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Stability analysis in quality protein maize (*Zea mays*) by Eberhart and Russell model, and GGE biplots

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ABSTRACT

An experiment was conducted during winter (*rabi*) 2019–20, 2020–21 and rainy (*kharif)* season of 2021 at Bihar Agricultural University, Sabour, Bihar to study the stability in Quality Protein Maize (QPM) (*Zea mays* L.). A total of 50 QPM inbred lines were screened during *rabi* 2019–20 out of which 14 inbred lines and 3 testers were selected as the promising genotypes. These lines and testers were hybridized to generate 42 crosses utilizing the line × tester fashion. The 61 genotypes (42 crossings, 14 lines, 3 testers and 2 checks) were assessed in three distinct environments viz. early *kharif* (sown on May 15), E_1; *kharif* (sown on June 30), E_2; and late *kharif* (sown on August 15), E_3 using a randomized complete block design (RCBD) with three replications. The data were recorded for 18 morphological and biochemical traits to draw conclusions on stability analysis using Eberhart and Russell model, and GGE biplots. The Eberhart and Russell model's estimations of stability study for grain yield showed that 7 hybrids, viz. $L_5 \times T_3$, $L_6 \times T_2$, $L_6 \times T_3$, $L_7 \times T_1$, $L_{13} \times T_3$, $L_{14} \times T_1$ and $L_{14} \times T_2$ were stable in a range of environmental circumstances. Similarly, using GGE biplots three hybrids ($L_5 \times T_3$, $L_6 \times T_3$ and $L_{13} \times T_3$) were found as the stable ones and the late *kharif* environment ranked the best for identifying the high-yielding genotypes.

Keywords: Crosses, Eberhart and Russel model, Environment, GGE biplots, QPM, Stability

Maize (*Zea mays* L.) falls within the Maydeae tribe of the Poales order, Poaceae family, Panicoideae subfamily, and *Zea* genus (Perera *et al*. 2014). It holds the third position in production, following wheat and rice. It is called the "Queen of cereals" because of its remarkable capacity for high productivity and its ability to thrive in various environmental conditions (Wang *et al*. 2018). Maize is grown in almost all 28 states of India, but 60% of its area is concentrated in 7 major states including Bihar as one of the major maize-producing state in the country, holding about 28.8% of total Indian maize production (Singh *et al.* 2023). It is cultivated in three seasons, viz. *kharif, rabi* and *zaid* and a total production of 3.19 million tonnes with productivity of 4771 kg/ha was recorded during 2018–19 (IIMR 2020). Although the average productivity of Bihar is higher than the national average, there is still room to raise maize production to a global level (Kumar and Singh 2017). Apart from aiming for high yields, newly designed hybrids must also exhibit consistent performance and adaptability across diverse environmental conditions (Nyirenda *et al*. 2021). While assessing new hybrids across multiple locations, their performance for a specific trait seldom remains uniform, posing challenges in identifying consistently superior genotypes. An essential prerequisite for effective selection lies in the establishment of a dependable method for estimating stability (Leon *et al*. 2016). The Eberhart and Russell model is an older method, whereas GGE biplots represent a more modern and comprehensive approach. Both methods are used to assess genotype × environment interaction (GEI) as well as the stability of genotypes across environments, but they differ in terms of their complexity and insights they provide. The GGE biplots (Genotype plus Genotype-by-Environment interaction biplots) are advanced graphical tools that offer a visual representation of complex genotype-environment relationships (Yan *et al*. 2007). It explains both the main effects of genotypes and the genotype-by-environment interactions, allowing for a more comprehensive assessment of stability and adaptability (Yan and Hunt 2002), identifying specific mega-environments and the genotypes that performs best in each mega-environment. Keeping these points in view an experiment was planned for stability analysis in quality protein maize by Eberhart and Russell model, and GGE biplots.

