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RESEARCH ARTICLE

Combined analysis of genotype × environment interaction by fixed and mixed effect models in the selection of stable and productive maize (*Zea mays* L.) inbreds

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Abstract

Assessment of genotype × environment interaction for stability and the performance of yield and its attributes of inbred lines across the environments serve as an important pre-breeding step in maize for selection of parental lines. A set of 70 inbred lines was evaluated for grain yield and other yield traits in multi-location trials under diverse environments to analyze Additive Main Effects and Multiplicative Interaction Effects, genotype and genotype × environment (GGE) biplots and best linear unbiased predictor (BLUP). A joint analysis of variance revealed significant differences across environments for the studied traits. The biplot analysis revealed that inbred lines, UMI 1260 and UMI 1266 performed well in Coimbatore (E_1) and Bhavanisagar (E_2), whereas IMR 108200 and UMI 1257 performed well in Vagarai (E_3). GGE and WAASBY biplot analysis showed that Coimbatore and Vagarai environments were found discriminative and may be suitable for selecting genotypes for specific adaptation, while Bhavanisagar is ideal for selecting genotypes with broad adaptability. Multiple stability indices, such as yield stability index (YSI), the harmonic mean of the relative performance of genetic values, and the weighted average of absolute scores of BLUPs (WAASBY) facilitated the selection of stable inbred lines in terms of grain yield, while multi-trait selection index aided in the selection of genotypes based on multiple traits. Positive selection gains were observed for yield and related traits, whereas negative gains were observed for plant height and ear height. The coincidence index at 20% selection intensity revealed a high level of coincidence between WAASBY and HMRPGV, indicating selection efficiency when either of the indices was used. Inbred lines, UMI 1286, UMI 1276, and UMI 1266 ranked consistently across all the stability indices and hence can serve well as potential inbred lines in hybrid breeding. The use of multiple stability indices enhances the accuracy of selecting inbreds or genotypes by compensating for the limitations of individual indices, providing a more balanced and reliable evaluation.

Keywords: Inbreds, stability, linear mixed models, adaptability, genotype × environment interaction.

Introduction

Maize (*Zea mays* L.) is an important cereal crop for its high productivity and versatile uses as food, feedstock, and industrial raw material (Murdia et al. 2016). It exhibits broad genetic variability and geographical adaptability due to its cross-pollinated nature. The need for crop improvement is a major requirement in the current global scenario to

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produce high quality and quantity to meet the increasing food demand. The cornerstone of a successful maize hybrid breeding program is the availability of genetically diverse inbred lines. It is achieved predominantly through heterosis breeding, which involves the development of high-yield hybrids for cultivation (Paterniani 2001). The success of any commercial hybrid production program relies not only on the yield potential of the hybrid alone but also on the yield potential of the parental inbred lines. Inbreds with high seed production potential could substantially reduce the costs associated with hybrid seed production (Pinnisch et al. 2012). Hence, the evaluation of available germplasms and identification of their yield potential as a step in breeding programs is of paramount importance.

The performance of a genotype is shaped by its genetic structure, the environment and their interaction, i.e., the genotype × environment interaction (GEI) (Malosetti et al. 2013). The GEI impacts both the expression and stability of a given trait across environments. Multi-environment testing is vital for evaluating genotypes for performance and stability, allowing breeders to identify those with consistent performance under diverse conditions (Mafouasson et al. 2018). A genotype is said to be stable when the difference in the degree of expression of a particular trait is minimal across environments. Several statistical analyses are available for assessing the stability of the genotypes and for partitioning the GEI. These include conventional models of stability analysis (Finlay and Wilkinson 1963; Wricke 1964; Eberhart and Russell 1966; Shukla 1972). Principal components analysis-based models such as additive main effects and multiplicative interaction effect (AMMI) models (Gauch 1988), which operate on fixed effects and genotype-by-environment interaction (GGE) biplot analysis (Yan 2001) evaluating environments have been widely adopted for GEI analysis.

Mixed effect models such as linear mixed models (LMMs) can improve the predictive accuracy of random effects through the estimation of best linear unbiased prediction (BLUPs) (Smith et al. 2005). Several stability models employing LMMs have been proposed for GEI estimation. BLUP-based selection indices, such as the harmonic mean of the relative performance of genetic values (HMRPGV) are utilized for selecting genotypes with stability and adaptability across different environments (Atroch et al. 2013; Candido et al. 2018). The weighted average of absolute scores from the singular value decomposition of the matrix of BLUPs (WAASB) index is another such stability index that also allows weighing between stability and performance for a trait (Olivoto et al. 2019a), which is not possible in AMMI-based methods. The multitrait selection index (MTSI) considers multiple traits, their stability and assigned weights, thereby enabling the selection of superior genotypes on the basis of a predefined ideotype (Olivoto et al. 2019b).

In light of the above considerations, the present study was designed to evaluate the performance of maize inbreds in multiple locations to quantify the GEI using multiple stability indices and select stable and productive inbreds as an important pre-breeding step in the maize hybrid program.

Materials and methods

Plant materials

A set of 70 inbred lines of maize were evaluated at three experimental research stations (E1: Coimbatore; E2: Bhavanisagar and E3; Vagarai) across Tamil Nadu, India has diverse environments with variable rainfall and soil type, etc. in the cropping season of *kharif* 2023. The inbred lines, their pedigrees and sources of origin are given in Supplementary Table S1. The experiments were conducted in a randomized block design (RBD) with two replications. Planting was carried out in 3 m rows with spacing of 60 × 25 cm, with a population of 12 plants/row. The recommended package of practices was followed throughout the crop period.

Observations Recorded

Phenological traits such as days to 50% anthesis (DA) and days to 50% silking (DS) were recorded on a plot basis when 50% of the plants presented anthesis and silking, respectively. Biometric traits such as plant height (cm) (PH) and ear height (cm) (EH) were measured from the base of the plant to the node bearing the flag leaf and ear-bearing node, respectively. Postharvest traits, *e.g.,* ear length (cm) (EL), ear girth (cm) (EG), kernel row number (KRN), and number of kernels per row (NKR), were recorded after harvesting. Data for all traits were recorded from five random plants per genotype per replication. The grain yield (g) (GY) was calculated on a plot basis and was expressed as the single-plant yield.

Statistical Analysis

The data from all the environments under study were subjected to a joint analysis of variance (ANOVA) following the methodology of Farshadfar et al. (2011) to determine the significance of the genotype, environment and GEI. This analysis was performed to analyze the additive main effects and multiplicative interaction effects (AMMIs) on grain yield. This model first explains the additive variance through ANOVA and then the non-additive variance through principal component analysis (Gauch 1988). AMMI biplot 1 and AMMI biplot 2 were generated by plotting PC_1 values against grain yield and PC_1 against PC_2, respectively. The AMMI stability value (ASV) (Purchase et al., 2000) and yield stability index (YSI) were also calculated. The GGE biplots based on site regression analysis, SREG and singular value decomposition (SVD) (Cornelius et al. 1996; Crossa et al. 2002) were used to construct "Mean vs Stability", "Which-Won-

Where", and "Representativeness vs Discriminativeness" biplots (Yan and Tinker 2006).

