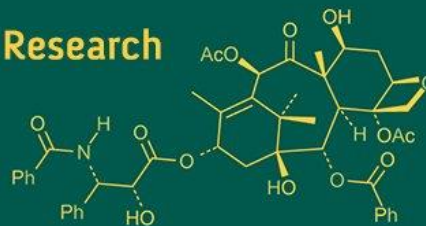
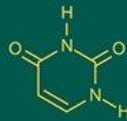
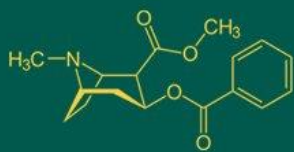


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Deciphering the interactions between genetic elements and environmental factors in rice (*Oryza sativa* L.) genotypes: Valuable perspectives unveiled through AMMI modeling and GGE biplots analysis

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Abstract

This study examined genotype-environment interaction among 45 rice genotypes during the 2020-21 & 2021-22 Kharif seasons in Chhattisgarh, India. Genotype main effect plus genotype by environment interaction analysis was employed to assess the interplay between genotype and environment, highlighting Genotype-environment interaction as the primary source of variation. The first two principal components of the GGE bi-plot explained substantial variability. Additive main effect and multiplicative interaction analysis identified Environment 1 as the most influential for yield. High-performing genotypes (G26, G60, G3, G37, G46, G49, G36, G14 and G6) were consistent across diverse environments, holding significance for rice breeding programs. These findings are valuable for selecting potential candidates for commercial cultivation to enhance both yield and stability across varying conditions, facilitating targeted trait improvement and robust rice cultivar development.

Keywords: AMMI analysis, GGE biplot, yield performance, stability assessment, multi-environmental trials (mets)

Introduction

Genotype-environment interaction significantly influences plant growth and traits (Annicchiarico 2002) [2]. Understanding this interaction is vital for plant biology and adaptation (Karimizadeh *et al.* 2012) [13]. Multi-environment trials (MET) are essential for evaluating genotypes across diverse conditions, revealing genotype-environment (GE) interactions (Yan *et al.* 2007) [23]. Evaluating GE interactions is crucial because it shows how genotypes perform differently in various environments, helping select suitable genotypes for specific conditions (Ebdon and Gauch, 2002) [4]. This approach enables breeders to tailor recommendations for specific needs (Gauch 2006) [7]. A deep understanding of genotype-environment interaction is vital for assessing adaptability and stability (Ahmadi *et al.* 2012) [1]. Researchers like (Jeberson *et al.* 2017) [12]. Have explored this field using biometric models, following methodologies advocated by (Yan *et al.* 2007; Goncalves *et al.* 2020) [23, 10].

Materials and Methods

Table-1 three-year study (2020-2021&2022) assessed 45 rice genotypes in Chhattisgarh (ENV1) Raipur, (ENV2) Bilaspur, and (ENV3) Bhatapara regions, developed from a cross between parent plants IGKV-R1 (P1) and CGR-52 (P2) to enhance protein content and yield performance across diverse environments in genotypes. It employed the Environmental Regression (ER) model, AMMI analysis, and GGE biplot analysis as per (Yan *et al.* 2000) [22]. The Multi-Location Trial (MLT) used an Augmented Block Design with standard planting densities. Each plot covered one square meter and followed best agricultural practices, including weed, insect, and disease management, and recommended fertilizer doses (N:P:K,120:60:40).

Statistical analysis

We used statistical analysis (ANOVA) to study differences in quantitative traits among genotypes, locations, and seasons. We also examined combinations like genotype by location, genotype by season, and genotype by location by season (genotype by environment). This analysis was performed using the R-package for multivariate analysis. To understand the interaction between genotype and environment ($G \times E$), we conducted a visual multivariate stability analysis using GGE biplot and AMMI in R studio, which is a simplified version of R statistical software developed by the R Core Team. For GGE biplots, we utilized the GUI package in R studio, and for AMMI, we employed the Agricolae package, which incorporates two important concepts the biplot concepts (Gabriel *et al.* 1971)^[5]. And (Yan *et al.* 2007)^[23]. As well as the GGE concept (Yan *et al.* 2000)^[22].

AMMI model

AMMI model analyzes how genotypes and environments interact in agriculture. It has additive parts for direct effects and a multiplicative component showing how genotypes behave in different environments. AMMI model, an advanced form of mean regression, effectively understands how genotypes and environments interact. Developed by (Gollob 1968)^[9], (Mandel 1971)^[15], and (Gabriel 1978)^[6]. It includes ANOVA, PCA, and regression. This equation evaluates crop impacts, aiming for a simple grasp of combined effects on yield and productivity in different situations.