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MATERIALS AND METHODS

An experiment was conducted during winter (*rabi*) 2019–20*,* 2020–21 and rainy (*kharif)* season of 2021 at Bihar Agricultural University, Sabour (25° 7'– 25° 30' N and 86° 37'–87° 30' E and at an altitude of 42.9 m amsl), Bihar. Total of 50 Quality Protein Maize (QPM) lines were screened for diverse morphological and quality traits during *rabi* 2019–20. The better performing 14 lines (females) and 3 testers (males) were selected as parents and crossing was performed in line × tester mating design during *rabi* 2020–21. Each entry of lines and testers was sown in plant-to-plant spacing of 25 cm and row-to-row spacing of 75 cm in four rows in a crossing block. The harvesting of the crosses [i.e. lines (14) × testers (3) = 42 crosses] and the parents was done separately. The suggested practices were done to raise a good maize crop. In *kharif* 2021, 42 F_1s were examined with 14 lines, 3 testers and 2 checks. The evaluation was performed in a randomized block design (RBD) with 3 replications in 3 dissimilar sowing dates. The environmental conditions were, early *kharif* (Date of sowing: 15th May, 2021) E_1; normal *kharif* (30th June, 2021), E_2; and late *kharif* (15th August, 2021), E_3. The morphological traits, viz. days to 50% tasselling as well as days to 50% silking were studied when 50% of plants in the individual plot have begun to emerge tassel and silk, respectively. Anthesis-silking interval was estimated as the difference between anthesis and silking. Days to 75% brown husk was recorded when 75% of plants in a plot display brown husk ear. Plant height was taken as a measurement in centimetres from the ground to the tip of five representative plants and ear height was measured as the average height of the representative plants from the ground to the uppermost node-bearing cob. Cob length was recorded after removing the husk cover and measuring from base to the tip. Cob girth was measured with a thread in the middle of dehusked cob. Kernel rows per cob and kernels per row were recorded as the count of kernels rows along with the number of kernels in a row, respectively in the cobs of representative plants. 1000-grain weight was measured as average from five representative plants of each plot. With 15% grain moisture, the grain yield (kg/ha) was calculated using a conventional process and method.

$$\text{Yield (kg/ha)}=\left[\frac{(100-\text{M})\times\text{Cob weight}\times 0.9411}{100}\right]\left[\frac{10000}{\text{Plot size}}\right]$$

where, M, Moisture percent of grains for every plot measured with a digital moisture meter; Cob weight, Ears from each plot were weighed (kg); Plot size, 4.8 m^2; 0.9411 is constant to adjust yield at 15% moisture level.

Apart from 12 morphological traits, five biochemical traits were studied in the experiment. Kernel starch content (%) was calculated by the anthrone reagent method (Hodge *et al*. 1962). Kernel protein content (%) was estimated by calculating the nitrogen content through standard micro Kjeldahl method (AOA 2001.11) following multiplication with a factor of 6.25. Kernel oil content (%) was extracted through ultrasound-assisted oil extraction method (Li *et al*. 2016) with some modifications. Tryptophan content (%) was calculated using papain hydrolysis method (Hernandez and Bates 1969) and the lysine content (%) in kernel protein was calculated by multiplying four into the percent tryptophan in kernel protein. Kernel vitreocity (%) was recorded by using torchlight, for which kernels were placed against a bulb, on glass. The passage of light through kernels was graded from 1–5 scale where 1=1–20%; 2=20–40%; 3=40–60%; 4=60–80%; and 5=80–100%.

The procurement of all 14 lines and 3 testers was carried out from International Wheat and Maize Improvement Centre (CIMMYT), Hyderabad, India. The 14 lines were VL109404, VL109353, VL1017524, VL109359, VL109584, VL1016010, VL1016951, VL109282, VL1016416, VL1016422, VL111366, VL192367, VL121100 and VL109378. The 3 testers were VL192366, VP-191, VQL-1 and the two checks were HQPM-5 and VQPM-9. The ANOVA table (using Eberhart and Russel model) was generated with Window stat software and the GGE biplot analysis was performed using PB Tools 2013 software.

RESULTS AND DISCUSSION

For every character under study, it was discovered that the mean squares resulting from environment (linear) and genotypes were considerably significant. With the exception of traits, viz. kernel protein content, tryptophan content, and lysine content, the mean squares resulting from genotypes, environment + genotype × environment, were shown to be highly significant for every attribute examined. Similarly, for every character under study, the mean squares resulting from genotypes × environments (linear) were extremely significant, with the exception of the anthesis silking interval, tryptophan content, and lysine content. The characters with the most statistical significance determined to have mean squares resulting from pooled deviation were anthesis silking interval, ear heightand 1000-grain weight (Table 1). These results are in close conformity with the findings of Kumari (2018), Oliveira *et al*. (2019), Singh *et al*. (2021) and Swapnil *et al*. (2021).