The HMRPGV is based on the BLUP/REML values and was employed to estimate the stability and adaptability of genotypes (Resende 2007). The WAASBY index was calculated for yield, assigning a higher weight to performance over stability at 60:40 ratios (Olivoto et al. 2019a). The MTSI is based on factor analysis scores, which are then converted into a genotype–ideotype distance index (Olivoto et al. 2019b). A selection intensity of 15% was applied to identify the inbreds that were nearest to the ideotype. A coincidence index was computed between all the indices to compare the selection efficiency of the indices, as proposed by Hamblin and Zimmermann (1986). All the statistical analyses were carried out via the 'metan' package (Olivoto and Lúcio, 2020) in R studio. R version 4.3.2 (R core team, 2023).

Results and discussion

Joint ANOVA and mean performance

The joint analysis of variance revealed significant differences among genotypes, environments and their interaction for all studied traits (Table 1). The proportion of variance explained for a trait by each source was calculated using the source total sum of squares. Contribution by genotypes to the total variance ranged between 44.45 (EH) to 75.82% (DS) with 64.34% contribution in the total variance for GY. The variability accounted for by GEI ranged between 14.46 (DA) to 42.30 (EL) with a contribution of 31.59% to the variance of GY. Environments explained 3.22 (NKR) to 17.37% (EH) of the variance, contributing 4.07% to the variance of GY. The proportionate contributions to the total sum of squares by the genotypes, GEI and environments indicate that genotypes were the primary drivers of observed variability followed by GEI. Higher contributions by GEI over environments indicated the effectiveness of the test environments in distinguishing the genotype's response to different conditions. Comparable findings were reported by Singh et al. (2024) in winter maize hybrids and Akter et al. (2014) in hybrid rice.

The overall mean of the genotypes for the studied traits across the environments is shown in Supplementary Table S2. The average grain yield across environments ranged from 33.60 (G16) to 112.00 g (G41) with an overall mean of 67.18 g. Among the environments, E_1 showed the highest mean yield (71.40 g) followed closely by E_3 with a location mean yield (69.60 g) with E_2 being the least productive environment with a mean grain yield of 60.6 g.

GEI analysis and AMMI biplots

The AMMI analysis for grain yield (GY) revealed significant differences between environments, genotypes and GEI. The GEI variance was subjected to the Principal Component Analysis part of the AMMI analysis. The GEI sum of squares was explained by two PCs, with PC_1 explaining 56.0% and PC_2 explaining 44.0% of the total GEI variance. PC_1 scores were plotted against mean yield to generate AMMI1 biplot (Fig. 1) to visualize the relationships between the genotypes and the environments. E_1 and E_3 showed longer vectors indicating these environments are highly interactive, whereas E2 has a shorter vector implicating its less interactive nature. Genotypes positioned near the origin are considered stable across environments (Esan et al. 2023). The genotypes IMR 108308 (G7), UMI 1285 (G70), IMR 110014 (G29), UMI 1270 (G45), and IMR 109580 (G15) are placed closer to the origin indicating their broad adaptability across environments with higher yields, while genotypes such as G4 (IMR 108200), G2 (UMI 1257), G42 (UMI 1267) are considered interactive or unstable as they placed farther from the origin. Moreover, genotypes with negative PC_1

Table 1. Joint ANOVA for nine agronomic traits evaluated across three environments

Source	ENV (df = 2)		GEN (df = 69)		GEN: ENV (df = 138)		Residuals (df = 207)
Trait	MSS	%(G+E+GEI)	MSS	%(G+E+GEI)	MSS	%(G+E+GEI)	MSS
DA	310.06**	10.78	62.33**	74.76	6.03**	14.46	3.057
DS	269.51**	9.46	62.59**	75.82	6.08**	14.72	2.972
PH	9514.42**	9.30	1551.15**	52.28	570.00**	38.42	100.121
EH	6031.65**	17.37	447.48**	44.45	192.24**	38.19	22.176
EL	36.59**	3.36	17.142**	54.34	6.67**	42.30	2.72
ED	6.01**	10.76	0.95**	58.83	0.24**	30.41	0.052
KRN	29.866**	4.53	11.98**	62.76	3.12**	32.71	0.296
NKR	144.34**	3.22	71.86**	55.36	26.88**	41.42	7.392
GY	4692.97**	4.07	2152.63**	64.34	528.44**	31.59	13.92

** = $p<0.05$; ENV/E = Environment; GEN/G = Genotype; GEN:ENV/GEI = Genotype x Environment Interaction; DA= Days to 50% Anthesis; DS= Days to 50% Silking; PH = Plant Height (cm); EH = Ear Height (cm); EL = Ear Length (cm); ED = Ear Diameter (cm); KRN = Kernel Row Number; NKR = Number of Kernels per Row; GY = Grain yield (g) values and high mean yields are suitable for environments classified as selective environments (Koundinya et al. 2019, 2021).

AMMI2 biplot was obtained by plotting PC_1 scores against PC_2 scores, which illustrates the interactions of different genotypes with the environment. A polygon connecting the outer genotypes was drawn which highlights the interactive genotypes. The polygon view of the AMMI 2 biplot efficiently captures GEI variance and identifies genotypes adapted to specific environments as it contains information from two PCs. From the polygon view of the biplot, genotypes UMI 1267 (G42) and IMR 108321 (G8) performed well in E_1, whereas UMI 1257 (G2) showed higher yield in E_3 and UMI 1261 (G36) in E_2.

AMMI Stability Value (ASV)

Although the graphical approaches for stability analysis, such as AMMI and GGE biplots, provide valuable insights into the nature of environments, specifically adapted genotypes and interactive (or) unstable genotypes, index-based methods offer a practical tool for genotype selection for further use. The use of stability-based selection indices enables in accurate selection of stable and productive genotypes. The AMMI stability value (ASV) (Purchase 2000), acts as a selection index for the selection of stable genotypes. Lower ASV values indicate the stability of genotypes. The yield stability index (YSI) is the summation of ASV ranks and yield ranks of each genotype. According to the YSI, genotypes UMI 1285 (G70), UMI 1255 (G61), UMI 1254 (G62), UMI 1288 (G66), UMI 1264 (G39), and UMI 1286 (G60) are stable and high yielding (Table 2).

GGE biplot analysis

GGE biplot analysis provides valuable insights by visualizing crossover interactions and facilitating the classification of environments (Luo et al. 2015). The GGE biplot analysis for grain yield (g) was constructed using a site regression (SREG) model (Cornelius et al. 1996; Crossa et al. 2002), which explains only the main effects of genotype and the GEI. The biplots are constructed via environment-based centering (centering = 2), no scaling (scaling = 0) and genotype-based single-value partitioning (SVP = 1). The first PC (PC_1) accounted for 67.81% of the total variation explained by the GEI, whereas PC_2 accounted for 18.22%, with a cumulative explained variance of 86.03%.

