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RESEARCH ARTICLE

Unveiling genotype × environment dynamics for grain yield in QPM hybrids through AMMI, GGE Biplot, and MTSI approach

Ramesh Kumar*#, Santosh Kumar[1#], G.K. Chikkappa[2], Abhijit Kumar Das, Somnath Dhonde[3], Yashmeet Kaur, Sushil Kumar, Shanu Shukla and Sujay Rakshit[4]

Abstract

The study meticulously assessed 77 experimental and eight commercial checks across three distinct Indian locations employing statistical tools: AMMI (additive main effects and multiplicative interaction), GGE [genotype main effect+ (G×E)], and MTSI (multi-trait stability index) to identify the best-suited hybrids and for the mega-environment. The environment predominantly shaped hybrid performance, influencing 4.24 to 68.12% of the variance, while genotype and genotype-environment interaction ranged from 13.43 to 37.76% and 18.45 to 57.99%, respectively. GGE biplots identified hybrids G77 (DQL 2490 × DQL 2709) and G85 (DQL 2513 × DQL 2709) as promising in yield and stability, surpassing commercial checks. Impressively, the MTSI technique highlighted these hybrids, alongside 11 others, as elite performers, aligning closely with anticipated stability and mean values. Furthermore, environmental stratification amalgamated the test locales into a singular mega-environmental category. AMMI-derived Yield Stability indices unequivocally endorsed hybrids G77 and G85 for their stability profiles. Crucially, these high-yielding, resilient hybrids not only promise to fortify food and nutritional security but also resonate with the Sustainable Development Goals (SDGs), exemplifying their pivotal role in advancing national nutritional objectives and broader global sustainability targets.

Keywords: QPM, genotype-environment interaction, mega environment, winning genotype, MTSI

Introduction

It is projected that by 2050, the worldwide demand for food will increase twice so global food production will have to increase by 70% to meet the projected population growth (Dijk et al. 2021). The increased demand can be met by enhancing the productivity of major food crops, namely rice, wheat, and maize (corn), by a factor of two or more. The scope and pressure to enhance productivity are greater in developing countries than in developed countries, as the yield level in most of the developed countries is already high and has reached a plateau in some of the developed countries. Enhancing the productivity of major food crops in developing countries is much more challenging under changing climate scenarios and low-input-driven agriculture, especially in regions like East Africa and South Asia (Chivasa et al. 2021). In 2020, nearly one out of three people lacked regular access to adequate food (United Nations 2022).

Quality Protein Maize (QPM) commercialization can combat the food and nutritional insecurity that is common in some developing nations of the world (Bankole et al.

ICAR-Indian Institute of Maize Research, Ludhiana 141004, India.
[1]ICAR-Indian Agricultural Research Institute, Barhi 825 406, Jharkhand, India.
[2]ICAR-Indian Institute of Maize Research, Unit Office, New Delhi 110 012, India.
[3]Mahatma Phule Krishi Vidyapeeth, Rahuri 413 722, Maharashtra, India.
[4]ICAR-Indian Institute of Agricultural Biotechnology, Ranchi 834 003, Jharkhand, India.
#Both the authors' contributed equally.
***Corresponding Author:** Ramesh Kumar, ICAR-Indian Institute of Maize Research, Ludhiana 141 004, India, E-Mail: rk.phagna@gmail.com

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2023). Asia is the world's second-largest maize producer; ~31% of the total maize in the world is harvested from ~34% of the world's total maize-growing land in Asia (Zaidi et al. 2016). The worldwide production of maize was around 1123.07 million metric tons (MMT) in 2020-2021 (Food and Agriculture Organization 2022). However, enhancing maize yields under changing climate scenarios is a daunting task, as projections indicate that a 1°C increase in global mean temperature would decrease maize yields by 7.4 percent (Zhao et al. 2017). Maize plays a vital role in ensuring the food security of several countries in Latin America, Africa, and a few Asian countries. On the contrary, maize also plays a significant role in the sustenance of several food and non-food-based industries in several developing countries. In India, maize has occupied the third most important cereal food crop and replaced sorghum in 2007-08 and pearl millet in 2012-13 as the most widely grown cereal food crop after rice and wheat. Maize is cultivated in a broad spectrum of climatic conditions, from extremely arid to humid environments; at sea level to above 4,000 meters in altitude, in irrigated to semi-arid circumstances during the rainy and summer seasons.

As compared to field corn, quality protein maize (QPM) ensures the food and nutrition security of communities that rely heavily on maize. QPM has a higher biological value and has double the levels of the important essential amino acids lysine and tryptophan as compared to field corn. Lysine and tryptophan have important secondary roles as neurotransmitters in addition to their primary activities as protein building blocks. Because field corn generally possesses a low concentration of two important essential amino acids, namely lysine and tryptophan, the discovery of the endosperm mutant *opaque2*, has led to the development of Quality Protein Maize (QPM) germplasm with acceptable agronomic performance. The biological value of protein available in QPM is almost equal to that of milk, which can effectively reduce protein malnutrition (Vasal 2000). The nutritional advantage of QPM has led to increased investment in research and development of QPM developing countries across Latin America, Africa, and Asia. The approximate total area under QPM in the world is currently ~9 million acres.

The development of maize hybrids that are nutritionally superior, like QPM, and can also perform better under changing climate scenarios is a top priority to simultaneously ensure food, feed, and nutritional security. The term "genotype-environment interaction" (GEI) pertains to how different environments impact the expression of different genotypes (Finlay and Wilkinson 1963). Understanding the proportion of genotype, environment, and GEI on the overall phenotype is crucial not only for exercising selection to bring improvement in any trait but also for the identification of stable genotypes across environments. The selection efficiency depends on the correlation between genotype and phenotype. The higher correlation between phenotype and genotype indicates higher heritability with low GEI or environmental influence on the trait, thus indicating a higher response to selection (Yan and Kang 2003). The presence of higher GEI not only affects the selection efficiency but also necessitates repeated evaluation at more locations and/or years to identify genotypes with stable performance across the locations. Thus, it is essential to select the most stable genotypes based on multi-environment trials (MET) for a given environment in the presence of high GEI both to stabilize yield and also minimize yield losses under unfavorable environmental conditions.