A variety is defined as a stable variety if it is having high mean yield in the desirable direction, regression coefficient (bi≃1) and minimum deviation from regression (S^2di = 0). Here, bi measures the varietal response to the environments and S^2di measures its stability. Not every environment is equally conducive to the genotypes grown in it performing well. The variation in performance of 61 genotypes (14 lines, 3 testers, 42 crosses, and 2 checks) for grain yield and its attributes were observed and evaluated over 3 environmental conditions. The study of stability parameters for grain yield (Table 2) was performed using Eberhart and Russel model which indicated that 7 hybrids, viz. $L_5 \times T_3$, $L_6 \times T_2$, $L_6 \times T_3$, $L_7 \times T_1$, $L_{13} \times T_3$, $L_{14} \times T_1$ and $L_{14} \times T_2$ displayed non-significant deviation from regression and regression coefficient nearly equal to unity with higher mean values than the population mean. The hybrids which showed consistent performance across environments for traits associated with grain yield were $L_5 \times T_3$ for traits like kernel rows per ear,

Table 1 Analysis of variance for stability of 18 quantitative characters in QPM

Sources of variation	Genotype	E + (G × E)	Environments (Linear)	G × E (Linear)	Pooled deviation	Pooled error
Df	60	122	1	60	61	360
Days to 50% tasseling	11.30**	44.98**	5306.07**	2.49**	0.54	5.44
Days to 50% silking	9.449**	36.08**	4155.74**	2.56*	1.51	6.27
Anthesis silking interval (days)	1.10**	0.97**	70.26**	0.38	0.41**	0.03
Days to 75% brown husk	8.69**	82.54**	9651.73**	5.54**	1.4	13.1
Plant height (cm)	458.98**	201.37**	24037.18**	6.66**	2.14	38.37
Ear height (cm)	478.44**	326.83**	34909.39**	68.23**	14.26**	8.44
Kernel rows/ ear	21.35**	12.61**	1457.10**	0.90**	0.44	0.35
Kernels/row	77.37**	26.68**	3031.92**	3.23**	0.47	0.85
1000-grain weight (g)	14485.62**	650.66**	40403.91**	478.01**	168.78**	97.39
Cob length (cm)	20.31**	7.06**	770.53**	1.11**	0.406	0.372
Cob girth (cm)	6.61**	1.66**	169.79**	0.41**	0.13	0.23
Grain yield (kg/ha)	12315730.00**	379330.00**	30144550.00**	229866.70**	38388.71	29719.98
Kernel starch content (%)	34.66**	1.99*	5.12*	2.77**	1.18	4.78
Kernel oil content (%)	0.54**	0.06**	5.94**	0.02**	0.01	0.02
Kernel protein content (%)	0.36**	0.00	0.01**	0.00	0.00	0.11
Tryptophan content (%)	0.01**	0.00	0.01**	0.00	0.00	0.01
Lysine content (%)	0.23**	0.01	0.11**	0.01	0.01	0.01
Kernel vitreosity (%)	0.28**	0.09**	10.49**	0.00*	0.00	0.02

*, ** significance at 5% and 1% level of probability. E, Environment; G, Genotype; QPM, Quality Protein Maize.

Table 2 Estimation of mean and stability parameters for grain yield in QPM crosses