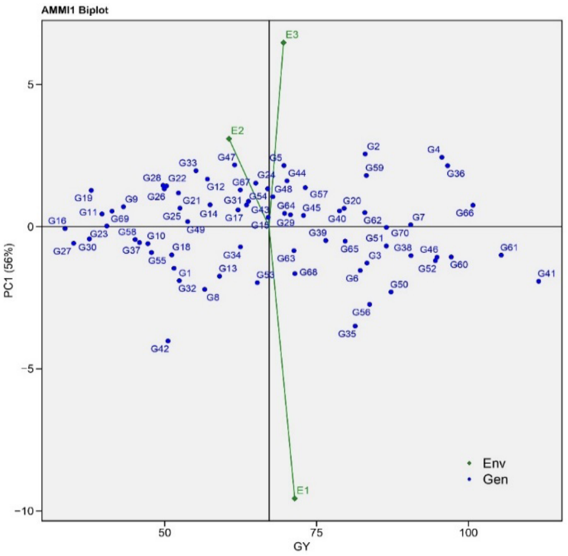

Fig. 1. AMMI 1 biplot for grain yield

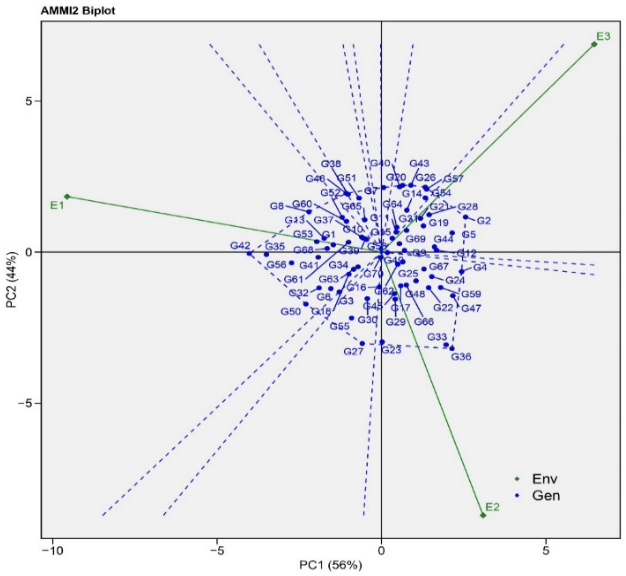

Fig. 2. AMMI 2 biplot for visualizing genotype × environment interactions

Mean vs Stability Biplot

The mean vs stability visualizes the stability and performance of the genotype based on their PC_1 and PC_2 scores and the average environment coordinate was drawn across the origin to visualize genotype stability. Genotypes with shorter projections from the AEC axis are considered more stable (Li et al. 2018) (Fig. 3). The performance of genotypes closer to the AEC is stable (Li et al. 2018). Inbred lines such as UMI 1285 (G70), IMR 108308 (G7), UMI 1254 (G62), UMI 1270 (G45), IMR 109736 (G20), IMR 109580 (G15), UMI 1264 (G39), and UMI 1253 (G65) have shorter projections from the AEC axis indicating that these inbred lines are stable for grain yield (g) (GY). UMI 1260 (G35), UMI 1267 (G42), and UMI 1281 (G56) had longer vectors and hence can be interpreted as interactive genotypes. Importantly, genotypes UMI 1255(G61), UMI 1288 (G66), UMI 1286 (G60), and UMI 1271 (G46) presented both shorter projections from the AEC and high mean yields.

Which-won-where biplot

The biplot identifies the winning genotypes in each environment. A polygon was constructed connecting the outer genotypes. The polygon is bisected by perpendicular dotted lines originating from the origin. This division

Table 2. Genotype ranking based on different stability indices

GEN	Y	Y_R	ASV	ASV_R	YSI_R	HMRPGV	HMRPGV_R	WAASBY	WAASBY_R
G1	51.5	53	1.88	32	85	0.733	52	24.2	66
G2	83	17	3.45	65	82	1.18	19	53.9	24
G3	83.3	15	2.1	36	51	1.21	15	64.8	11
G4	95.6	6	3.17	63	69	1.38	6	50.8	31
G5	69.6	32	2.81	58	90	0.994	33	56.5	21
G6	82.2	19	2.3	45	64	1.19	17	36.7	54
G7	90.5	10	2.14	40	50	1.31	9	79.1	4
G8	56.6	47	3.11	60	107	0.745	50	29.9	60
G9	43.2	63	0.891	11	74	0.64	62	38.7	51
G10	47.2	60	0.908	12	72	0.684	57	40.5	48
G11	39.7	66	0.869	9	75	0.573	65	33.8	56
G12	57	46	2.13	39	85	0.823	45	41.7	44
G13	59	44	2.26	43	87	0.837	44	41.1	46
G14	57.5	45	1.7	28	73	0.822	46	41.3	45
G15	67	34	0.621	3	37	0.993	34	58.5	16
G16	33.6	70	1.16	15	85	0.493	69	27.6	62
G17	62.1	42	1.34	20	62	0.918	40	47.8	35
G18	51.2	54	1.46	22	76	0.745	51	37.9	52
G19	37.9	67	1.84	30	97	0.528	67	49	34
G20	79.5	22	2.36	47	69	1.13	21	55.4	23
G21	52.3	52	1.88	31	83	0.746	49	35.2	55
G22	50.3	56	2.17	41	97	0.722	53	30.4	58
G23	40.5	65	2.97	59	124	0.515	68	21.3	67
G24	65	37	2.11	38	75	0.946	36	44.9	39
G25	52.5	50	0.889	10	60	0.781	48	44	42
G26	49.9	57	2.74	57	114	0.658	59	25.8	65
G27	35	69	3.12	62	131	0.372	70	13.5	70
G28	49.7	58	2.22	42	100	0.698	56	30.2	59
G29	70.7	29	1.65	26	55	1.04	27	59.1	15
G30	37.6	68	1.63	24	92	0.533	66	26.1	64
G31	63.5	39	1.21	16	55	0.933	37	50.4	32
G32	52.4	51	2.69	55	106	0.717	55	28.8	61
G33	55.1	48	3.96	67	115	0.721	54	18.3	68
G34	62.5	40	1.03	14	54	0.923	39	51.3	30
G35	81.4	20	4.45	69	89	1.12	24	46.9	36
G36	96.6	5	4.2	68	73	1.36	8	52.5	27
G37	45.9	61	0.848	8	69	0.665	58	39.9	50
G38	90.5	9	2.31	46	55	1.3	10	62.2	14
G39	76.5	24	0.759	7	31	1.13	22	65.8	9

