypes; ASV: AMMI stability value; ASV_R: Genotype rank based on AMMI stability value; YSI: Yield stability index; MTSI: Multi-trait stability index; MTSI_R: Genotype ranking based on MTSI values; WAASBY: Weighted average of absolute scores of best linear unbiased predictors × yield; WAASBY_R: Genotype ranks based on WAASBY values

Projections were drawn from the origin, dissecting the polygon perpendicularly and dividing the biplot into six sectors (Fig. 4). E_1 (Coimbatore) and E_2 (Bhavanisagar) constitute a single mega environment, while E₃ (Vagarai) alone constitutes a mega environment. Genotype H15 is the winning genotype in mega environment E_1 , while H29 is the winning genotype in mega environment E₃. Genotypes H28, H17, H5 and H7 are not winning any environment, indicating their mediocre to poor performance across the environments.

Fig. 3. Mean vs stability biplot for plot yield (t/ha). **Fig. 4.** Which-Won-Where view of the GGE biplot for plot yield (t/ha) to select the winning genotypes in each environment.

Discriminativeness vs representativeness biplot

The discriminativeness vs representativeness biplot reveals the environments' ability to select genotypes that are widely adapted or specifically adapted genotypes. The angle between the environments indicates the relationship between them. An angle of <90° indicates a positive correlation, while an angle of >90° indicates a negative correlation. An angle equal to 90° indicates no relation between environments. The length of the environment vector determines the nature of the environment;

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The longer the vector, the more discriminative the environment is, while a shorter vector length indicates that the environment is a representative environment (42). Additionally, the angle formed between the vector and average environment axis indicates the discrimination or representativeness of the environment. Smaller angles between the vector and the axis indicate a higher representativeness of the environment (43). Environment E_2 (Bhavanisagar) has the shortest vector and is closer to the average environment axis, indicating that it is a representative environment and can be used to test widely adapted genotypes (Fig. 5). Meanwhile, E_3 (Vagarai) has the longest vector and a higher angle with the axis, indicating that this environment is more discriminative and can be used to test specifically adapted genotypes. The angle between all the environments is less than 90°, indicating that all the environments are positively correlated.

Fig. 5. Discriminativeness vs representativeness view of biplot for plot yield (t/ha) to study environmental relationships.

WAASBY index

The WAASBY index was calculated for the hybrids with more weightages given to yield than stability (60:40), as comparative selection for higher yield is preferred over stability. Lower WAASB scores indicate genotype stability and good performance. The WAASB scores are plotted against the plot yield (t/ha) and the genotypes and environments are represented on the biplot. The WAASBY plot for plot yield was divided into four quadrants (17). Quadrant I consists of unstable genotypes with yields lower than the mean yield (Fig. 6). The environments in this quadrant are highly discriminative. Environments E_2 (Bhavanisagar) and E_3 (Vagarai) are placed in quadrant I, along with six hybrids. Quadrant II comprises highly productive yet unstable genotypes.

Table 5. Principal component analysis for multi-trait stability index (MTSI)

Fig. 6. WAASBY biplot for plot yield (t/ha) plotted against WAASB scores.

The environments in this quadrant are of special importance as they provide good discriminative ability while expressing higher magnitudes of the response variable (plot yield (t/ha)). The environment E₁ (Coimbatore) falls under this quadrant. Quadrant III comprises stable but low-productive genotypes, while quadrant IV comprises of high-productive and stable genotypes. Quadrant IV is particularly important when selecting for stable and high-yielding genotypes, whereas quadrants I and II are significant when studying the test environments. Similarly, the WAABY was used to evaluate and select maize genotypes by other researchers (44, 45). Based on the WAASBY values, the topranking genotypes are H15, H30, C1, C2 and H16 (Table 4).