Several parametric and non-parametric statistical tools have been suggested for analyzing GEI. The stability methods proposed by Finlay and Wilkinson (1963) and Eberhart and Russell (1966) are extensively used and tend to be univariate but the response of a genotype in different environments is multivariate (Lin et al. 1986; Gauch 1988). Thus, advanced statistical techniques like AMMI (additive main effects and multiplicative interaction) and the GGE [Genotype main effect+(G×E)] biplots are considered more useful. The principal component analysis of GEI, along with the analysis of variance, is used to find the main effects of genotype and environment in the AMMI model (Gauch and Zobel 1997) but it does not include a quantitative stability metric, which is necessary for ranking genotypes according to yield stability. Later, Purchase (2000) introduced the AMMI stability value (ASV), based on the IPCA1 and IPCA2 scores, for each genotype to show how stable that genotype is. Subsequently, GGE biplot analysis was proposed, which combines both the main effects of genotype and GEI (Yan et al.2000). The GGE biplot analysis aids in assessing genotypes for mean performance as well as their stability across different environments. It simplifies and also completes the process of selecting the best-suited genotype that is influenced by both the individual's genetic makeup and their environment (Yan and Tinker 2006). However, the GGE biplot can best be exploited while dealing with a single trait. Therefore, Olivoto et al. (2019a) proposed a multi-trait stability index (MTSI) to select high-performing, stable genotypes in METs using a multi-trait approach. MTSI offers a unique selection procedure that enables the fine consideration of mean performance and stability while dealing with more than one trait (Olivoto et al. 2019b). The current study was conducted with the objective of identifying the best-performing QPM hybrids in terms of mean yield and environmental stability using AMMI, GGE, and MTSI.

Materials and methods

The experimental material comprises 85 single cross-Quality Protein Maize hybrids, which include eight checks: IQPMH 1601 (G134), IQPMH 1705 (G135), IQPMH 18-2 (G136), BIO

9544 (G137), DKC 9224 (G138), DKC 8221 (G139), DKC 8211 (G140), and DKC 7204 (G141) (Supplementary Table S1). The experimental hybrids were generated by crossing 8 inbred lines from heterotic group (HG) B with 24 inbred lines from HG-A. Two inbreds *viz*., CML 161 and CML 165 collected from CIMMYT, Mexico, were used as testers to classify these inbreds into distinct heterotic groups. Out of 192 possible cross combinations as per line × tester mating design, 77 crosses were generated depending on the synchronization in flowering between selected inbred lines. The 77 cross combination included the four different sets of crosses generated between 10 lines (DQL 2011, DQL 2099-1, DQL 2192, DQL 2325, DQL 2487, DQL 2490, DQL 2513, DQL 2646, CLQRCY 40)of HG-Aand three lines (DQL 2700, DQL 2709, DQL 2751)from HG-B; 11 lines (DQL 2167, DQL 2192, DQL 2246, DQL 2290-4, DQL 2306, DQL 2325, DQL 2543, DQL 2631, DQL 2642, DQL 2668, CLQRCY 40) from HG-A and two lines (DQL 2698, DQL 2699)from HG-B; six lines (DQL 2021-1, DQL 2192, DQL 2631, DQL 2646, DQL 2668, CLQRCY 40) from HG-A and two lines (DQL 2743, DQL 2753) from HG-B; 13 lines (DQL 2099-1, DQL 2167, DQL 2192, DQL 2325, DQL 2370, DQL 2452, DQL 2471, DQL 2487, DQL 2513, DQL 2543, DQL 2545, DQL 2572, DQL 2642) from HG-A and one line (DQL 2691) from HG-B. The details of inbred lines, i.e., pedigree, developing institution, grain type and grain color are given in Supplementary Table S2. The hybrids were evaluated at three locations, namely Ludhiana in Punjab, Hazaribagh in Jharkhand, and Rahuri in Maharashtra, during the rainy season 2021. The coordinates of Ludhiana, Jharkhand and Rahuri are 30°54′N 75°51′E, 23.98°N 85.35°E, 19.38°N 74.65°E and have an altitude of 244, 613 and 657 m above mean sea level, respectively.

The trial was conducted in a lattice (Patterson and Williams 1976) design with three replicates at each location. The entries were sown in two rows of 4 m with a between-row spacing of 0.6 meters and a plant-to-plant distance within the row of 0.2 m at all locations. Trials were managed at all three locations by following optimal agronomic and cultural procedures to raise a healthy crop. The observations were recorded on days to 50% anthesis (DA), days to 50% silking (DS), field weight (FW), grain weight (GW), and grain yield (GY in q/ha). The details of the crosses, along with their allotted genotype numbers, are given in Supplementary Table 3. The crosses have been represented by their corresponding genotype number in the result and discussion parts.

Statistical analysis

Analysis of variance

The data from each testing site was subjected to analysis of variance using the general linear mixed model (GLMM), under the assumption that genotypes were fixed and environments, replications, and blocks within a replication were random (Rusinamhodzi et al. 2020; Mhlaba et al. 2019).

The genotype-by-environment interaction (GEI) was studied to understand the behavior of experimental hybrids and to identify stable hybrids across locations using different approaches like the additive main effects and multiplicative interaction (AMMI) and genotype plus genotype-by-environment (GGE) analysis (Gollob 1968; Gauch and Zobel 1988; Vargas and Crossa 2000; Yan et al. 2000; Yan 2001, 2002; Yan and Kang 2003; Yan and Tinker 2006; Zerihun 2011). Additionally, AMMI's stability values (ASV) and yield stability index (YSI) were used to rank the experimental hybrids (Purchase et al. 2000). Finally, the multi-trait stability index (MTSI), a quantitative value on the stability of each genotype, was generated by using a linear mixed-effect model, the weighted average of absolute scores (WAASB) (Olivoto et al. 2019a), for the selection of hybrids.

The statistical analysis, namely, descriptive statistics, genetic variability parameters, individual and combined ANOVA across locations and genotypes by environment interaction, and stability parameter analysis, including AMMI (Gauch 1988), GGE (Yan et al. 2000; Yan 2001, 2002; Yan and Kang 2003; Yan and Tinker 2006), and MTSI (Olivoto et al. 2019a), were performed by using the 'metan' package version v1.15.0 (Olivoto and Lúcio 2020), and biplots were constructed by the 'ggplot2' version 3.3.4 package in RStudio (RStudio 2021).

Results and discussion

ANOVA and mean performances of experimental hybrids

The result showed that the mean sum of squares for each studied trait differed significantly among genotypes, environments, and GEI. The combined analysis of variance exhibited highly significant variation for all the traits (Table 1). Based on the results of the study, the environmental (E) factor accounted for the highest percentage of the total variability (G + E + GEI) for the traits DS, FW, and GW, while the GEI factor recorded the highest proportion of total variability for the traits DA and GY. The environmental contribution was recorded highest for FW, followed by GW, DS, GY, and DA, whereas the percent contribution of genotype was about 37.76% for DA, followed by GY (34.12%), DS (18.09%), GW (13.8%), and FW (13.43%). GEI contributed the most to DA, followed by GY, DS, GW, and FW towards total variation. There was a wide variation in GY, ranging between 24.3 q/ha and 92.5 q/ha, with 55.67 q/ha being the average GY. The mean value and ranges for the studied traits, namely DA (54.56; 48–65), DS (54.75; 50–68), FW (1.12; 0.11-2.04), and GW (0.88; 0.03–1.56), show wide variation (Supplementary Table S4).