G40	78.8	23	2.27	44	67	1.12	23	55.8	22
G41	112	1	2.45	49	50	1.64	1	84.8	1
G42	50.5	55	5.12	70	125	0.578	64	16.8	69
G43	63.8	38	2.49	52	90	0.883	42	40.4	49
G44	70.1	30	2.06	35	65	1.02	31	52.3	28
G45	72.9	26	1.46	23	49	1.08	25	57.1	18
G46	94.8	7	2.38	48	55	1.37	7	64.9	10
G47	61.5	43	3.12	61	104	0.855	43	32.8	57
G48	67.8	33	1.64	25	58	0.999	32	50.2	33
G49	53.8	49	0.227	2	51	0.801	47	44.6	41
G50	87.3	11	3.39	64	75	1.23	14	51.6	29
G51	86.5	13	1.98	34	47	1.25	12	62.5	13
G52	94.6	8	1.91	33	41	1.38	5	68.9	8
G53	65.2	36	2.53	53	89	0.928	38	44.9	40
G54	66.9	35	2.47	50	85	0.949	35	41.9	43
G55	47.8	59	2.47	51	110	0.646	61	26.8	63
G56	83.7	14	3.5	66	80	1.19	18	53.5	26
G57	73.2	25	2.72	56	81	1.03	29	44.9	38
G58	45.1	62	0.711	4	66	0.657	60	40.5	47
G59	83.2	16	2.57	54	70	1.21	16	56.7	20
G60	97.2	4	1.69	27	31	1.43	4	72.8	5
G61	105	2	1.32	19	21	1.56	2	84.3	2
G62	82.9	18	0.748	6	24	1.23	13	71.5	6
G63	71.3	28	1.22	17	45	1.05	26	57.1	19
G64	69.7	31	1.01	13	44	1.03	30	57.3	17
G65	79.7	21	1.25	18	39	1.17	20	63.1	12
G66	101	3	1.45	21	24	1.49	3	80.6	3
G67	62.4	41	1.74	29	70	0.916	41	46.2	37
G68	71.4	27	2.11	37	64	1.04	28	53.7	25
G69	41.3	64	0.747	5	69	0.609	63	69.2	7
G70	86.5	12	0.181	1	13	1.29	11	37.9	53

Y = Yield, Y_R = Yield rank, ASV = AMMI Stability Value, ASV_R = AMMI Stability Value Rank, YSI_R = Ranks based on yield stability index, HMRPGV = Harmonic mean of the relative performance of genetic value and, WAASBY = Weighted Average of Absolute Scores of BLUPs

helps visualise high-performing genotypes in specific environments (Gauch 2013). E_1 and E_2 together comprised one mega environment, while E_3 constituted a separate mega environment (Fig. 4). Genotypes UMI 1266 (G41) and UMI 1260 (G35) are the winning genotypes in mega-environment $E_{1,}$ whereas UMI 1257 (G2) and IMR 108200 (G4) are the winning genotypes in $E_{2,}$ indicating their high performance in those particular environments (Yan and Tinker 2006).

Discriminativeness vs Representativeness

The discriminativeness vs representativeness biplot was constructed via the column metric preserving method (environment) of single value partitioning to study the test environments (Fig. 5). The length of the vectors of the environments is proportional to their discriminative ability. Environments with shorter vector lengths indicate that they are less interactive and provide an ideal environment for genotype selection for broad adaptation (Badu-Apraku et

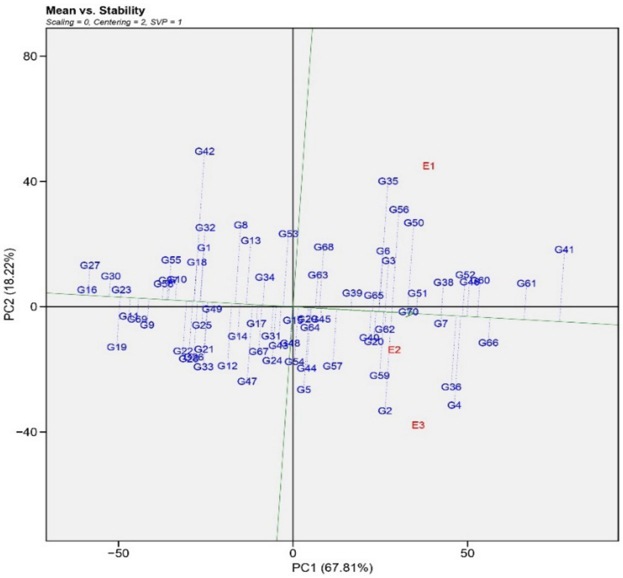

Fig. 3. Mean vs. stability biplot for grain yield of 70 genotypes across three locations

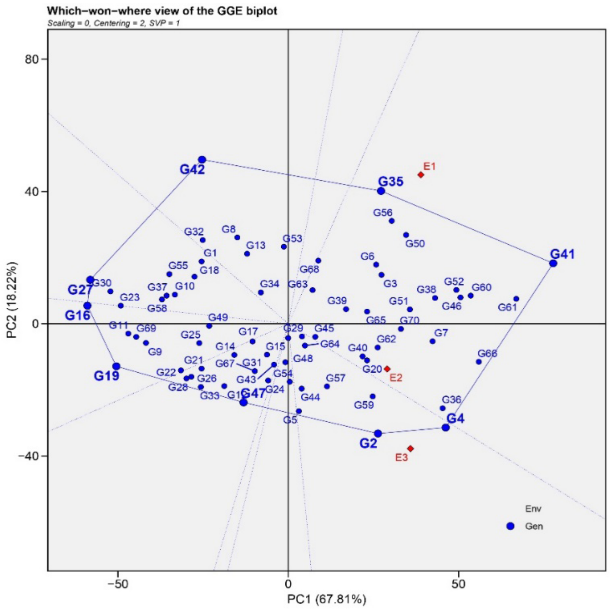

Fig. 4. Which-Won-Where view of the GGE biplot for a grain yield of 70 to identify winning genotypes across locations

al. 2012). E_2 is placed close to the AEC abscissa, indicating that it is a representative environment. The longer vector lengths and larger angles formed with the AEC axis indicate the highly discriminative nature of environments E_1 and E_3)to differentiate the performance of specifically adapted genotypes. These environments are useful for the identification of unstable genotypes or genotypes with specific adaptability to these conditions. The cosine angle between all the environments presented acute angles (<90°), indicating a positive correlation among them. Pramitha et al. (2020) reported similar findings in identifying stable maize genotypes via GGE biplot analysis. These findings highlight the significance of crossover interactions, which result in differential rankings of the genotypes across environments, which are crucial for breeders to consider and select genotypes suited across all environments or to a specific environment.

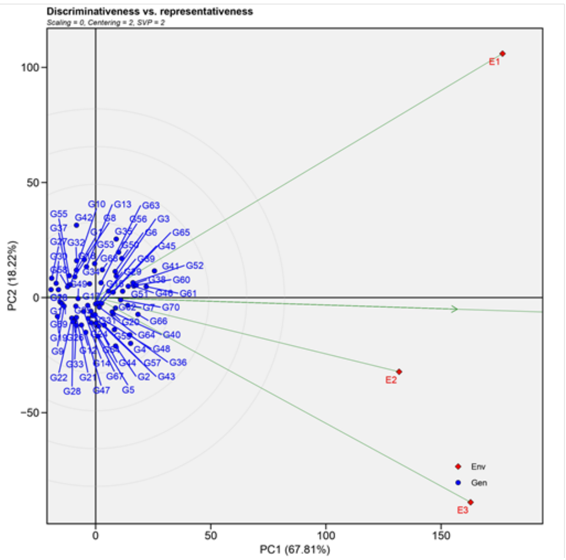

Fig. 5. Discriminative vs. representative view of the GGE biplot for environment classification

Harmonic mean of the relative performance of genotypic values

The HMRPGV formulated by Resende (2004) is based on a mixed model approach using the REML/BLUP method. Lower standard deviation in genotypic performance across the locations resulted in higher HMRPGV values and reflected in simultaneous selection for mean performance, stability and adaptability and accounts for heterogeneous variances among environments (Rodovalho et al. 2015). According to the HMRPGV values, the genotypes UMI 1266 (G41), UMI 1255 (G61), UMI 1288 (G66), UMI 1286 (G60), and UMI 1277 (G52) were found to be stable with high HMRPGV values (Table 2). HMRPGV has been effectively utilized in maize by several researchers for analyzing GEI and selecting stable genotypes across multiple environments (Rodovalho et al. 2015; Elias et al. 2016; Oliveria et al. 2017).