Model's equation

$$Y_{ij} = \mu + g_i + e_j + \sum_{k=1}^n \lambda_k \alpha_{ik} \gamma_{jk} + \varepsilon_{ij}$$

Where

Y_{ij} : Represents the mean yield of genotype (i) in Environment (j)

μ : Signifies the overall mean yield across all genotypes and environments

g_i : Denotes the effect of the (i) genotype

e_j : Represents the effect of the j environment

$\sum_{k=1}^n \lambda_k \alpha_{ik} \gamma_{jk} + \varepsilon_{ij}$: Capture the multiplicative interaction effect

λ_k : is a singular value α_{ik} is a singular value of the (i) genotype and γ_{jk} is the singular value of the (j) environment and ε_{ij} Represents the experimental error

Results and Discussion

The table-2 presents an analysis of various sources contributing to the variation observed in the dataset. Environmental factors (ENV) show statistical significance (p-value = 0.000142) with a contribution of 0.85% to the total variation. REP (ENV) (Replication within Environment) is also statistically significant (p-value = 0.00001) and contributes 1.42%. The most influential source is GEN (Genotype) with a highly significant p-value of 0.00001 and a substantial contribution of 85.07% to the total

variation. GEN: ENV (Genotype and Environment Interaction) is statistically significant (p-value = 0.006619) and contributes 6.39%. Principal Components, PC1 and PC2 represent mathematical combinations of the original data. PC1 is statistically significant (p-value = 0.015857) and contributes 3.39% to the total variation. However, PC2 is non-significant with a p-value of 1, indicating a limited contribution (0.05%) to the total variation. PC2's weak impact stems from minimal influence on dataset variability, possibly due to weak correlations or a complex structure. Genetic factors dominate outcomes, followed by GEN: ENV and PC1, while ENV and REP (ENV) play minor roles. PC2 lacks significance (Oladosu *et al.* 2017)^[16]. And (Yan & Kang, 2007; Dehghani *et al.*, 2006)^[23,3]

Biplot pattern for elucidation of multivariate analysis

Multi-environment trials (MET) address genotype-environment ($G \times E$) interactions in crop farming (Oladosu *et al.* 2017)^[16]. Genotype (G) and $G \times E$ interactions are the primary sources of variation in MET (Yan *et al.* 2000)^[22]. The biplot technique aids in interpreting MET data, revealing the "which-won-where" pattern for GEI, assessing genotype stability and mean performance across environments, and evaluating test environment representativeness and discrimination. A biplot graphically represents genotypes and environments on a 2D scale using PC1 and PC2 scores. High PC1 values indicate better yield potential, while low PC2 values signify stability. Genotypes, linked to the encompassing polygon, are positioned away from the biplot's center, revealing expected environments, and promising genotypes at polygon vertices.

GGE biplot ('which-won-where' pattern)

Figure 1 depicts the GGE biplot pattern for plant yield in square meters, where genotype (G) and genotype-environment interaction ($G \times E$) jointly explain 96.29% of yield variation. The biplot segregates environmental indicators into two distinct sections, each favoring specific genotypes. This emphasizes the significant interplay between genotype and environment for a trait. Using 45 rice genotypes across three environments, the GGE biplot divides into 12 clockwise fan-shaped sections for yield. A genotype positioned near a vertex of the polygon indicates high performance in a specific environment, signifying optimal suitability for those conditions. Such genotypes excel in particular environments, showcasing optimal performance. This information aids in identifying superior-performing genotypes, crucial for applications like plant breeding and genotypic selection. Genotypes far from the polygon's center have weaker performance, indicating below-average results. A genotype in the opposite direction suggests lower performance in that specific environment compared to others. Our result Genotype G26 excelled in yield and stability in ENV1, while G5, G37, G1, G137, and G82 performed well in both ENV1 and ENV2. G60 and G36 exhibited the highest yield and stability in ENV2. In ENV3, G6, G49, and G14 excelled in yield with high stability, while G21, G40, G19, G42, G46, and G120 performed best in ENV3. These genotypes (G97, G109, G16, G116, G124, G99, G28, G117, G118, G164, and G50) are consistently positioned away from the center of the polygon and in the opposite direction of the environment. This indicates their comparatively lower performance,

especially when assessed in the context of specific environmental conditions. Our findings, aligned with (Hashim *et al.* 2021) ^[11]. And (Oladosu *et al.* 2017) ^[16], investigate genotype performance across diverse environments. Grouping environmental indicators implies consistent performance, while scattered indicators indicate variations.

GGE biplot pattern of ‘mean vs. stability’ analysis and ideal genotype assessment

Biplot analysis is a vital statistical method for evaluating genotypic performance in diverse environments. The AEC Abscissa, a vertical line in the biplot, gauges mean performance. Genotypes close to this line, especially in the arrow's direction, signify superior average performance across various environments. The AEC Ordinate, a horizontal line, assesses stability, with genotypes near it indicating reduced variability in performance across diverse conditions (Yan & Rajcan 2002) ^[20]. Our GGE biplot showed that 96.29% of the variation in plant yield per square meter resulted from genotype (G) and genotype-environment interaction ($G \times E$) (Fig. 2). G26 excelled in both yield and stability in ENV1, followed by G6, G3, G37, G46, G1, and G120. In ENV3, G6, G21, G49, G14, and G40, and in ENV2, G5, G60, and G36, exhibited high yields but lower stability. G82 and G188 excelled in stability but had lower yields in ENV1, while G33, G56, and G137 (ENV2) and G19, G88, G219, and G42 (ENV3) displayed high stability but lower yields. Genotypes G6, G21, G49, G14, G40, G5, G60, and G36 yielded well but had low stability, evident from their placement away from the AEC line. These trends align with prior studies by (Oladosu *et al.* 2017; Hashim *et al.* 2021; Sabri *et al.* 2020) ^[16, 11, 17].