Genotype	Mean	bi	S^2di	Genotype	Mean	bi	S^2di
$L_1 \times T_1$	3721.93	3.43	238705	$L_8 \times T_1$	5171.81	1.26	-45432
$L_1 \times T_2$	6127.29	1.82	72280	$L_8 \times T_2$	7238.31	2.23	39204
$L_1 \times T_3$	5599.55	0.79	-47012	$L_8 \times T_3$	5521.79	0.52	-21638
$L_2 \times T_1$	5816.20	0.46	21544	$L_9 \times T_1$	5771.76	1.37	-12574
$L_2 \times T_2$	7388.29	2.15	-28972	$L_9 \times T_2$	5549.56	0.83	15555
$L_2 \times T_3$	6210.67	0.18	-44858	$L_9 \times T_3$	5344.02	1.18	-18696
$L_3 \times T_1$	4360.76	1.35	645612	$L_{10} \times T_1$	5432.90	1.43	-1071
$L_3 \times T_2$	4671.85	1.29	13692	$L_{10} \times T_2$	5582.88	0.74	-45141
$L_3 \times T_3$	4977.36	0.64	-46249	$L_{10} \times T_3$	8144.16	2.30	-46762
$L_4 \times T_1$	5144.98	0.42	-42385	$L_{11} \times T_1$	6060.62	0.84	-46159
$L_4 \times T_2$	5449.56	2.26	-22220	$L_{11} \times T_2$	6338.38	1.71	-44051
$L_4 \times T_3$	4429.84	0.47	-31115	$L_{11} \times T_3$	5864.13	0.39	-47008
$L_5 \times T_1$	5921.75	1.39	121522	$L_{12} \times T_1$	7382.74	1.36	-47114
$L_5 \times T_2$	5432.71	0.39	-27633	$L_{12} \times T_2$	6959.28	0.84	-47199
$L_5 \times T_3$	6904.84	0.94	23296	$L_{12} \times T_3$	8092.20	1.98	-45887
$L_6 \times T_1$	5671.77	1.24	-43294	$L_{13} \times T_1$	5371.79	2.37	-9249
$L_6 \times T_2$	4971.82	1.05	12912	$L_{13} \times T_2$	5821.75	0.37	-46981
$L_6 \times T_3$	6282.83	1.03	42299	$L_{13} \times T_3$	5821.57	0.86	13132
$L_7 \times T_1$	4955.16	1.14	-46785	$L_{14} \times T_1$	5049.59	0.92	-38060
$L_7 \times T_2$	8016.02	2.16	-33628	$L_{14} \times T_2$	5182.92	1.06	-28818
$L_7 \times T_3$	4860.72	0.64	-38970	$L_{14} \times T_3$	7060.54	1.56	-27694

bi, Varietal response to the environments; S^2di, Measures stability; QPM, Quality Protein Maize.

kernels per row, cob length and kernel vitreosity; the hybrid $L_6 \times T_2$ for days to 50% silking, plant height and kernels per row; the hybrid $L_6 \times T_3$ for kernel rows per ear, kernels/row and cob length; the hybrid $L_7 \times T_1$ for ear height, kernel rows per ear and cob girth; the hybrid $L_{13} \times T_3$ for days to 75% brown husk, kernels per row, cob girth and kernel vitreosity; the hybrid $L_{14} \times T_1$ for days to 50% tasseling, days to 50% silking, kernels per row, cob length and kernel vitreosity; the hybrid $L_{14} \times T_2$ for plant height and cob length. Hence, these hybrids were identified as stable genotypes for early *kharif,* normal and late *kharif* environment. Therefore, it might be advised to cultivate these hybrids generally throughout the three test conditions. Total 16 hybrids, viz. $L_1 \times T_3$, $L_2 \times T_1$, $L_2 \times T_3$, $L_3 \times T_3$, $L_4 \times T_1$, $L_5 \times T_2$, $L_7 \times T_3$, $L_8 \times T_3$, $L_9 \times T_2$, $L_{10} \times T_2$, $L_{11} \times T_1$, $L_{11} \times T_3$, $L_{12} \times T_2$, $L_{13} \times T_2$, C_1 and C_2 recorded non-significant deviation from regression, below-average response (bi<1) and higher mean values than the population mean. Non-significant deviation from regression (S^2di), below average response (bi<1) and mean values in the desirable direction as compared to the population mean suggests a stable performance of genotypes under unfavourable environment. Total of 17 hybrids, viz. $L_1 \times T_2$, $L_2 \times T_2$, $L_4 \times T_2$, $L_5 \times T_1$, $L_6 \times T_1$, $L_7 \times T_2$, $L_8 \times T_1$, $L_8 \times T_2$, $L_9 \times T_1$, $L_9 \times T_3$, $L_{10} \times T_1$, $L_{10} \times T_3$, $L1_1 \times T_2$, $L_{12} \times T_1$, $L_{12} \times T_3$, $L_{13} \times T_1$, and $L_{14} \times T_3$ showed higher mean value as compared to the population mean, above-average response (bi>1) and non-significant deviation from regression (S^2di). Non-significant deviation from regression (S^2di), above average response (bi>1) and mean values in desirable direction as compared to the population mean depicts a stable performance of genotypes under favourable environmental conditions.The results are agreed with Bhartiya *et al*. (2017), Pramitha *et al*. (2022) and Kumawat *et al*. (2023).

GGE biplot study: The significant mean sum of squares of G × E interaction for grain yield over the three test environments showed a meaningful role to evaluate the performance of genotypes in different locations. For the identification of accurate performance of the genotypes, the GGE biplot is the most effective method. In the biplot analysis, the horizontal axis (PC_1) represented the main effect of the genotypes and explained the variation among the yield. However, the vertical axis (PC_2) specified the G × E interaction depicted the genotype stability over the environments. Numerical values of PC_1 and PC_2 were found as 91.7% and 6.8%, respectively and these two principal components were depicted as 98.5% of the entire variation in yields (Fig. 1).