The results showed that environment is the major factor that influences the performance of the genotypes. The contribution of environment, genotypes and genotype-environment interaction in total variance ranged from 4.24

Table 1. Sum of squares oftraits studied and percentage contribution towards total variation

Source of variation	Environment		Genotype		GEI		Rep. (Env.)	Residuals
Degree of freedom	2		84		168		3	252
Traits	Sum squares	% (G+E+GEI)	Sum squares	% (G+E+GEI)	Sum squares	% (G+E+GEI)	Sum Squares	Sum squares
DA	75**	4.24	666.7**	37.76	1023.7**	57.99	10.7	867.3
DS	2495.3**	55.18	818.2**	18.09	1208.4**	26.72	23.2	1014.8
FW	60.86**	68.12	12.00**	13.43	16.48**	18.45	0.20	8.14
GW	37.55**	67.35	7.70**	13.8	10.50**	18.83	0.17	5.17
GY	29015**	29.83	33191**	34.12	35064**	36.05	29	1301

** p<0.01

to 68.12%, 13.43 to 37.76% and 18.45 to 57.99%, respectively (Table 1). All three components of the variance were statistically significant. Approximately equal proportions of genotype and genotype by environment interaction contributed to the total variance; together, they accounted for >60 percent. Therefore, it has become imperative to apply appropriate statistical tools like AMMI and GGE to identify stable genotypes across environments and/or location-specific genotypes to maximize yield potential and farmers' profitability. The present results are not an exception but are expected more often in multi-environment trials (MET). The underlying reasons are obvious due to variations in environmental factors like precipitation, temperature, and soil conditions. Balstre et al. (2009) also reported similar findings in maize. Rakshit et al. (2012) reported a 59.3–89.9% contribution of location to the total variation in sorghum. Kaya et al. (2006) reported that nearly 81% of the variation was explained by the environment in bread wheat multi-environment data. Kumar et al. (2023) reported highly significant differences for environments, genotypes as well as for Genotype × Environment interactions (G×E). Sum of squares due to G × E interactions was high, may be due to the large differences in environmental mean for yield. While doing barley yield studies in Iran, Dehghani et al. (2006) observed the same thing. Campbell and Jones (2005) reported environmental variation of 23, 14, and 13% for the traits gin torn out, fiber strength, and fiber elongation, respectively, and genotypic variation ranging from 2 to 84% in cotton. In the present study, G explained a relatively lower proportion of the variation than GE for all the traits under study. A lower proportion of G as compared to GE indicates that the performance of hybrids under evaluation relies more on environmental factors (Mohammadi et al. 2009). The environmental component for DS, FW, and GW was more than 50%, indicating that the environment highly influences the expression of these traits. Thus, it becomes inevitable to identify either stable genotypes across environments or the best-adopted genotypes for the particular environment to stabilize and/or enhance the yield levels across the environments (Table 1).

GEI analysis

AMMI ANOVA (Table 2) for grain yield confirmed that environment, genotype, and GEI had a statistically significant (p <0.001) effect. Environmental factors alone significantly affected about 29.83% of the total sum of squares (G + E + GEI) alone. The contribution of genotypic effects and GEI to the total sum of squares for grain yield was 34.12 and 36.05%, respectively. According to the results, PC1 accounted for roughly 64.6% and PC2 for approximately 35.4% of the GEI sum of squares (Table 2).

AMMI biplot

AMMI biplots were used to visually reflect the yield potential of the genotypes, stability levels, and interaction of test environments. Quantification of grain yield against PC1 scores (AMMI 1) provides insight into the interaction between QPM maize hybrids and test environments (Fig. 1A). Hazaribagh and Ludhiana, both located far from the origin and marked by long vectors, are regions of significant interaction, while Rahuri, located close to the origin and with shorter vectors, reveals very modest interaction. The superior genotype exhibits a higher yield along the horizontal axis, while IPC1, representing the first interaction item, has a minimum value and is near zero on the vertical axis. In addition to maximizing grain performance, it is important to consider genotypes that display stability. Stable genotypes are located close to this line, indicating minimal G×E interaction. In poor and weak locations, genotypes with below-average grain yield performance can still be recommended if they possess a positive value for IPC1.

The experimental hybrids, namely G85, G77, G84, G82, G131, G124, G34, and G50, positioned on the right side of the vertical axis and in proximity to the horizontal axis, clearly indicate their superior performance and stability across multiple locations. These hybrids consistently outperformed all three Quality Protein Maize (QPM) checks and the three normal corn checks in terms of mean grain yield. Seventeen hybrids are clustered closer to their points of origin, indicating that they are either widely adaptable

Table 2. AMMI analysis of variance for grain yield

Source	Df	SS	MSS	Total variation explained (%)	GEI contributed (%)
Environment	2	29015	14507.7	29.83	
Genotype	84	33191	395.13	34.12	
GEI	168	35064	208.71	36.05	
PC1	85	22667	266.67	-	64.6
PC2	83	12396	149.35	-	35.4
Residuals	252	1301	5.16	-	0.0

SS= Sum of squares, MSS= Mean sum of squares, and GEI= Genotype-environment interaction

or environmentally neutral, with normal to slightly above-average mean yields. In the AMMI2 biplot, the first two main components (PC1 and PC2) accounted for 66.5% and 33.5% of the total variation in GEI, respectively (Fig. 1B). The genotype-environment interaction was represented by projecting the genotype onto the environmental vector at right angles. The figure revealed that entries G73, G22, and G63 had poor stable performance across the environments despite having higher or lower grain yields. Quantification and classification of the genotypes were accomplished by calculating the yield stability index (YSI), which is a combination of the rank of AMMI's stability values and the mean grain yield (Table 3). To find the most productive and stable QPM hybrids, YSI was employed for the screening. The lower absolute PC1 scores were found to be the best predictor of genotype stability as reported earlier by Asfaw et al. (2009).

The hybrids with the lowest YSI (Yield Stability Index) ratings are the most robust and productive. According to rYSI, the best hybrids in terms of stability and grain yield were G77, G85, G120, G104, G82, G137, G84, G76, G117, G14 and G21, followed by checks G138, G140, and G139. Poor productivity and reduced stability were also noted in genotypes G16, and G63, followed by G29, and G11 (Table 3). The check hybrids, viz. G138 (Check DKC 9224), G139 (Check DKC 8221), and G140 (Check DKC 8211) with higher projection displayed greater variability, i.e., were not stable across the location and had strong interactions with the specific environments, suggesting the presence of higher variance among the hybrids in response to environmental conditions. This also implies the higher sensitivity of hybrids to the surrounding environmental conditions, leading to narrow adaptation (Ebdon and Gauch 2002).