Genotype ranking: best and ideal genotype assessment.

The genotype ranking biplot (Fig. 3) is a useful tool for identifying ideal genotypes. Genotypes like G26, G3, G37, and G6 excel as top performers due to their proximity to the arrowhead in the circle representing plant yield (Fig. 3). Ideal genotypes are typically within the innermost circle, closer to the arrow's head at the circular ring's center. In cases with no genotype in the inner circle, those closest to it are considered ideal (Oladosu *et al.* 2017) ^[16]. An ideal genotype should show both high mean and stability (Yan & Tinker 2006) ^[20], it's often represented by a ring at the head of the arrow on the horizontal AEC abscissa axis (Oladosu *et al.* 2017) ^[16]. Breeders use data from yield performance evaluations for genotype selection in multi-environment trials (Lin & Binn 2010) ^[14]. Genotypes close to the ideal genotype are promising (Fig. 3): G26 > G6 > G3 > G37 > G46 > G21 > G5 > G60 > G1 > G19 > G17 > G36 > G40. These results align with (Oladosu *et al.* 2017) ^[16].

‘Discriminativeness vs. representativeness’

The GGE biplot is vital for selecting ideal test environments for superior genotypes and gauging discriminativeness and representativeness (Oladosu *et al.* 2017) ^[16]. In our study (Fig. 4), ENV1, characterized by a short vector for plant yield, stood out as an exceptional research location, while longer vectors denoted a greater impact in discriminating rice genotypes. The ideal test environment features a long vector forming a shorter angle with the AEC abscissa line, indicating high representativeness and discriminative power, as exemplified by ENV1, which excelled in plant yield (Fig. 5). ENV2 and ENV3 were less suitable for genotype selection. Optimal environments depend on genotype considerations, balancing representativeness and discriminative capacity. Prior studies (Hashim *et al.* 2021; Oladosu *et al.* 2017) ^[11, 16]. Also identified yield-based ideal environments, facilitating environment selection based on genotype-environment correlations approximated by the cosine of the angle between AEC and environment vectors.

Ranking environment

The ideal test environment should be the most discriminating (informative) and also most representative of the target environment. Figure 5 defines an “ideal test environment”, which is the center of the concentric circles. It represents the apex of the AEA in the positive direction, symbolizing the most representative point with a distance from the biplot origin equivalent to the longest vector across all environments, thus embodying the highest level of informativeness. E1 is closest to this point and is, therefore, best, whereas E2 and E3 were the poorest for selecting cultivars adapted to the whole region.

Relationships among Test Environments

Yan's 2002 elucidation ^[18] highlights that the relationship between genotype performance is significantly influenced by the angle between vectors representing two test environments. An angle less than 90° suggests a positive connection, indicating consistent performance across environments. An angle of 90° signifies orthogonality and a weak association, while an angle exceeding 90° indicates a negative connection, revealing an inverse relationship in genotype performance between the environments. In our study, E1, E2, and E3 showed positive correlations (Figure-6). All Environments are an acute angle and no right angle or obtuse angle was observed. If two environments consistently correlate closely across years, one might be eliminated without substantial genotype information loss. (Lin & Binns 2010) ^[14], stated that the effect of the environment on genotype is highly influenced by unpredictable (e.g., weather) and predictable (e.g., soil) factors. The soil is a fixed factor due to its persistence from year after year and is noted as a predictable component.

Table 1: Materials 45 rice genotypes cross of (P1) IGKV-R1 and CGR-52 (P2)

S. N	Genotype	S.N	Genotype	S.N	Genotype
1	G114	16	G159	31	G120
2	G109	17	G33	32	G14
3	G16	18	G42	33	G40
4	G130	19	G188	34	G17
5	G50	20	G56	35	G36
6	G111	21	G133	36	G49
7	G118	22	G75	37	G1
8	G124	23	G24	38	G60
9	G99	24	G219	39	G5
10	G116	25	G119	40	G21
11	G87	26	G137	41	G46
12	G164	27	G82	42	G37
13	G117	28	G88	43	G3
14	G28	29	G102	44	G6
15	G97	30	G19	45	G26

Table 2: Additive main effect and multiplicative interaction (AMMI) analysis of variance for grain yield

Source of variation	Df	Sum Sq	Mean Sq	p-value	variation in %
ENV	2	47064.85	23532.42**	0.000142	0.85
REP(ENV)	3	78575.47	26191.82**	0.00001	1.42
GEN	45	4690657.15	104236.82**	0.00001	85.07
GEN:ENV	88	352455	4005.17**	0.006619	6.39
PC1	46	186821.63	4061.33**	0.015857	3.39
PC2	44	2834.63	64.42	1	0.05
ERROR	131	324898.66	2480.14		
Total	359	5514076.48	15359.54		