Multi-trait stability index (MTSI)

Unlike traditional approaches that consider only a single trait for genotype selection, the MTSI offers a method to select genotypes that are stable and desirable across multiple traits (21). This provides a more holistic approach to the selection of genotypes compared to single-trait stability and selection indices (46). The MTSI is calculated based on the WAASB values, with higher weightage given to yield traits. An exploratory factor analysis was conducted to capture the variation into eight PCs. The first three PCs scored eigenvalues >1, explaining a cumulative variance of 78.7% of the total variation (Table 5). The communality, which measures the shared variance accommodated in the significant PCs after varimax rotation, ranged between 0.249 (EG) to 0.939 (DS), with a mean communality value of 0.787 indicating that the variance has been fitted well (Table 6). At 15% selection intensity, five genotypes, namely H30, H15, C1, C2 and H19, have been selected with MTSI values closer to zero (Table 4) (Fig. 7). The selection differential percentage (SD_{perc}) of the selected lines over the population ranged between 1.54% (CG) and 17.3% (PY),

Fig. 7. Multi-trait stability index plot of selected genotypes at 15% selection intensity.

indicating a positive direction in selection. Other traits such as DT (-0.78%), DS (-1.48%) and PH (-0.19%) showed negative but marginal SD_{perc} values, while EA (2.86%) exhibited positive SD_{perc} values (Table 6).

Identification of stable hybrids through different indices

At a selection intensity of 15%, the top five ranked genotypes, excluding the checks, were selected based on three stability indices: YSI, WAASBY and MTSI. Hybrids MH-TN-15, MH-TN-24 and MH-TN-30 consistently ranked highly across all three indices, highlighting their superior and stable performance (Fig. 8). In addition to these, hybrids MH-TN-16 and MH-TN-1 were ranked in the top five for YSI; MH-TN-24 and MH-TN-2 for WAASBY; and MH-TN-19 and MH-TN-29 for MTSI. Notably, MH-TN-15 and MH-TN-30 showed higher plot yields than the check varieties CoH(M) 8 and S6668, making them strong candidates for cultivation.

Conclusion

For successful commercial cultivation of a hybrid, it must be adapted to all the growing conditions with minimal yield plasticity. The usage of linear fixed models or linear mixed models provides valuable insights into the genotypeenvironment interactions, which also aids in the selection of widely adapted and specifically adapted genotypes for respective environments with high yields. Instead of relying on a

Fig. 8. Venn diagram of top five ranking genotypes excluding checks across three indices (YSI: Yield Stability Index; WAASBY: Weighted Average of Absolute Scores of BLUPs × Yield; MTSI: Multi-Trait Stability Index).

single metric or analysis, using multiple statistical models such as AMMI, GGE biplots, WAASBY and MTSI addresses the limitations of individual methods, enhancing the precision and efficiency of genotype selection. In this study, the combined use of these models highlighted the importance of integrating different metrics for robust evaluation. Genotype rankings based on multiple indices suggest that MH-TN-15 and MH-TN-30 are strong candidates for further testing, as they demonstrated consistently higher yields than the commercial checks among the thirty hybrids studied.

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Authors' contributions

HDM, SS and KVN conceptualized the research work and designed the experiment. SS and KVN contributed the experimental material. HDM, SS, KV and SSKRV executed the field experiments and collected the data. HDM carried out the statistical analysis and interpreted the results. HDM, SS and KV prepared the manuscript. SN, UD and SA reviewed and approved the final draft of the manuscript. All authors read and approved the final manuscript.

Table 6. Factor loading of each trait after varimax rotation and selection differential between the base population and selected hybrids

DT: Days to 50% tasselling; DF: Days to 50% silking; PH: Plant height (cm); EA: Ear altitude (cm); EL: Ear length (cm); ED: Ear diameter (cm); NKE: Number of kernels per ear; PY: Plot yield (t/ha); FAs: Factor analysis components; Xo: Population mean; Xs: Mean of selected genotypes; SD: Selection differential; SD: Selection differential percentage

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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