The ranking based on the YSI ranked hybrids 77 and 85 as having the highest yield. Genotypic responses varied among locations due to differences in testing environments and genetic heterogeneity among the hybrids evaluated. Taking into account the interplay between the genotype and its environment, stability analysis techniques like GGE and AMMI are considered beneficial for maize breeders to identify high-yielding and stable hybrids.

GGE biplots

A graphical representation of the combined variance of genotype and genotype by environment would aid in the identification of location-specific genotypes in the event of the absence of stable genotypes. The GGE biplot helps to exploit both genotype and environmental variance. GGE analysis can be applied to maximize yield levels through the exploitation of GGE variance. Another part of the GGE biplot that helps find the best genotypes based on stability is the AEC ordinate, which is a thick solid line that runs perpendicular to the AEC and shows variability (poor stability) in both directions. The GGE biplots site regression (SREG) model was utilized to produce genotype and genotype plus environment (GGE) biplots, which account for genotype main effects and genotype by environment interaction (GEI) effects while excluding the influence of random error. As per GGE analysis, the first two principal components (PC1 with 63.23 percent and PC2 with 25.53 percent) account for 88.76 percent of the total variation in GEI (Fig. 1C). The GGE biplots of this study are ideal for which-won-where pattern analysis, genotype, and test environment evaluation. The data used in this study was environment-centered (centering = 2), not scaled (scale = 0), and all GGE biplots either used column singular value partitioning (SVP = 2) or genotypic-metric preserving (SVP = 1) (Yan et al. 2017; Yan 2002). The graphical representation of biplots also facilitates the interpretation of the interaction between testing environments through the angle between their vectors. The long environment vectors indicated the higher discriminatory power of environments (Mebratu et al. 2019). The line connecting the marker of an environment to the origin of the biplot represents the vector of that environment, and the cosine of the angle between two vectors indicates their correlation (Yan and Tinker 2006). Based on the cosine of the angle between the two environmental vectors, Yan (2002) concludes that this angle describes the relationship between the two. If the two angles were close together, it meant that the hybrids' performance in both environments was comparable; if they were perpendicular to one another, it meant that there was no correlation between the two environments,

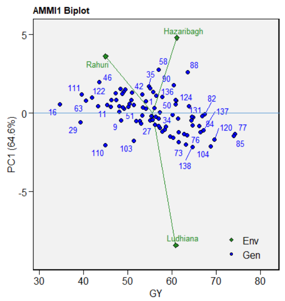

Fig. 1A. PC1 (AMMI 1) scores against grain yield

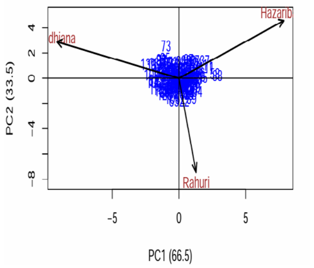

Fig. 1B. Scatter plot of IPC1 vs. IPC2 in AMMI

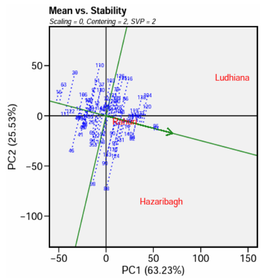

Fig. 1E. The average-environment coordination view

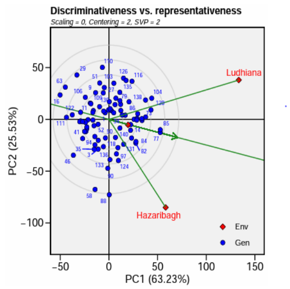

Fig. 1F. Discrimitivenes vs. representativenss of test environments

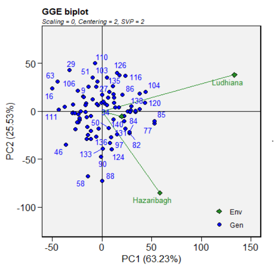

Fig. 1C. The environment-vector view of the GGE biplot

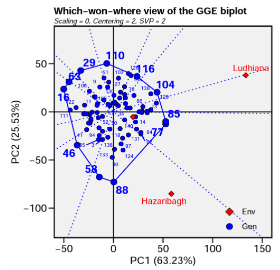

Fig. 1D. The which-won-where view of the GGEbiplot for grain yield

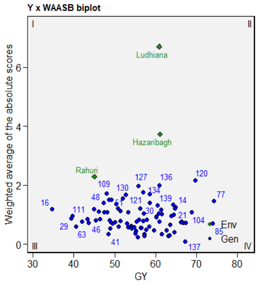

Fig. 1G. The interaction between mean grain yield and WAASB values (Y× WAASB biplot)

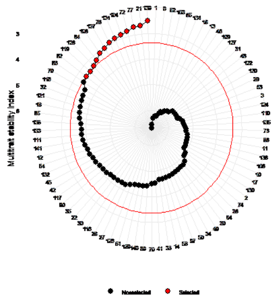

Fig. 1H. Genotypes with their respective MTSI values

and the performance of hybrids was negatively correlated with one another at obtuse angles. Analysis for the trait GY revealed acute vector angles representing all three locations: Hazaribag, Ludhiana, and Rahuri, as depicted in Fig. 1C. The angle between Rahuri and both Hazaribag and Ludhiana was found to be much smaller. Acute vector angles indicate a closer relationship between environments (Yan and Tinker, 2006). Therefore, all three locations exhibited a strong correlation. The closer relationships among different locations indicate the absence of crossover GE, indicating consistent genotype rankings across locations (Rao et al. 2011). This phenomenon can be attributed to the fact that some genotypes are highly responsive to environmental variation, while others display stability due to the combined properties of their gene combinations. Consequently, the three testing locations could be grouped into one mega-environment.

The strong correlations indicated that the test environments were similar in terms of genotype performance (Makumbi et al. 2015; Sserumaga et al. 2016). Many times, the biological association between different traits of economic importance and yield in particular, also needs to be considered during the selection of genotypes. Sometimes, there is a need to strike a balance between different traits. For example, earliness is often associated with reduced yields. However, earliness is an important trait for developing hybrids that fit different cropping systems and moisture-dense regions. Therefore, the objectives of breeding vary between different breeding programs. Therefore, breeding strategies also need to consider the selection of stable genotypes based on the performance of more than one desired trait. The utility of different stability measures like parametric, non-parametric, the AMMI stability model, and GGE biplots has been demonstrated and reported in several studies. However, as statistical techniques evolve, refinement of earlier techniques and/or the development of novel techniques will also continue.