"NS: Not statistically significant, *: Statistically significant at a ($p < 0.05$) 5% significance level, **: Statistically significant at a ($p < 0.01$) 1% significance level"

Table 3: Mean performance and their yield comparisons of 45 lines of rice genotypes

Genotype	Mean (gm/m ²)	Genotype	Mean(gm/m ²)	Genotype	Mean (gm/m ²)
G114	109.47	G159	315.38	G120	418.19
G109	118.25	G33	317.06	G14	431.47
G16	126.59	G42	322.97	G40	428.81
G130	121.88	G188	321.03	G17	433.41
G50	141.47	G56	317.66	G36	423.91
G111	136.06	G133	302.30	G49	448.22
G118	148.97	G75	317.63	G1	452.91
G124	145.75	G24	326.91	G60	448.09
G99	146.75	G219	322.31	G5	454.16
G116	147.94	G119	318.50	G21	461.44
G87	152.06	G137	322.06	G46	468.22
G164	145.15	G82	324.56	G37	471.25
G117	158.00	G88	325.56	G3	488.69
G28	161.94	G102	318.56	G6	521.31
G97	163.72	G19	327.41	G26	532.69
Descriptive statistics					
Mean	306.81	Min. yield	G114(109.47)		
SE	8.56	Max. yield	G26(532.69)		
SD	140.43				
CV	45.85				

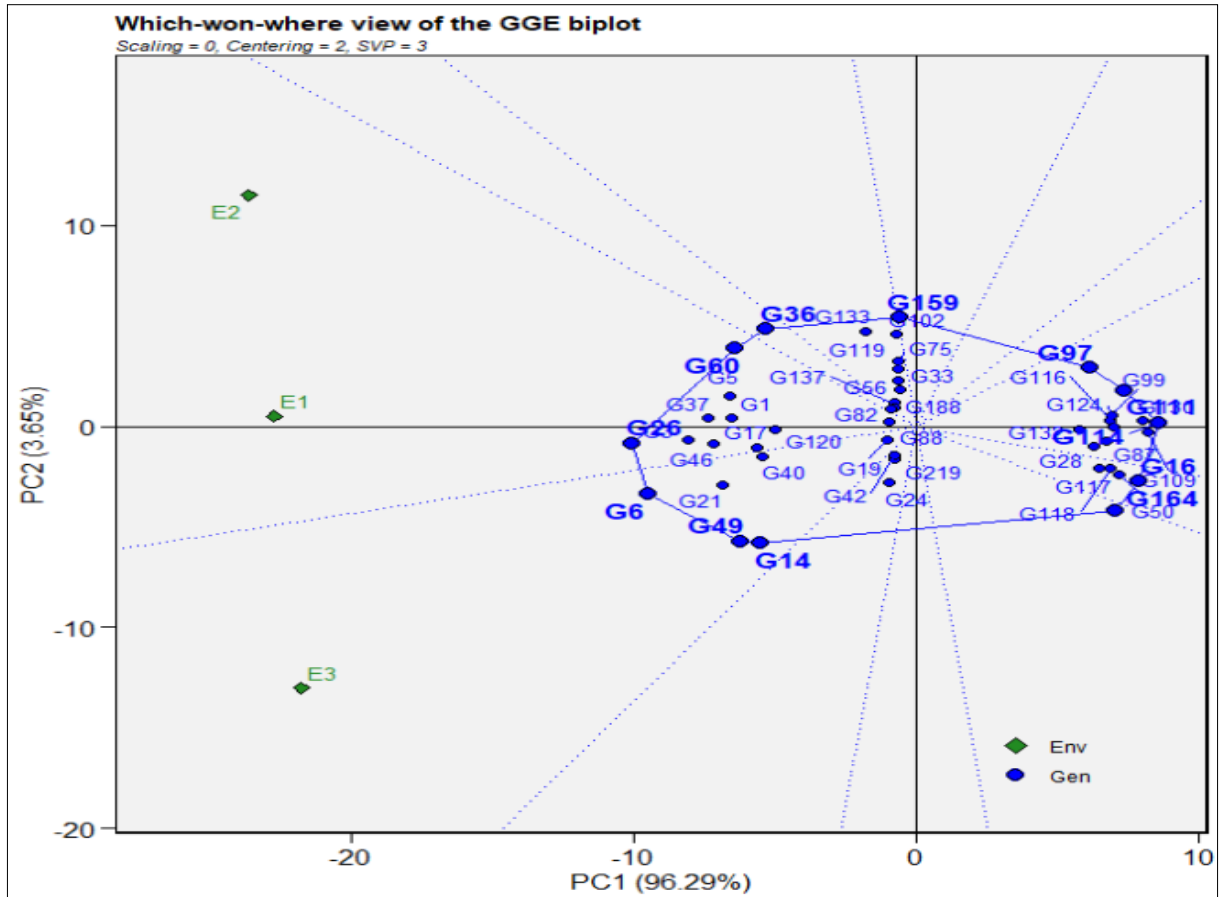


Fig 1: “Which-won-where” pattern of GGE biplot polygon view displaying the genotype main effect plus $G \times E$ interaction effect of 45 rice genotypes in three seasons in three locations for plant yield. The biplots were based on Centering = 2, SVP = 3, Scaling = 0.