In case of this experiment, three lines split the biplot into three particular divisions but all the three environments (E_1, E_2 and E_3) were under the two divisions. The genotypes at the vertex are considered as the superior ones. They are the hybrid genotypes, viz. G_{18} ($L_1 \times T_1$), G_{29} ($L_4 \times T_3$), G_{23} ($L_2 \times T_3$), G_{53} ($L_{12} \times T_3$) and G_{47} ($L_{10} \times T_3$) (Fig. 2). All the three environments (marked in red) can be visualised in the first environment and 14 hybrids were identified suitable for this mega environment, viz. G_{48} ($L_{11} \times T_1$), G_{30} ($L_5 \times T_1$), G_{35} ($L_6 \times T_3$), G_{19} ($L_1 \times T_2$), G_{49} ($L_{11} \times T_2$), G_{59} ($L_{14} \times T_3$), G_{51} ($L_{12} \times T_1$), G_{32} ($L_5 \times T_3$), G_{52} ($L_{12} \times T_2$), G_{40} ($L_8 \times T_2$), G_{22} ($L_2 \times T_2$), G_{37} ($L_7 \times T_2$), G_{53} ($L_{12} \times T_3$) and G_{47} ($L_{10} \times T_3$). This suggests that these hybrids were performing best in all the three environments. On the other hand, the rest of the hybrids were not suitable for any environment. These hybrids may have less adaptation ability to the experimental areas. Similar reports have been suggested by, Kunwar *et al*. (2016), Maniruzzaman *et al*. (2019), Mumtaz *et al*. (2019), Ahmed (2020) and Pramitha *et al*. (2022).

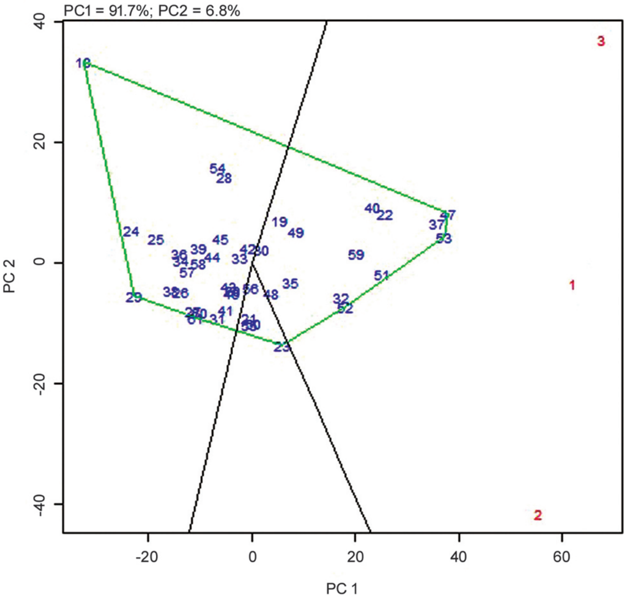

Fig. 1 "Which-won-where pattern" GGE biplot of the genotype × environment interaction.

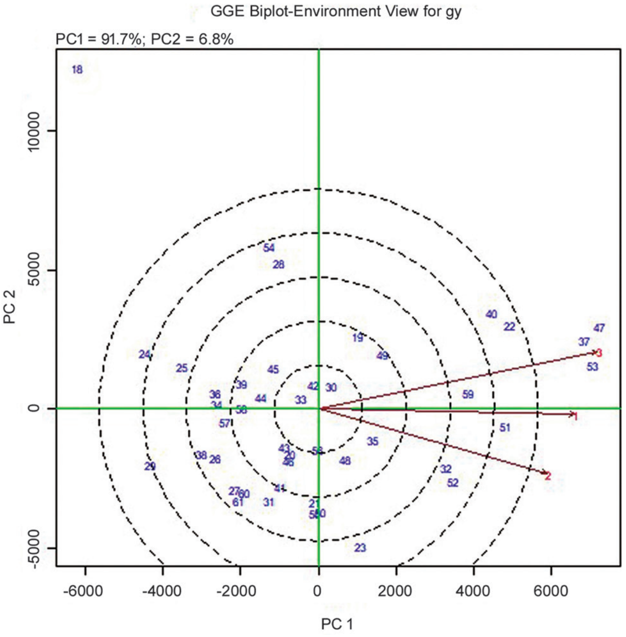

Fig. 2 GGE biplot representing relationship among environments. Note: Environment and genotypes are marked in red and blue colours, respectively.