Which-won-where and what

The "Which-won-where" pattern biplot is appropriate for the visualization of mega-environment through grouping of similar kinds of locations (Gauch and Zobel 1997; Badu-Aprakuet al. 2012; Abakemal et al.2016; Vaezi et al. 2019). This pattern (Fig. 1D) makes it possible to visually classify environments according to the degree to which they favor high-yielding genotypes that have been subjected to GEI. PC1 and PC2 accounted for 88.76% of the total variance, which was deemed to be adequate for model fitting and GGE biplot generation (Yang et al. 2009). The biplot displayed one mega-environment. The plot showed that hybrids G85 (DQL 2513 x DQL 2709) and G77 (DQL 2490 x DQL 2709) situated on the vertex were the winning hybrids in terms of mean grain yield in all three locations. The vertex hybrids in the sector with no environment were G88, G58, G46, G16, G63, G29, G110, G116, and G104. This means that these hybrids would be the lowest-yielding hybrids in any environment. Similar

Table 3. Mean grain yield (t/ha), AMMI stability values (ASV), multi-trait stability index (MTSI) and ranking orders of the 144 maize hybrids tested across three environments

Genotype	ASV	YSI	rASV	rYSI	Means	MTSI
G1	1.539511	86	35	51	54.06667	6.689037
G2	0.846714	58	11	47	55.145	4.999937
G3	2.335262	111	57	54	53.345	5.469082
G9	1.117982	89	20	69	48.56333	6.559716
G10	0.866852	88	12	76	46.165	5.14913
G11	0.818473	89	9	80	43.29167	5.187074
G12	1.503956	84	34	50	54.10667	3.878278
G13	2.186951	83	51	32	58.39667	5.970102
G14	0.579873	14	4	10	64.86667	4.64044
G16	1.195221	109	24	85	34.65667	6.037122
G17	1.291134	90	25	65	49.23333	5.145726
G18	0.578835	49	3	46	55.355	3.684689
G20	1.71152	116	41	75	46.38333	4.738691
G21	1.473566	44	33	11	64.745	2.66905
G22	3.148827	137	73	64	49.43333	4.207007
G26	1.135315	83	21	62	50.395	4.807964
G27	1.836663	98	43	55	53.03667	4.234409
G28	1.596404	110	37	73	47.38833	4.233139
G29	1.464658	115	31	84	39.28333	5.565816
G30	1.580718	67	36	31	58.63	4.212691
G31	1.140276	99	22	77	45.325	5.759109
G32	2.785201	85	66	19	62.73333	3.63679
G33	0.72708	74	7	67	48.87333	4.636651
G34	0.428621	30	2	28	60.175	4.703679
G35	3.096896	120	71	49	54.755	4.162184
G41	2.815669	138	68	70	48.43833	4.534372
G42	2.579432	123	63	60	50.98167	4.055155
G45	0.640468	43	6	37	57.06667	4.038354
G46	3.815794	159	80	79	43.59	5.967531
G48	2.487721	135	61	74	47.25833	5.606701
G49	2.457339	125	59	66	49.07667	4.727894
G50	1.008135	41	16	25	60.77667	4.702665
G51	2.108176	106	49	57	51.91833	4.373851
G52	1.450687	81	29	52	53.42667	6.315096
G54	0.762067	49	8	41	56.37833	3.94925
G58	5.243071	123	85	38	57.00833	4.655521
G63	2.786621	149	67	82	40.54333	5.564876
G65	1.666546	100	39	61	50.77167	6.211697
G70	1.436274	86	28	58	51.42833	4.513891
G72	1.142404	68	23	45	55.63333	2.788219
G73	4.325841	101	83	18	63.135	5.276836
G74	2.511025	130	62	68	48.77667	4.98078
G76	2.330746	64	56	8	66.415	2.935055
G77	2.709501	66	65	1	74.30833	2.695162

G79	2.227769	89	55	34	57.77333	3.602574
G82	0.831716	15	10	5	67.45	3.363178
G84	1.025033	24	17	7	66.86167	3.224243
G85	2.886333	71	69	2	73.93833	3.727558
G86	2.190221	73	52	21	61.53667	5.271492
G88	4.976965	100	84	16	63.51667	3.39963
G89	0.28173	54	1	53	53.35667	4.40632
G90	3.336797	103	77	26	60.46667	4.140853
G94	2.91341	118	70	48	55.045	4.744848
G97	2.183305	86	50	36	57.255	4.667851
G103	3.200684	135	76	59	51.41	6.236182
G104	3.84869	85	81	4	68.82833	2.866424
G106	1.916037	116	45	71	48.11667	5.157917
G108	2.00123	81	46	35	57.30667	3.087001
G109	0.638891	77	5	72	48.05333	5.846348
G110	3.729447	157	79	78	44.92333	5.362152
G111	2.199473	136	53	83	39.59667	3.831392
G116	3.514143	98	78	20	61.57833	4.223724
G117	1.465828	41	32	9	66.205	4.123885
G118	2.102953	88	48	40	56.48333	3.61344
G119	2.694369	81	64	17	63.48167	3.358699
G120	3.106808	75	72	3	69.67833	5.583844
G121	1.63614	77	38	39	56.95	3.66212
G122	1.87242	125	44	81	41.95667	5.601771
G124	2.210065	77	54	23	60.935	5.389776
G126	3.153688	103	74	29	59.66	3.316651
G127	2.463546	104	60	44	55.81333	5.77591
G128	1.073279	40	18	22	61.40333	4.308008
G129	1.3531	68	26	42	56.16667	4.380266
G130	1.459514	86	30	56	52.69333	5.131184
G131	0.954975	29	14	15	63.91333	2.90542
G132	0.964989	78	15	63	50.11833	3.970279
G133	2.454739	91	58	33	57.77667	3.824587
G134	1.681497	70	40	30	58.64667	6.200345
G135	3.163042	102	75	27	60.26833	5.332132
G136	1.831972	66	42	24	60.90667	5.172828
G137	2.070273	53	47	6	67.18833	3.044627
G138	4.009454	94	82	12	64.665	3.80833
G139	0.871491	27	13	14	64.34	2.506993
G140	1.093984	32	19	13	64.49167	4.385314
G141	1.395541	70	27	43	56.00333	3.859653

kinds of studies were conducted by Yan and Rajcan (2002) for the identification of mega-environments. Samonte et al. 2005, Yanet al.2001 and Laurie and Booyse (2015) all reported that the SREG model can make a biplot pattern that shows yield and stability in two dimensions using PCA1 and PCA2 scores. The present study corroborates previous studies showing that the GGE biplot methodology can be used to identify stable and high-yielding maize hybrids (Alwala et al. 2010; Oyekunle et al. 2017).