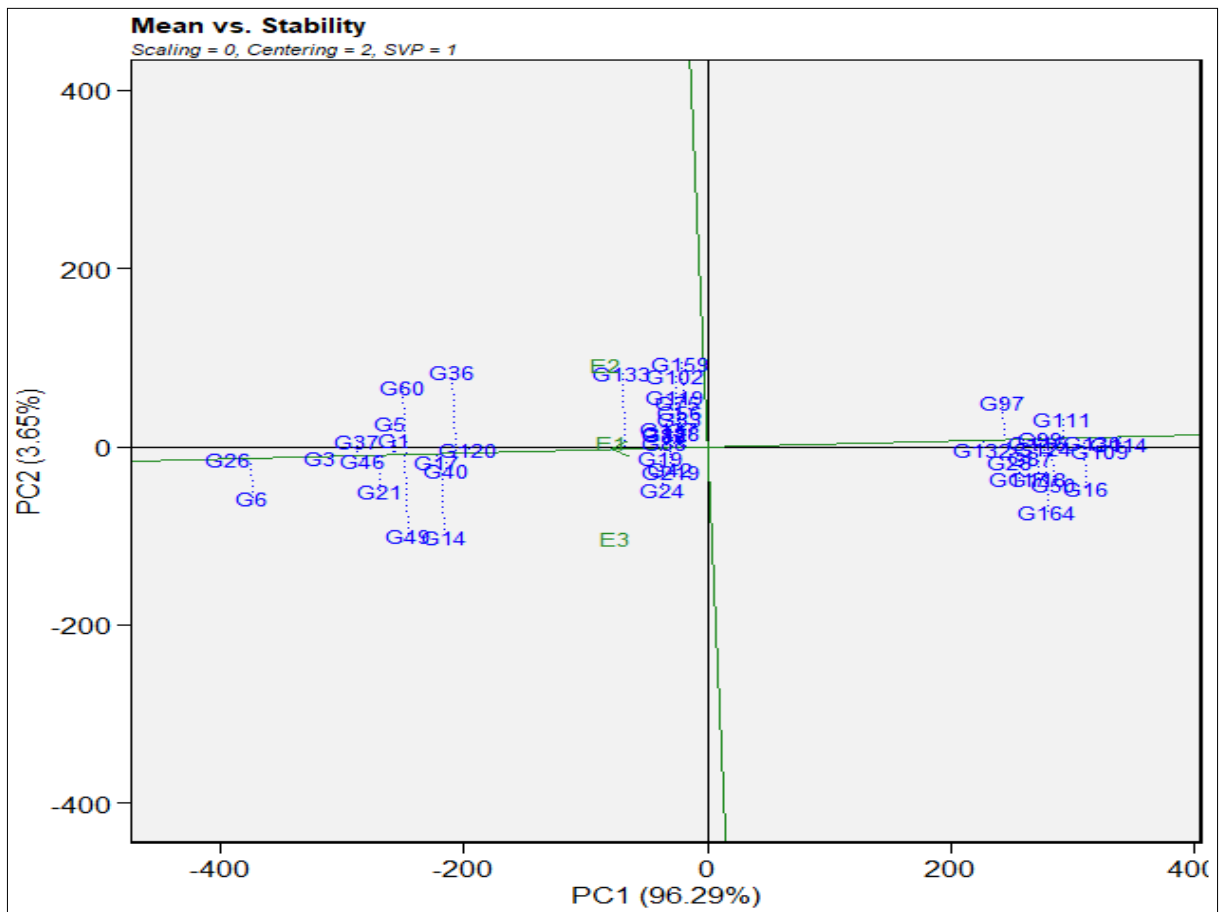


Fig 2: ‘Mean vs. stability’ pattern of GGE biplot illustrating interaction effect of 45 lines of rice Genotype under three seasons three locations for plant yield. The biplots were created based on Centering = 2, SVP = 1, Scaling = 0