WAASB × Y biplot (WAASBY)

The WAASBY is a stability-based selection index that allows weighing between the trait of interest, particularly grain yield (GY) and genotype stability (WAASB value) (Olivoto et al. 2019a). WAASBY values are calculated with a weight of 60:40 for the mean performance and WAASB. The WAASBY scores serve as a selection index for genotype selection, while the biplot enables visualization for both genotype and environmental classification. The WAASB×Y biplot is constructed by plotting WAAS scores against

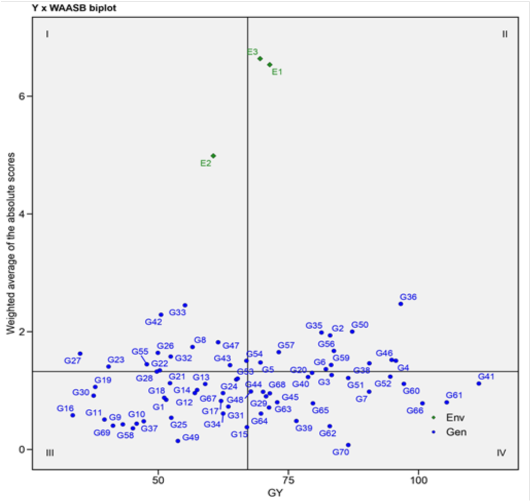

Fig. 6. WAASB × Y plot for grain yield (g) at 60:40 weight to yield and stability

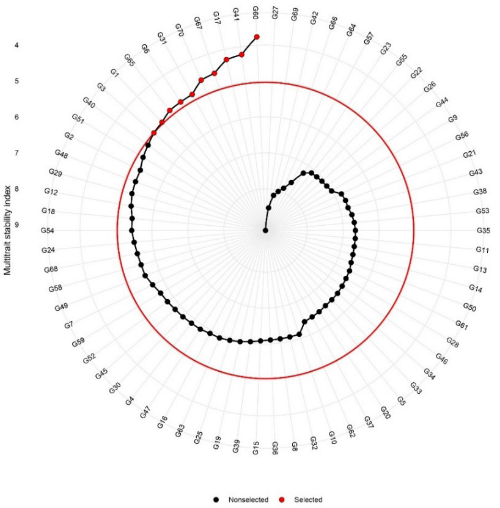

Fig. 7. Genotype ranking based on the multiple trait stability index at 15% selection intensity for the genotypes under study

mean yield. Lower WAASB scores are indicative of the stable performance of the genotypes. The WAASBY biplot is divided into four quadrants (Fig. 6). The first quadrant comprises highly unstable genotypes and is the major contributor to GEI. The environments lying in this quadrant are considered highly discriminative environments and the environment E_2 was placed in this quadrant along with the twelve genotypes. The second quadrant comprises unstable genotypes with high mean performance. The environments present in this quadrant have good discriminating ability apart from influencing higher mean yields. Environments E_1 and E_3 are placed in the second quadrant along with the twelve genotypes and the second quadrant is important for the selection of environments. The third quadrant comprises low-yielding but stable genotypes with 25 genotypes placed in this quadrant. The fourth quadrant, containing 21 genotypes, comprises high-yielding and stable genotypes, making it crucial for genotype selection. Genotypes UMI 1266 (G41), UMI 1255 (G61), UMI 1288 (G66), IMR 108308 (G7), and UMI 1286 (G60) presented relatively high WAASBY values (Table 2). Studies have demonstrated that WAASBY can effectively guide the selection of stable genotypes and classify environments on the basis of their interactive ability (Singamsetti et al. 2021; Patel et al. 2023).

Multi-trait Stability Index (MTSI)

The selection of a stable genotype on the basis of a single trait often overlooks the complex interactions between the trait of interest and other traits, which could aid in selection for improvement. The multi-trait stability index (MTSI) provides a holistic approach to genotype selection by considering multiple traits and their stable expression simultaneously (Olivoto et al. 2019b). By incorporating multiple traits, breeders can identify genotypes with desirable trait combinations, increasing selection efficiency and potential genetic gains across the studied traits (Balbaa et al. 2022; Patel et al. 2023; Perišić et al. 2023). An ideotype must be defined for in calculation of the MTSI index. In this study, the ideotype was assumed to be medium-maturing with high yield and short stature. The data was subjected to exploratory factor analysis (EFA) which captured the variation into nine FAs. The first three FAs with eigenvalue>1, cumulatively explained 74.9% of the total variation. The average communality of 0.748 after varimax rotation indicates that approximately 75% of the variance has been explained by the three FAs and that only a small portion of the variance is left unexplained (Supplementary Table S3).

At a selection intensity of 15%, ten genotypes were selected that were closer to the proposed ideotype. Selection differentials were computed for both stability (WAASB) and general mean performance across all traits for the selected genotypes. The selection differential percentage (SDperc) ranged between 4.75% (DFS) and 30.2% (EH) for WAASB, whereas it ranged between -10.8 (EH) and 16.1 (GY) in mean performance. Negative selection gains were observed for PH (-3.99) and EH (-6.71). Positive selection gains were observed for all the yield and yield attributing traits, with GY exhibiting the highest gains (12.1%) (Table 3). Lower MTSI scores indicate how closely the selected genotype resembles the proposed ideotype across all studied traits. Among the ten selected genotypes, UMI 1286 (G60) is closest to the ideotype, followed by UMI 1266 (G41), IMR 109659 (G17), IMR 108790 (G67), UMI1285 (G70), IMR 110053 (G31), IMR 108271 (G6), UMI 1253 (G65), UMI 108799 (G9) and UMI 1258 (G3) (Fig. 7). The MTSI scores for all the genotypes are given in Supplementary Table S4.