Mean grain yield and stability of hybrids

Another part of the GGE biplot that helps find the best genotypes based on stability is the AEC ordinate, which is a thick solid line that runs perpendicular to the AEC and shows variability (poor stability) in both directions. In order to visualize the hybrids' mean grain yield and stability, an AEC representation of the biplot was constructed (Fig. 1E). Genotypes' yield was indicated by the AEC abscissa (shown as a single arrowed line) pointing towards a higher mean yield. Thus, hybrids G77 and G85 had maximum yield stability across environments, followed by G137, G84, G76, G117, G14, G21, G138, G139, while G16, G63, followed by G29, and G111 were recorded as least yielders. The AEC ordinate, which was a thick, solid line perpendicular to the AEC, indicated more variability (poor stability) in both directions. Thus, hybrids G77 and G85, with minimal projection from the AEC abscissa, were highly stable in terms of mean grain yield across the location. These two hybrids were outperforming all eight checks. The check hybrids, viz., G138 (Check DKC 9224), G139 (Check DKC 8221), and G140 (Check DKC 8211), with higher projection, displayed greater variability, i.e., were not stable across the location. The "discrimination and representativeness" approach (Fig. 1.F) provides a handy visual for analyzing the degree to which each of the three testing environments can be distinguished from one another. In a study carried out by Singh et al. 2024, testing of 24 single cross hybrids and four checks across three environments was done, which revealed the significant GEI for all the studied traits. Both GGE biplot and AMMI analysis revealed the three high-yielding hybrids with average stability.

Multi-trait stability index (MTSI)

All of the attributes assessed in the study indicated strong ($p< 0.05$) GEI, as indicated by the p-values for likelihood ratio tests of the traits. Recently, Olivoto et al. (2019a) calculated a weighted average of absolute scores (WAAS) using linear mixed models to characterize the stability of genotypes across a range of environmental conditions. The computation of WAASB (stability alone) and WAASBY (stability and all other examined features) allowed simultaneous selection based on all the traits studied or traits of absolute necessity**.** For the WAASBY values, a Pearson's correlation matrix was calculated, and the retrieved high-magnitude interactions were categorized as common factors. Extracting WAASBY values from each individual character enabled the classification of seven attributes into three factors (FA). Under FA1, the characteristics associated with FW, GW, and GY were categorized; under FA2, PH and EH were grouped; and under FA3, DA and DS were classified (Table 4). EPA scores were used for selecting individuals based on their genotype and ideotype distance (Euclidian). Table 4 presents the mean performance and WAASBY index selection differential (SD) for the component traits. The WAASBY index had a positive standard deviation for all traits, and its mean SD% was 7.21%, with the DA and DS having the lowest (-0.82 and -0.73%) and the GY having the highest (16.08%). The mean performance of the traits, DA and DS, had a negative SD% (Table 4).

The study grouped the genotypes into two different categories based on the interaction between mean grain yield and WAASB values (Y × WAASB biplot), facilitating the simultaneous selection of genotypes with optimized stability and mean performance (Fig. 1G). Y x WAASB biplot approach grouped the genotypes into four categories, each represented by a separate quadrant (Fig.1.G). The approach was adopted in the present study, and the approach proved effective in choosing the most productive and stable genotypes, as evidenced by the positive SD% of WAASBY scores for the traits FW, GW, and GY and the anticipated negative SD% for the mean performance of the flowering traits, viz., DA and DS (Table 4). One environment (Ludhiana) was plotted in quadrant II of the biplot with no genotype, while Hazaribagh was in quadrant IV with 36 genotypes and Rahuri in quadrant III with 49 genotypes. The WAASBY

Table 4. Selection differential for the waasby

	VAR	Factor	Xo	Xs	SD	SDperc	h2	SG	SGperc	Sense
1	FW	FA 1	1.121149	1.287538	0.166389	14.84097	0.313774	0.052209	4.656717	Increase
2	GW	FA 1	0.877816	1.012756	0.134941	15.37233	0.3172	0.042803	4.876096	Increase
3	GY	FA 1	55.67104	64.62551	8.954474	16.08462	0.471784	4.22458	7.588469	Increase
4	PH	FA 2	185.6657	190.2859	4.620211	2.488457	0	0	0	Increase
5	EH	FA 2	98.41529	101.6513	3.235988	3.288095	0	0	0	Increase
6	DA	FA 3	54.56471	54.11538	-0.44932	-0.82347	0.232258	-0.10436	-0.19126	Decrease
7	DS	FA 3	54.74902	54.34615	-0.40287	-0.73584	0.261554	-0.10537	-0.19246	Decrease

FW= Field weight, GW= Grain weight, GY= Grain yield, PH= Plant height, EH= Ear height, DA= Days to 50% anthesis, DS= Days to 50% silking,Xo= Original population mean, Xs= Mean of selected genotypes, SD= Selection differential, h2= heritability, SDperc= Selection differential percentage, SG= Selection gain, SGperc= Selection gain percentage, and Sense= Desired selection sense index was used in conjunction with an LMM to perform a unified selection for both average performance and stability. The genotypes and environments that fall under quadrant I are those that are most likely to change over time, have a high discrimination ability and have lower productivity than the grand mean. There were unstable genotypes in Quadrant II, but their grain yield was above the mean. Because of their high grain yields and discriminatory abilities, the environments located in this sector demand special attention. Quadrant III genotypes are less productive than the grand mean, but they are more likely to remain stable due to their lower WAASB levels. The environments shown in this quadrant were seen as being under-whelming productive and lacking in discrimination abilities. Due to the high magnitude of productivity and strong stability performance as projected lower values of WAASB, the genotypes included in quadrant IV are widely adapted and have a high degree of productivity. In the present study, 36 hybrids fell in quadrant IV, depicting these hybrids as having higher productive ability and stable across the location.

The most stable hybrids with the highest mean performance across all assessed traits were selected at a selection intensity of 15%, as indicated by lower MTSI values. Genotypes viz., G139, G21, G77 followed by G72, G104, G131, G76, G137, G108, G84, G126, G119 were selected as the best hybrids. Selection intensity placed genotype G82 (DQL 2513 × DQL 2691) with MTSI 3.36 at the cut point (Fig. 1.H; red circle). The hybrids G1, followed by G9, G52, and G103, were identified with a high MTSI value reflecting sub-par performance and weaker stability (Fig. 1.H, genotypes nearer to the origin). In a study carried out by Patel et al. (2023), the performance of 45 sweet corn genotypes were assessed under three environments and the multi-trait stability index identified seven genotypes with higher phenotypic stability and mean performance for all interactive traits.