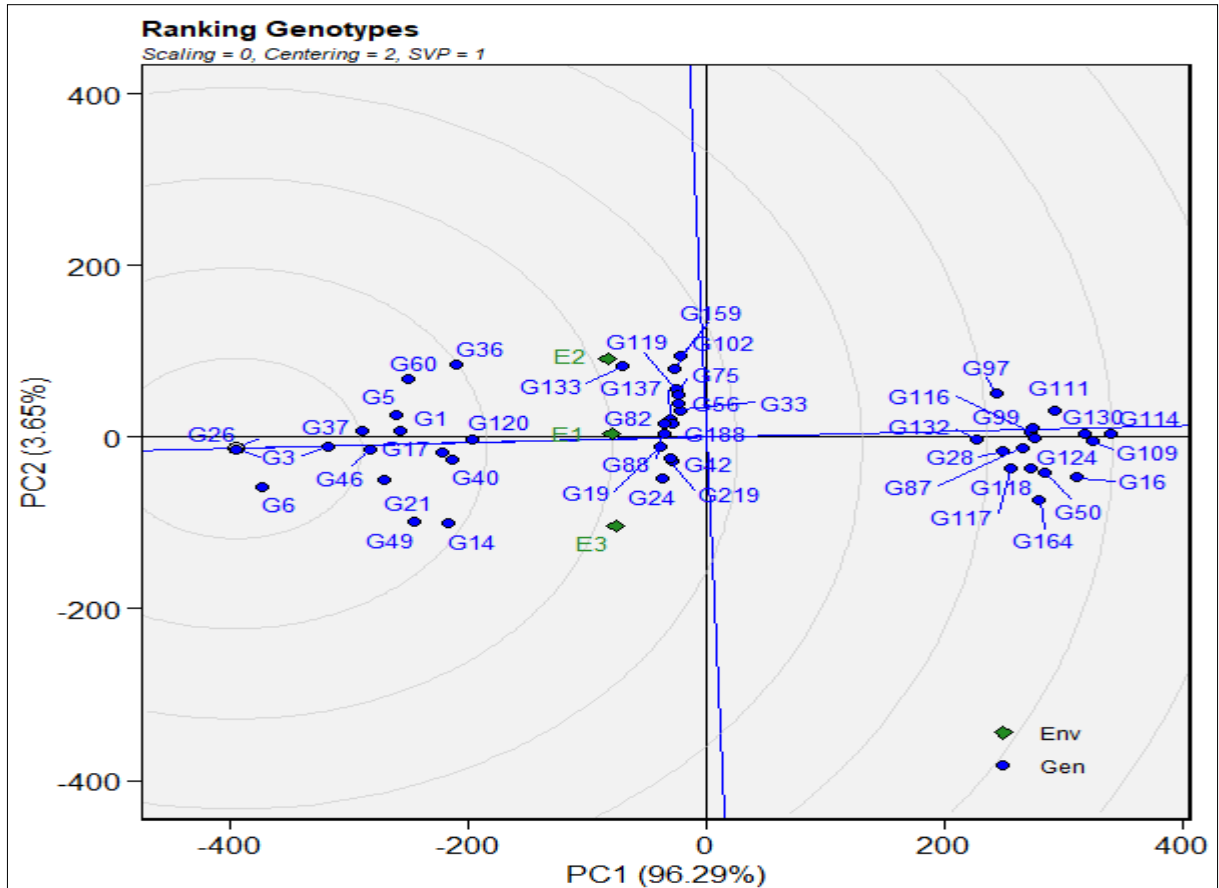


Fig 3: The GGE biplot ‘genotypes ranking’ pattern for genotype comparison with ideal genotype showing G + G × E interaction effect of 45 lines of rice genotypes. Under three season three locations for plant yield. The biplots were created based on Centering = 2, SVP = 1,