Identification of Stable Genotypes

The top fifteen stable genotypes identified by each stability index *viz.*YSI, HMRPGV, WAASB, and MTSI were analyzed using a Venn diagram to determine the genotypes

Table 3. Selection differential and selection gains for 9 different traits of 70 maize genotypes evaluated across three environments

		WAASB			Mean performance				
VAR	Factor	Xo	Xs	SD_{perc}	Xo	Xs	SD_{perc}	SG	SG_{perc}
EL	FA1	49.8	63.0	26.5	12.6	13.1	3.29	0.254	2.01
ED	FA1	58.9	72.7	23.4	3.81	4.14	8.64	0.244	6.41
KRN	FA1	50.4	64.1	27.1	13.1	14.6	11.4	1.1	8.43
NKR	FA1	63.1	71.9	13.9	22.3	24	7.61	1.06	4.76
GY	FA1	48.4	60.3	24.6	67.2	78	16.1	8.16	12.1
DFA	FA2	62.6	66.5	6.29	55	55.6	1.17	0.581	1.06
DFS	FA2	62.6	65.6	4.75	57.8	58.3	0.886	0.462	0.8
PH	FA3	53.1	66.9	26.0	117	109	-6.32	-4.66	-3.99
EH	FA3	52.8	68.7	30.2	60.2	53.7	-10.8	-3.71	-6.17

FA = Factors; Xo = population mean; Xs = Selected genotypes mean; SD_{perc} = Selection differential percentage; SG = Selection gains; SG_{perc} = Selection gain percentage; DA= Days to 50% Anthesis; DS= Days to 50% Silking; PH = Plant Height (cm); EH = Ear Height (cm); EL = Ear Length (cm); ED = Ear Diamater (cm); KRN = Kernel Row Number; NKR = Number of Kernels per Row; GY = Grain yield (g)

commonly occurring across multiple indices (Fig. 8). Three genotypes namely, UMI 1286 (G60), UMI 1276 (G51), and UMI 1266 (G41) ranked consistently across all four stability indices. UMI 1285 (G70) was ranked consistently across HMRPGV, YSI and MTSI, whereas UMI 1253 (G65) also occurred consistently across WAASBY, MTSI, and YSI. UMI 1258 (G3) commonly occurred under MTSI, WAASBY and HMRPGV, whereas five genotypes, UMI 1255 (G61), UMI 1254 (G62), UMI 1288 (G66), UMI 1277 (G52), and IMR 108308 (G7), were commonly ranked in the WAASBY, HMRPGV and YSI indices. Singamsetti et al. (2021) similarly employed multiple indices to stabilize maize hybrids across moisture regimes.

The coincidence index measures the alignment between different indices used to evaluate genotype stability and performance in plant breeding (Hannachi and Fellahi 2023). The coincidence rates between the indices are shown in Table 4. The highest rate of coincidence was observed between WAASBY and HMRPGV (66.24), followed by WAASBY and YSI (57.8) and HMRPGV and YSI (49.36). The MTSI recorded low levels of coincidence at 15.61, 15.61, and 24.05 with the YSI, HMRPGV, and WAASBY, respectively. The high levels of coincidence between HMRPGV and YSI indicate that the efficiency of selection utilizing either one of the indices is good. A higher coincidence percentage enables the use of any one of the indices to assist in the selection of desired genotypes (Smiderele et al. 2019; Casagrande et al. 2022). The high levels of coincidence for WAASBY with YSI or HMRPGV can be attributed to the use of a mixed model involving both AMMI and BLUP analysis for estimating the stability parameters of the genotypes. In contrast, MTSI showed lower coincidence rates with other indices likely because it integrates a broader selection approach considering multiple traits, stability, and desired selection direction.

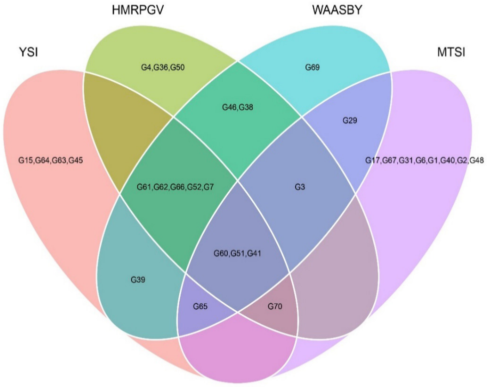

Fig. 8. Venn diagram showing genotype overlap across different stability indices

Table 4. Coincidence indices for genotype selection indices

	YSI	HMRPGV	WAASBY
HMRPGV	49.36		
WAASBY	57.8	66.24	
MTSI	15.61	15.61	24.05

Employing a combination of stability indices enhances the accuracy of genotype selection through a multifaceted approach that captures various aspects of genotype stability and adaptability, reducing the risk of selection bias inherent in single-model selection methods (Pour-Aboughadareh et al. 2022). A comparative analysis of the top fifteen ranked genotypes across the four models revealed that some genotypes are ranked similarly across the models. Genotypes UMI 1286, UMI 1276, and UMI 1266 ranked consistently across all the indices, indicating their stability, yield capacity and potential use as parental lines. The use of multiple stability parameters to identify stable genotypes has been used by many researchers in crops such as Maize (Singamsetti et al. 2021; Patel et al. 2023), Wheat (Al-Ashkar et al. 2023; Casagrande et al. 2023) and Ragi (Anuradha et al. 2022).

Authors' contribution

Conceptualization of research (SS, KV, HD); design of experiment (SS, KV, HD); contribution of experimental material (SS, KV, YKR); execution of field experiments and data collection (SS, KV, HD, SSKRV); analysis of data and interpretation (SS, KV, HD); preparation of manuscript (HD, SS, KV); review of the manuscript (SN, UD, BK, SA).

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Supplementary Table S1. Details of the pedigree and source of the 70 inbreds under study

Code	Genotype name	Pedigree	Source
G1	UMI 1256	WN/TN-52068	Dept. of Millets, TNAU
G2	UMI 1257	WN/TN-52099	Dept. of Millets, TNAU
G3	UMI 1258	WN/TN-52485	Dept. of Millets, TNAU
G4	IMR 108200	CM 117-3-4-1	WNC, ICAR-IIMR
G5	IMR 108242	CM 140	WNC, ICAR-IIMR
G6	IMR 108271	CM 210	WNC, ICAR-IIMR
G7	IMR 108308	CML141	WNC, ICAR-IIMR
G8	IMR 108321	CML 169	WNC, ICAR-IIMR
G9	IMR 108799	E 30	WNC, ICAR-IIMR
G10	IMR 108841	EC 672486⊗⊗⊗	WNC, ICAR-IIMR
G11	IMR 109242	IML15-202	WNC, ICAR-IIMR
G12	IMR 109246	IMLSB 1000-2	WNC, ICAR-IIMR
G13	IMR 109258	IMLSB 1043-3-2	WNC, ICAR-IIMR
G14	IMR 109534	JCY 3-7	WNC, ICAR-IIMR
G15	IMR 109580	MANIPUR LOCAL SEL-1-1	WNC, ICAR-IIMR
G16	IMR 109619	NAI 147⊗⊗	WNC, ICAR-IIMR
G17	IMR 109659	North east 4-3 (N)- ⊗-1-1-1-⊗-1-2	WNC, ICAR-IIMR
G18	IMR 109688	P66C0BBB26BBB	WNC, ICAR-IIMR
G19	IMR 109726	PFSR (Y)-C1-B ⊗-2-2-1-2-⊗-2-1⊗	WNC, ICAR-IIMR
G20	IMR 109736	PFSR 10109⊗⊗	WNC, ICAR-IIMR
G21	IMR 109747	PFSR 62-2⊗⊗⊗⊗⊗	WNC, ICAR-IIMR
G22	IMR 109844	SHD 1ER6	WNC, ICAR-IIMR
G23	IMR 109845	SHD 1ER6	WNC, ICAR-IIMR
G24	IMR 109866	SW 5-10-B*5-2-B*8	WNC, ICAR-IIMR
G25	IMR 109971	VH 9-1-2-1-1⊗	WNC, ICAR-IIMR
G26	IMR 110001	VS 78 - 2	WNC, ICAR-IIMR
G27	IMR 110007	WLS-F36-4-2-2-B-1-B*7	WNC, ICAR-IIMR
G28	IMR 110013	WNZDC2 × AEB(Y)C534-1	WNC, ICAR-IIMR
G29	IMR 110014	WNZPBTL 6	WNC, ICAR-IIMR
G30	IMR 110020	HEY Pool -2011-15-3-6-1-3-1ÄÄÄ	WNC, ICAR-IIMR
G31	IMR 110053	CAL14135	WNC, ICAR-IIMR
G32	IMR 110057	CAL1433	WNC, ICAR-IIMR
G33	IMR 110067	CAL186	WNC, ICAR-IIMR
G34	UMI 1287	1701-2-1S	Dept. of Millets, TNAU
G35	UMI 1260	701-13-1S	Dept. of Millets, TNAU
G36	UMI 1261	701-15-3S	Dept. of Millets, TNAU
G37	UMI 1262	701-2-3-3S	Dept. of Millets, TNAU
G38	UMI 1263	701-2-3-2S	Dept. of Millets, TNAU
G39	UMI 1264	701-2-3-5S	Dept. of Millets, TNAU