The primary benefit of MTSI's simultaneous selection for mean performance and stability is the capacity to fine-tune weights and rescale across traits in accordance with the breeder's needs. Based on their correlations, the exploratory factor analysis identified the number of latent variables or constructs that can be reduced to common factors (Ullman 2006). The estimation of final factor scores then enabled the management of multi-collinearity (Olivoto et al. 2017). When compared to commonly used indices, the WAASBY is unambiguous, and weights can be applied to prioritize mean performance above stability while selecting genotypes. It was determined that YSI stability measure is significantly correlated with other stability measures such as Eberhart and Russell's (S2 di), Wricke's (Wi), and Shukla, while Finlay and Wilkinson's (bi) and Linn and Binns' (Pi) correlation with any of the other approaches was only moderate (Purchase et al. 2000). Previous works that assessed the stability and mean performance of genotypes, taking into account a number of variables, could have gained from this approach, including intra-trait interactions (GET biplots) and yield × trait interactions (GYT biplots) (e.g., Yan and Fregeau-Reid 2018; Kizilgeci et al. 2019; Kendal 2019; Koundinya et al. 2019; Nduwumuremyi et al. 2017; Bocianowski et al. 2019). Thirteen best entries were selected with 15% selection intensity based on low MTSI values, which were selected as best hybrids. The ranking based on the YSI ranked hybrids G77 and G85 as having the highest yielders and surpassed commercial checks also. Considering the genotype x environment interaction, the selected hybrids may be tested under AICRP for further release and commercial cultivation.

Supplementary material

Supplementary Tables S1 to S4 are provided, which can be accessed at www.isgpb.org

Authors' contribution

Conceptualization of research (RK, SR); Designing of the experiments (RK, AKD, SK); Contribution of experimental materials (RK); Execution of field/lab experiments and data collection (RK, YK, SS, SK, SD); Analysis of data and interpretation (SK, CGK, RK, SR); Preparation of the manuscript (RK, AKD, SK).

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Supplementary Table S1. List of the checks used in the study

S. No.	Name of Hybrid	Grain type	Grain Colour	Year of release	Developing institution
1.	Bio 9544	Semi-dent	Orange-yellow	2014	Bioseeds India Ltd.
2.	DKC 7404	Semi-dent	Orange-yellow	2020	Bayer crop Sciences
3.	DKC 8211	Semi-dent	Orange-yellow	2021	Bayer crop Sciences
4.	DKC 8221	Semi-dent	Orange-yellow	Research Hybrid	Bayer crop Sciences
5.	DKC 9224	Semi-dent	Orange-yellow	Research Hybrid	Bayer crop Sciences
6.	IQPMH 1601 (IQMH 202)	Flint	Orange	2020	ICAR-IIMR, Ludhiana
7.	IQPMH 1705 (IQMH 203)	Flint	Orange	2020	ICAR-IIMR, Ludhiana
8.	IQPMH 18-2	Flint	Orange	Research Hybrid	ICAR-IIMR, Ludhiana

Supplementary Table S2. Details of inbred lines used for making crosses

S. No.	Inbred Line	Pedigree/source population	Developing institution	Grain type (flint/ dent/semi-dent)	Colour of the kernel (orange/yellow/white)	Heterotic Group (A/B/AB)
1.	DQL-2011	HKI-193-23-D-1-1-1-1	ICAR-IIMR, Ludhiana	Semi-dent	Orange	A
2.	DQL-2021-1	HKI-5072-2-2BT	ICAR-IIMR, Ludhiana	Flint	Orange	A
3.	DQL-2064	Seed Tech 2324	ICAR-IIMR, Ludhiana	Flint	Orange	A
4.	DQL-2099-1	HQPM-7	ICAR-IIMR, Ludhiana	Flint	Orange	A
5.	DQL-2167	Temp x Trop (Ho)QPM-23-BBB-1-1-1	ICAR-IIMR, Ludhiana	Flint	Orange	A
6.	DQL-2192	Su2Su2O2O2 Comp-R-BBB-27-B-B-1-1	ICAR-IIMR, Ludhiana	Flint	Orange	A
7.	DQL-2246	WNCQPM-10343-1	ICAR-IIMR, Ludhiana	Flint	Orange	A
8.	DQL-2290-4	DML-1230-1	ICAR-IIMR, Ludhiana	Flint	Orange	A
9.	DQL-2306	DML-2062	ICAR-IIMR, Ludhiana	Flint	Orange	A
10.	DQL-2325	CLQRCY 47-3-1-2-1-1	ICAR-IIMR, Ludhiana	Flint	Orange	A
11.	DQL-2370	DQL 2248 x DQL 2208-P-57	ICAR-IIMR, Ludhiana	Flint	Orange	A
12.	DQL-2452	DQL 2248 x DQL 2184-P-127	ICAR-IIMR, Ludhiana	Flint	Orange	A
13.	DQL-2471	DQL 2164 x DQL 2180-P -68	ICAR-IIMR, Ludhiana	Flint	Orange	A
14.	DQL-2487	DQL 2024 x DQL 2208-P -52	ICAR-IIMR, Ludhiana	Flint	Orange	A
15.	DQL-2490	DQL 2024 x DQL 2208 P-82	ICAR-IIMR, Ludhiana	Flint	Orange	A
16.	DQL-2513	DQL -621-1-1-4-13-1	ICAR-IIMR, Ludhiana	Flint	Orange	A
17.	DQL-2543	DQL 2054 x DQL 2231-P-57	ICAR-IIMR, Ludhiana	Flint	Orange	A

18.	DQL-2545	DQL 2054 x DQL 2231-P-60	ICAR-IIMR, Ludhiana	Flint	Orange	A
19.	DQL-2631	DQL 2054 x DQL 2096-P-76	ICAR-IIMR, Ludhiana	Flint	Orange	A
20.	DQL-2642	DQL 2054 x DQL 2096-P-117	ICAR-IIMR, Ludhiana	Flint	Orange	A
21.	DQL-2646	DQL 2063 x DQL 2231-P-14	ICAR-IIMR, Ludhiana	Dent	Orange	A
22.	DQL-2668	DQL 2063 x DQL 2231-P-122	ICAR-IIMR, Ludhiana	Flint	Orange	A
23.	DQL-2691	VL-174583	ICAR-IIMR, Ludhiana	Flint	Orange	B
24.	DQL-2698	VL-174593	ICAR-IIMR, Ludhiana	Dent	Orange	B
25.	DQL-2699	VL-174593-1	ICAR-IIMR, Ludhiana	Flint	Orange	B
26.	DQL-2700	VL-174596	ICAR-IIMR, Ludhiana	Flint	Orange	B
27.	DQL-2709	VL-174620-1	ICAR-IIMR, Ludhiana	Flint	Orange	B
28.	DQL -2743	VL-174877	ICAR-IIMR, Ludhiana	Flint	Orange	B
29.	DQL-2751	VL-174931	ICAR-IIMR, Ludhiana	Semi dent	Orange	B
30.	DQL-2753	VL-174932	ICAR-IIMR, Ludhiana	Flint	Orange	B