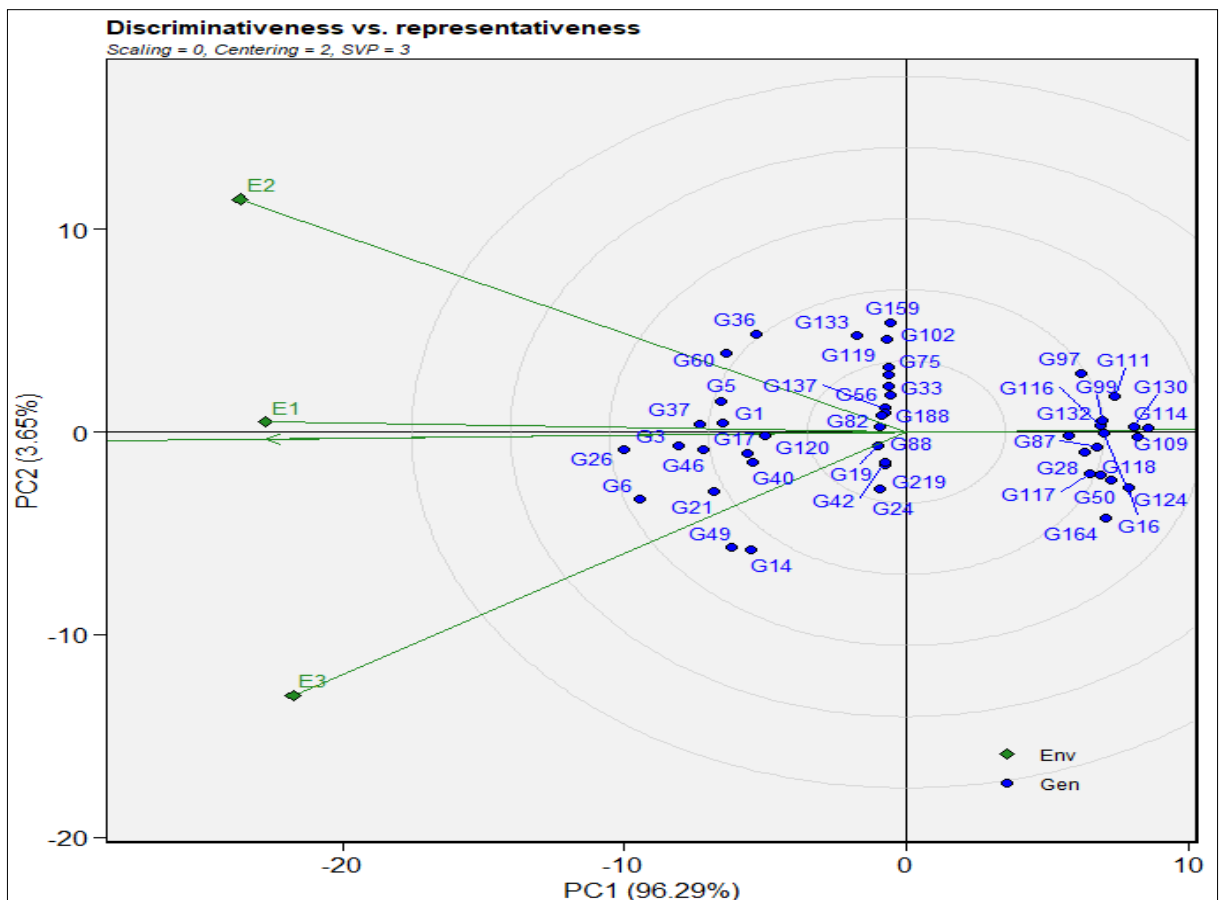


Fig 4: The GGE biplot ‘Discriminativeness vs. representativeness’ for genotype comparison with ideal genotype shows the G + G × E interaction effect of 45 lines of rice genotypes. Under three seasons three locations for plant yield. The biplots were created based on Centering = 2, SVP = 3, and Scaling = 0.

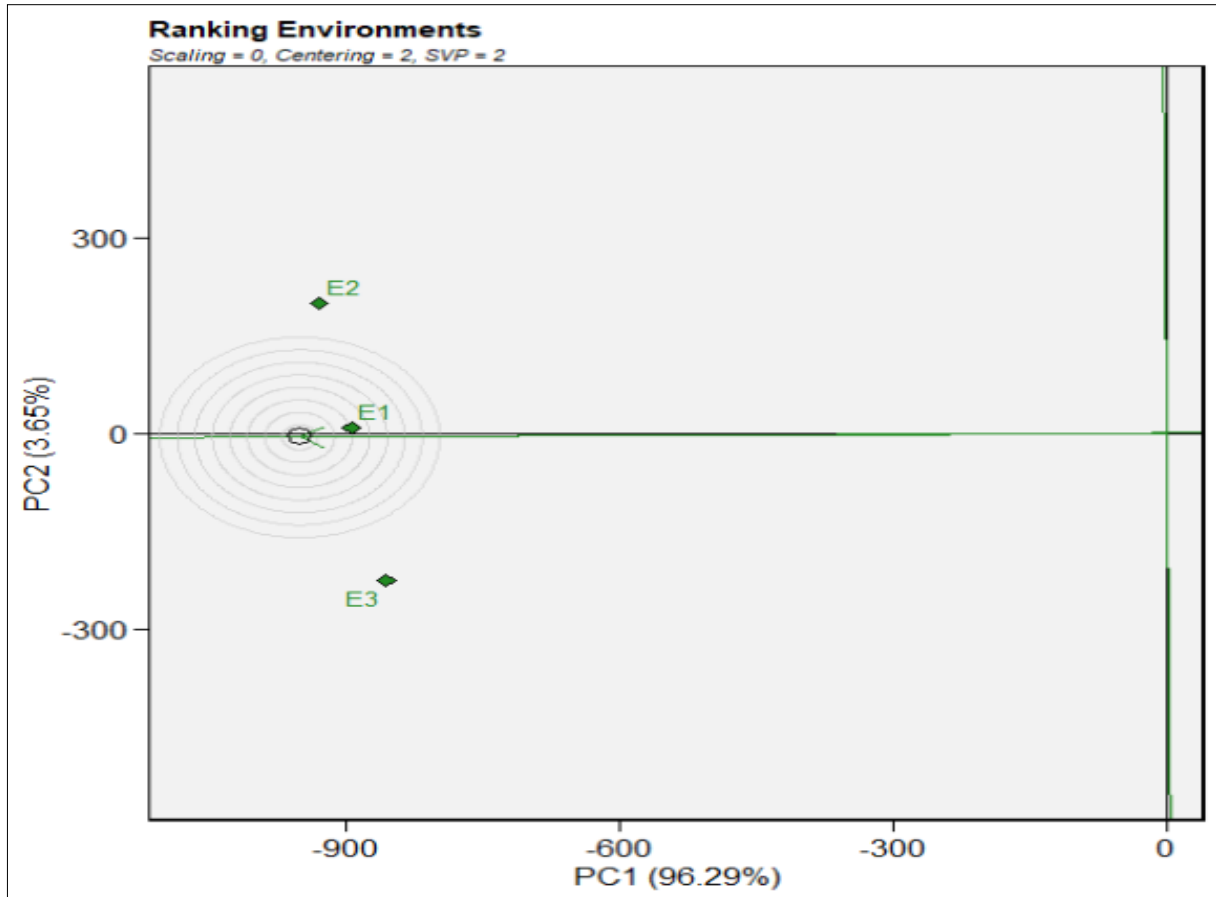


Fig 5: The GGE biplot ‘Environment ranking’ pattern for environment comparison with the ideal environment shows the G + G × E interaction effect of 45 lines of rice genotypes under three seasons and three locations for plant yield. The biplots were generated using Centering = 2, SVP = 2, and Scaling = 0 as the parameter settings