G40	UMI 1265	701-9-4-1S	Dept. of Millets, TNAU
G41	UMI 1266	704-2-1-6S	Dept. of Millets, TNAU
G42	UMI 1267	704-9-4S	Dept. of Millets, TNAU
G43	UMI 1268	705-4-5/1S	Dept. of Millets, TNAU
G44	UMI 1269	705-4-5/3S	Dept. of Millets, TNAU
G45	UMI 1270	706-17-2/2S	Dept. of Millets, TNAU
G46	UMI 1271	706-17-2/3S	Dept. of Millets, TNAU
G47	UMI 1272	706-17-2/4S	Dept. of Millets, TNAU
G48	UMI 1273	707-2-3/1S	Dept. of Millets, TNAU
G49	UMI 1274	707-9-4-2/5S	Dept. of Millets, TNAU
G50	UMI 1275	707-9-4-3/1S	Dept. of Millets, TNAU
G51	UMI 1276	707-9-4-4/1S	Dept. of Millets, TNAU
G52	UMI 1277	707-9-4-4/5S	Dept. of Millets, TNAU
G53	UMI 1278	711-13-4-3/2S	Dept. of Millets, TNAU
G54	UMI 1279	711-13-3/2S	Dept. of Millets, TNAU
G55	UMI 1280	711-13-5/5S	Dept. of Millets, TNAU
G56	UMI 1281	711-13-4/6S	Dept. of Millets, TNAU
G57	UMI 1282	711-13-6/5S	Dept. of Millets, TNAU
G58	UMI 1283	711-13-2/1S	Dept. of Millets, TNAU
G59	UMI 1284	711-13-2/2S	Dept. of Millets, TNAU
G60	UMI 1286	811-1-2/2S	Dept. of Millets, TNAU
G61	UMI 1255	N09-162	Dept. of Millets, TNAU
G62	UMI 1254	N10-105	Dept. of Millets, TNAU
G63	UMI 1251	NS-333-2-2-3	Dept. of Millets, TNAU
G64	UMI 1252	S8-2	Dept. of Millets, TNAU
G65	UMI 1253	S9-1	Dept. of Millets, TNAU
G66	UMI1288	WN/TN-DMRE63	Dept. of Millets, TNAU
G67	IMR 108790	DTPWC 9-F67-2-2-1-3-2-1-2-B*9	WNC, ICAR-IIMR
G68	UMI 1288	1701-2-5S	Dept. of Millets, TNAU
G69	IMR 108846	EC 672591⊗⊗⊗	WNC, ICAR-IIMR
G70	UMI 1285	711-13-1/4S	Dept. of Millets, TNAU

Supplementary Table S2. Mean performance of genotypes across environments

GEN	Code	DFA	DFS	PH	EH	EL	ED	KRN	NKR	GY
G1	UMI 1256	60	63	87.20	45.30	9.83	3.61	14	19	51.5
G2	UMI 1257	59	61	117.00	62.70	14.70	3.84	12	25	83.
G3	UMI 1258	60	63	115.00	57.20	12.00	3.93	13	22	83.3
G4	IMR 108200	58	61	149.00	65.80	14.20	4.12	13	24	95.6
G5	IMR 108242	48	51	82.30	45.50	12.00	3.75	13	23	69.6
G6	IMR 108271	54	56	102.00	54.00	12.70	4.75	15	26	82.2
G7	IMR 108308	58	62	138.00	72.80	14.30	3.82	13	24	90.5
G8	IMR 108321	55	58	116.00	54.20	11.70	3.61	13	21	56.6
G9	IMR 108799	55	58	99.50	40.70	10.80	3.29	13	20	43.2
G10	IMR 108841	56	59	95.20	55.20	9.67	3.40	11	17	47.2
G11	IMR 109242	54	57	105.00	53.80	11.80	2.76	11	16	39.7
G12	IMR 109246	57	60	127.00	69.70	12.20	3.47	12	20	57.0
G13	IMR 109258	57	60	132.00	70.20	12.30	3.68	12	19	59.0
G14	IMR 109534	53	56	134.00	65.50	11.30	3.48	12	18	57.5
G15	IMR 109580	57	60	132.00	58.30	12.30	3.63	13	18	67.0
G16	IMR 109619	61	63	118.00	59.70	10.50	3.03	12	17	33.6
G17	IMR 109659	59	62	95.30	57.50	12.80	3.77	14	23	62.1
G18	IMR 109688	60	63	93.70	48.30	11.20	3.67	14	22	51.2
G19	IMR 109726	61	64	92.30	54.80	10.30	3.08	11	15	37.9
G20	IMR 109736	60	62	144.00	67.50	13.20	3.87	11	25	79.5
G21	IMR 109747	61	64	104.00	55.20	12.50	4.03	13	20	52.3
G22	IMR 109844	50	52	82.70	47.70	11.70	3.72	14	19	50.3
G23	IMR 109845	53	57	70.00	39.80	9.67	3.21	13	16	40.5
G24	IMR 109866	62	65	133.00	56.80	11.70	3.40	11	22	65.0
G25	IMR 109971	52	55	95.00	51.80	11.70	3.42	12	23	52.5
G26	IMR 110001	59	61	115.00	55.00	11.00	3.11	10	21	49.9
G27	IMR 110007	48	51	111.00	51.50	10.00	3.24	11	15	35.0
G28	IMR 110013	57	60	143.00	72.50	10.50	3.43	13	17	49.7
G29	IMR 110014	57	60	129.00	58.50	14.10	3.96	12	27	70.7
G30	IMR 110020	58	61	109.00	53.20	9.83	3.49	15	20	37.6
G31	IMR 110053	53	56	114.00	51.80	14.00	4.25	15	22	63.5
G32	IMR 110057	58	61	101.00	50.30	13.50	3.58	12	22	52.4
G33	IMR 110067	56	59	99.20	56.50	13.20	3.54	12	22	55.1
G34	UMI 1287	55	58	108.00	60.80	14.50	3.50	12	21	62.5
G35	UMI 1260	55	58	113.00	66.80	15.50	3.66	12	27	81.4
G36	UMI 1261	53	55	119.00	63.80	16.70	4.67	15	24	96.6
G37	UMI 1262	58	60	126.00	63.30	11.00	3.40	13	20	45.9
G38	UMI 1263	52	55	137.00	66.00	13.70	4.06	13	26	90.5
G39	UMI 1264	51	54	121.00	59.50	12.00	3.96	13	23	76.5
G40	UMI 1265	54	57	108.00	58.20	14.00	3.93	14	22	78.8