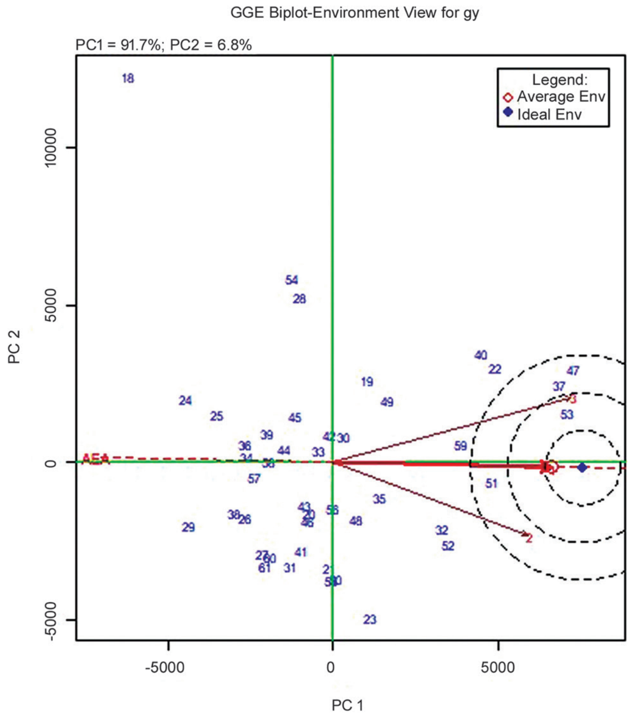

Fig. 3 GGE biplot for discriminating ability of the locations and representativeness.

In the GGE biplot, the vector represents the relationship among the different environments. The angle between the vectors of environments depicts the degree of association between them (Fig. 3). The smaller angle depicts the highest correlation among the environment. A positive correlation among all three environments was observed as the angles between them were less than 90°. Similar trends of relationships were also recorded by several other researchers like Maniruzzaman *et al*. (2019), Mumtaz *et al.* (2019) and Ahmed (2020). The vector of the GGE biplot depicts that the three environments were clustered into three groups i.e. E_3 constituted the first group and E_1 and E_2 constituted the other group. The environment-3 had long-vectors which depicts that this is the most discriminative environment carrying more information than the rest followed by environment-1. Environment-2 has the shortest vector which means it is an exclusive environment. It also creates a small angle with the AEC abscissa (Fig. 3) which means this environment might be excluded for the evaluation of better hybrid maize genotypes, but it could be used to remove unstable hybrids. All three environments remain close to each other and hence carry almost the same information about the genotypes. Environment 1 and 2 were highly correlated according to their ranking. Thus, the order of ranking the test environments according to their discriminating and representativeness are as follows: E_1, E_2 and E_3. Therefore, the potential single cross hybrids would be better for the expected yield in E_3 environment.

Also, the study of an adaptation biplot study showed that the genotypes G_{32} ($L_5 \times T_3$) followed by G_{35} ($L_6 \times T_3$) and G_{56} ($L_{13} \times T_3$) were high-response hybrids for grain yield in all the three environments and hence, considered stable hybrids with higher yields. However, some of the hybrids produced higher yields in a specific environmental condition. G_{47} ($L_{10} \times T_3$) followed by G_{53} ($L_{12} \times T_3$) and G_{37} ($L_7 \times T_2$) showed high yield as a response to Environment 3 (E_3).

The study of stability analysis through Eberhart and Russel model for grain yield identified 7 hybrids, viz. $L_5 \times T_3$, $L_6 \times T_2$, $L_6 \times T_3$, $L_7 \times T_1$, $L_{13} \times T_3$, $L_{14} \times T_1$ and $L_{14} \times T_2$ as stable genotypes under varying environmental conditions. In comparison to older models, GGE biplots provide accurate and in-depth graphical illustrations. Using this, three hybrids, viz. $L_5 \times T_3$, $L_6 \times T_3$ and $L_{13} \times T_3$ were identified as stable over all the environments. The environment, E_3 (late *kharif*) was ranked the most discriminative for accessing the high-yielding performance of genotypes. Apart from this, the superior genotypes under a single environmental condition (early *kharif*/normal *kharif*/ late *kharif*) were also identified using both methods.

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