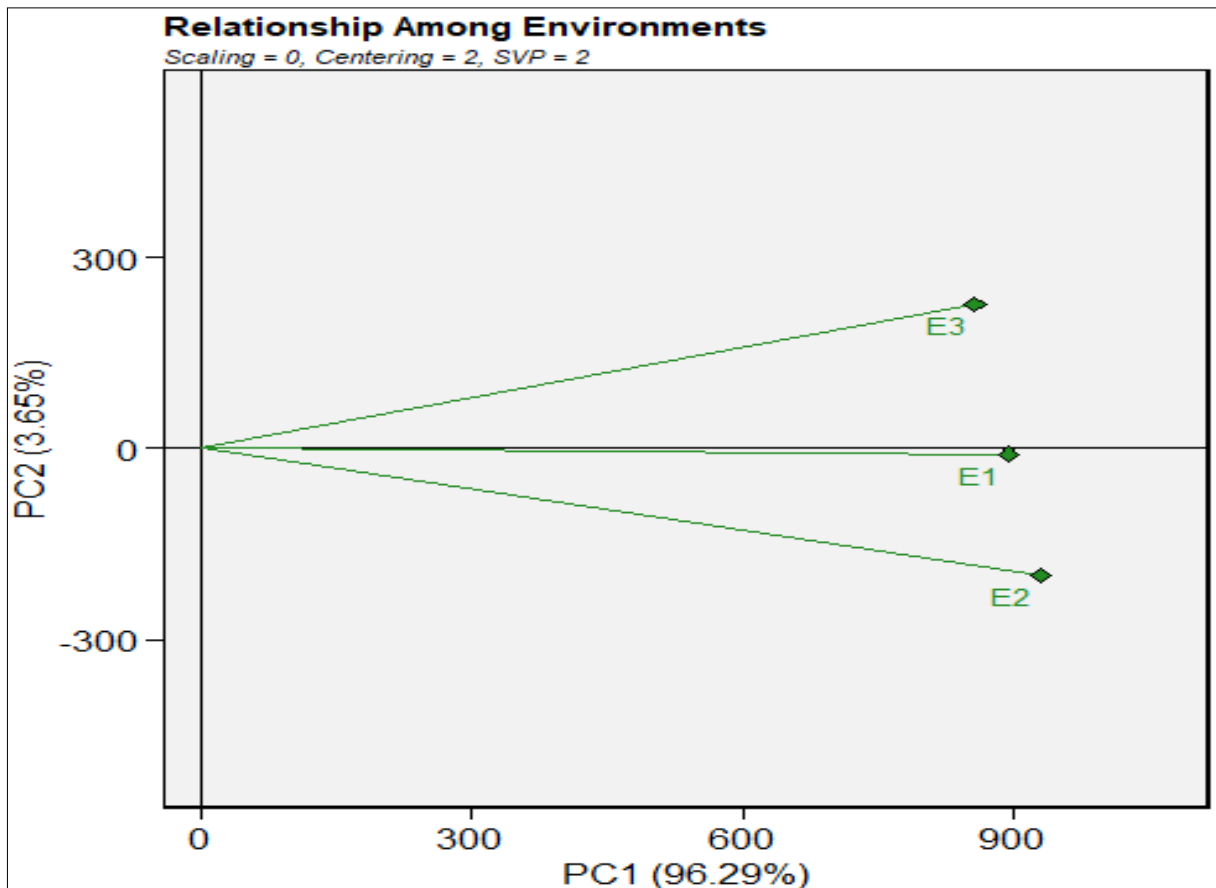


Fig 6: The environment-vector view of the GGE biplot shows similarities among test environments under three seasons and three locations for plant yield. The biplots were created based on Centering = 2, SVP = 2, Scaling = 0

Conclusion

This study evaluates rice genotypes across diverse environments, aiming to identify high-performing genotypes. Multi-environment trials (MET) assess adaptability and stability. Genotypes fall into three categories. Group one includes stable, high-yielding genotypes (G26, G3, G46, G120, G17, G46, G1, and G37) suitable for ENV1. Group two has genotypes with low stability but high yield per square meter (G6, G21, G49, G14 in ENV3, and G40, G5, G60, G36 in ENV2). The last group consists of genotypes with low yield but high stability (G19, G88, G188, G82, G33, G42, G219, and G56), ideal for targeted breeding. Some genotypes (G26, G6, G60, G49, and G14) performed consistently well, making them prime candidates for commercial cultivation in Chhattisgarh. These findings suggest the potential for improving rice genotypes, with several ideal candidates for cultivation in Chhattisgarh.

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