Supplementary Table S3. **List of the genotypes used in the study**

	Genotypes			Genotypes			Genotypes	
1	G1	DQL 2011 x DQL 2700	31	G49	DQL 2325 x DQL 2698	61	G111	DQL 2642 x DQL 2699
2	G2	DQL 2011 x DQL 2709	32	G50	DQL 2325 x DQL 2699	62	G116	DQL 2646 x DQL 2700
3	G3	DQL 2011 x DQL 2751	33	G51	DQL 2325 x DQL 2700	63	G117	DQL 2646 x DQL 2709
4	G9	DQL 2021-1 x DQL 2743	34	G52	DQL 2325 x DQL 2709	64	G118	DQL 2646 x DQL 2743
5	G10	DQL 2021-1 x DQL 2753	35	G54	DQL 2325 x DQL 2751	65	G119	DQL 2646 x DQL 2751
6	G11	DQL 2064 x DQL 2700	36	G58	DQL 2370 x DQL 2691	66	G120	DQL 2646 x DQL 2753
7	G12	DQL 2064 x DQL 2709	37	G63	DQL 2452 x DQL 2691	67	G121	DQL 2668 x DQL 2698
8	G13	DQL 2064 x DQL 2751	38	G65	DQL 2471 x DQL 2691	68	G122	DQL 2668 x DQL 2699
9	G14	DQL 2099-1 x DQL 2691	39	G70	DQL 2487 x DQL 2691	69	G124	DQL 2668 x DQL 2743
10	G16	DQL 2099-1 x DQL 2700	40	G72	DQL 2487 x DQL 2700	70	G126	DQL 2668 x DQL 2753
11	G17	DQL 2099-1 x DQL 2709	41	G73	DQL 2487 x DQL 2709	71	G127	CLQRCY 40 x DQL 2698
12	G18	DQL 2099-1 x DQL 2751	42	G74	DQL 2487 x DQL 2751	72	G128	CLQRCY 40 x DQL 2699
13	G20	DQL 2167 x DQL 2691	43	G76	DQL 2490 x DQL 2700	73	G129	CLQRCY 40 x DQL 2700
14	G21	DQL 2167 x DQL 2698	44	G77	DQL 2490 x DQL 2709	74	G130	CLQRCY 40 x DQL 2709
15	G22	DQL 2167 x DQL 2699	45	G79	DQL 2490 x DQL 2751	75	G131	CLQRCY 40 x DQL 2743
16	G26	DQL 2192 x DQL 2691	46	G82	DQL 2513 x DQL 2691	76	G132	CLQRCY 40 x DQL 2751
17	G27	DQL 2192 x DQL 2698	47	G84	DQL 2513 x DQL 2700	77	G133	CLQRCY 40 x DQL 2753

18	G28	DQL 2192 x DQL 2699
19	G29	DQL 2192 x DQL 2700
20	G30	DQL 2192 x DQL 2709
21	G31	DQL 2192 x DQL 2743
22	G32	DQL 2192 x DQL 2751
23	G33	DQL 2192 x DQL 2753
24	G34	DQL 2246 x DQL 2698
25	G35	DQL 2246 x DQL 2699
26	G41	DQL 2290-4 x DQL 2698
27	G42	DQL 2290-4 x DQL 2699
28	G45	DQL 2306 x DQL 2698
29	G46	DQL 2306 x DQL 2699
30	G48	DQL 2325 x DQL 2691
48	G85	DQL 2513 x DQL 2709
49	G86	DQL 2513 x DQL 2751
50	G88	DQL 2543 x DQL 2691
51	G89	DQL 2543 x DQL 2698
52	G90	DQL 2543 x DQL 2699
53	G94	DQL 2545 x DQL 2691
54	G97	DQL 2572 x DQL 2691
55	G103	DQL 2631 x DQL 2698
56	G104	DQL 2631 x DQL 2699
57	G106	DQL 2631 x DQL 2743
58	G108	DQL 2631 x DQL 2753
59	G109	DQL 2642 x DQL 2691
60	G110	DQL 2642 x DQL 2698
78	G134	Check IQPMH 1601
79	G135	Check IQPMH 1705
80	G136	Check IQPMH 18-2
81	G137	Check Bio 9544
82	G138	Check DKC 9224
83	G139	Check DKC 8221
84	G140	Check DKC 8211
85	G141	Check DKC 7204

Supplementary Table S4. **Relative dissection of the studied traits across the three environments**

S. No.	Parameters	DA	DS	PH	EH	FW	GW	GY
1.	Mean	54.56	54.75	185.67	98.42	1.12	0.88	55.67
2.	SE	0.1	0.15	0.75	0.56	0.02	0.02	0.62
3.	SD	2.28	3.3	16.98	12.65	0.44	0.35	13.9
4.	CV	4.18	6.04	9.15	12.87	39.08	39.46	25
5.	Min	48 (21 in Ludhiana)	50 (85 in Ludhiana)	130 (103 in Rahuri)	52 (136 in Ludhiana)	0.11 (88 in Ludhiana)	0.03 (88 in Ludhiana)	24.3 (110 in Rahuri)
6.	Max	65 (1 in Ludhiana)	68 (2 in Ludhiana)	252.3 (110 in Ludhiana)	141.3 (76 in Ludhiana)	2.04 (126 in Rahuri)	1.56 (128 in Rahuri)	92.5 (104 in Ludhiana)
7.	MinENV	Hazaribagh (54.02)	Hazaribagh (51.62)	Rahuri (181.56)	Ludhiana (96.14)	Ludhiana (0.72)	Ludhiana (0.55)	Rahuri (45)
8.	MaxENV	Rahuri (54.86)	Rahuri (56.42)	Ludhiana (188.04)	Rahuri (102.01)	Rahuri (1.56)	Rahuri (1.21)	Hazaribagh (61.11)
9.	MinGEN	86 (52)	86 (52.17)	86 (171.38)	103 (87.78)	29 (0.79)	29 (0.61)	16 (34.66)
10.	MaxGEN	1 (58)	11 (58.83)	21 (196.73)	51 (109.03)	77 (1.55)	77 (1.21)	77 (74.31)