G41	UMI 1266	54	56	118.00	60.80	12.30	4.72	18	27	112.0
G42	UMI 1267	53	56	132.00	62.70	12.00	3.61	12	19	50.5
G43	UMI 1268	54	57	122.00	63.50	13.20	3.58	13	19	63.8
G44	UMI 1269	55	58	113.00	62.50	12.50	4.03	14	24	70.1
G45	UMI 1270	55	58	123.00	66.80	14.50	3.87	11	26	72.9
G46	UMI 1271	49	52	136.00	75.20	12.90	4.25	12	29	94.8
G47	UMI 1272	55	57	131.00	65.20	13.40	4.14	12	21	61.5
G48	UMI 1273	54	57	122.00	68.80	12.80	4.28	14	24	67.8
G49	UMI 1274	54	57	117.00	63.70	13.50	3.69	13	22	53.8
G50	UMI 1275	53	56	114.00	59.00	13.30	4.16	14	24	87.3
G51	UMI 1276	53	56	123.00	60.70	15.30	4.60	14	29	86.5
G52	UMI 1277	54	58	125.00	62.80	13.80	3.82	15	24	94.6
G53	UMI 1278	52	55	115.00	59.30	10.80	3.79	13	20	65.2
G54	UMI 1279	55	59	116.00	66.20	13.00	4.03	14	22	66.9
G55	UMI 1280	54	57	104.00	57.00	9.83	3.72	14	19	47.8
G56	UMI 1281	50	53	129.00	69.30	16.10	3.98	13	27	83.7
G57	UMI 1282	51	54	135.00	75.50	13.30	4.28	14	24	73.2
G58	UMI 1283	57	60	114.00	52.80	10.20	3.76	13	19	45.1
G59	UMI 1284	51	55	123.00	59.80	12.00	4.14	16	26	83.2
G60	UMI 1286	54	57	118.00	54.50	15.10	4.11	13	28	97.2
G61	UMI 1255	53	56	131.00	71.30	14.30	4.25	14	29	105.0
G62	UMI 1254	52	55	117.00	66.20	13.10	3.98	14	22	82.9
G63	UMI 1251	55	57	114.00	60.00	12.00	3.93	13	26	71.3
G64	UMI 1252	51	54	130.00	74.80	11.20	4.03	13	22	69.7
G65	UMI 1253	56	58	108.00	51.70	15.00	3.96	14	25	79.7
G66	DMRE 63	50	53	142.00	74.50	14.30	3.91	15	26	101.0
G67	IMR 108790	53	55	101.00	40.80	11.30	4.03	15	23	62.4
G68	UMI 1288	57	60	131.00	71.00	13.80	4.17	15	23	71.4
G69	IMR 108846	55	57	124.00	75.80	14.00	3.40	11	24	41.3
G70	UMI 1285	55	57	134.00	63.20	15.50	4.25	15	25	86.5
E1	Coimbatore	55	58	115	59.6	13.0	3.95	13.3	22.9	71.4
E2	Bhavanisagar	56	59	110	54.0	12.1	3.57	12.5	21.1	60.6
E3	Vagarai	53	56	126	67.0	12.9	3.90	13.3	22.8	69.6

DFA = Days to 50% Anthesis; DFS = Days to 50% Silking; PH = Plant Height (cm); EH = Ear Height (cm); EL = Ear Length (cm); ED = Ear Diamater (cm); KRN = Kernel Row Number; NKR = Number of Kernels per Row; GY = Grain yield (g)

Supplementary Table S3. Factorial loadings after varimax rotation

VAR	FA1	FA2	FA3	Communality	Uniqueness
DFA	-0.0326	0.988	-0.00499	0.978	0.022
DFS	-0.0131	0.988	0.000732	0.976	0.024
PH	0.0190	-0.0919	-0.859	0.747	0.253
EH	-0.132	0.0981	-0.873	0.790	0.210
EL	0.735	-0.0672	0.0976	0.555	0.445
ED	0.825	0.105	-0.146	0.714	0.286
KRN	0.578	0.0233	-0.379	0.478	0.522
NKR	0.847	-0.0437	0.111	0.732	0.268
GY	0.846	-0.0884	0.215	0.770	0.230
Communality mean: 0.749					

DFA = Days to 50% Anthesis; DFS = Days to 50% Silking; PH = Plant Height (cm); EH = Ear Height (cm); EL = Ear Length (cm); ED = Ear Diamater (cm); KRN = Kernel Row Number; NKR = Number of Kernels per Row; GY = Grain yield (g)

Supplementary Table S4. Multi trait stability index (MTSI) values for 70 genotypes and their ranks

Genotype	MTSI	MTSI R	Genotype	MTSI	MTSI R	Genotype	MTSI	MTSI R
G1	5	9	G26	7.08	61	G51	5.2	12
G2	5.3	13	G27	9.16	70	G52	5.79	25
G3	5.04	10	G28	6.55	47	G53	6.68	54
G4	5.86	28	G29	5.31	15	G54	5.44	18
G5	6.48	43	G30	5.84	27	G55	7.11	63
G6	4.88	7	G31	4.86	6	G56	6.82	58
G7	5.68	23	G32	6.12	38	G57	7.64	65
G8	6.12	37	G33	6.49	44	G58	5.57	21
G9	7.02	59	G34	6.5	45	G59	5.76	24
G10	6.12	39	G35	6.66	53	G60	3.77	1
G11	6.65	52	G36	6.11	36	G61	6.57	48
G12	5.36	16	G37	6.43	41	G62	6.4	40
G13	6.65	51	G38	6.72	55	G63	5.91	31
G14	6.64	50	G39	6.04	34	G64	7.88	66
G15	6.09	35	G40	5.12	11	G65	4.89	8
G16	5.91	30	G41	4.22	2	G66	8.02	67
G17	4.28	3	G42	8.16	68	G67	4.56	4
G18	5.44	17	G43	6.78	56	G68	5.54	20
G19	5.99	33	G44	7.06	60	G69	8.53	69
G20	6.45	42	G45	5.82	26	G70	4.61	5
G21	6.78	57	G46	6.53	46			
G22	7.1	62	G47	5.87	29			
G23	7.25	64	G48	5.3	14			
G24	5.49	19	G49	5.59	22			
G25	5.95	32	G50	6.62	49			