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RESEARCH ARTICLE

AMMI and GGE biplot-based evaluation of yield and protein percentage in nutrient-rich rice genotypes across diverse environments

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Abstract

Stable, high-yielding and nutritionally enriched rice genotypes are essential to ensure food and nutritional security under changing climatic conditions. In this study, 12 nutrient-rich rice genotypes, along with standard checks, were evaluated for grain yield and protein content across four diverse and contrasting environments in Chhattisgarh, India, during kharif season of 2024–25, using a randomized complete block design (RCBD) with two replications. Although based on a single year, the use of multiple test sites and robust genotype × environment (G × E) interaction analysis through additive main effects and multiplicative interaction (AMMI) and genotype (G) plus genotype × environment interaction (GE) biplot (GGE biplot) models enabled reliable genotype assessment. Combined analysis of variance (ANOVA) based on the AMMI model revealed significant effects of environmental factors and G × E interactions (GEIs) on both traits. In the GGE model, the first two principal components explained 91.6 % of the variation in yield and 81.5 % of the variation in protein content. The "which-won-where" biplot identified G9 as superior for yield across environments, while G9 and G3 excelled in protein content in E1 and E2 respectively. The most discriminating environment for yield was E3, whereas E1 and E3 were highly discriminating for protein content. The most representative environments were E1 for yield and E4 for protein content. The ideal environments identified were E4 for yield and E2 for protein content. Genotypes G9 and G3 emerged as promising candidates for commercial cultivation. These nutrient-rich lines may also serve as valuable parental material for breeding programs targeting enhanced yield and nutritional quality, contributing to the development and release of biofortified rice varieties.

Keywords: AMMI analysis; discriminative and representative environment; GGE biplot analysis; stability

Introduction

Rice (*Oryza sativa* L.) is the most widely cultivated and consumed staple food crop globally, feeding nearly half of the world's population. Asia produces about 80 % of the world's total rice supply. Despite having the largest area under rice cultivation (44.1 Mha) and an annual production of 165.3 Mt, India's average productivity remains relatively low at 3.78 t ha^{-1} (1). In addition to its caloric value, rice is a rich source of protein, comprising four major fractions-prolamin, glutelin, globulin and albumin-each with distinct solubility properties. These proteins are nutritionally important due to their favourable amino acid profile and functional properties. The widespread availability and digestibility of rice protein make it an easily accessible and valuable dietary protein source with various health benefits.

Because of these attributes, the food industry is increasingly exploring rice protein as a sustainable and hypoallergenic alternative to animal-based and gluten-containing proteins (2). Owing to its high nutritional value and excellent digestibility, rice is often referred to as the "queen of cereals" (2). Among cereal grains, rice exhibits the highest net protein utilization (3). Moreover, rice protein isolates are particularly recommended for infants and the elderly due to their digestibility, nutritional quality and low allergenicity, further establishing rice as a promising alternative to animal-derived proteins (4).

Ensuring the sustainable production of safe and nutritious food remains a significant global challenge. Rising demand for increased crop productivity, particularly with improved protein content, has raised significant concerns regarding global food and nutritional security. Rice productivity is constrained by several factors, including the limited availability of superior varieties—such as early-maturing, high-yielding, biotic- and abiotic-stress-tolerant genotypes—along with suboptimal soil fertility and complex genotype × environment interactions (GEIs), which collectively impede effective selection and breeding progress (5). Although numerous rice varieties have been released, many fail to remain in cultivation due to inconsistent performance across diverse agro-ecological zones. Only a limited number of genotypes with stable and reliable performance continue to be cultivated over time (6).

To overcome these limitations, plant breeders employ multi-environment trials (METs) to assess genotype performance, adaptability and stability across variable conditions. Stable genotypes are those capable of delivering high yields under both optimal and suboptimal environments. Understanding the adaptability and stability of rice cultivars is crucial for selecting genotypes that perform consistently across regions and seasons (5).

The expression of agronomic traits is determined by the combined effects of genotype (G), environment (E) and their interaction (G × E). To identify genotypes with consistent performance in terms of grain yield and protein content across years and locations, it is essential to study yield stability and GEIs (6). A comprehensive understanding of GEIs serves as a vital decision-making tool in the final stages of variety release. It helps breeders assess patterns of adaptation, select promising breeding lines and define recommendation domains for released varieties.

Although the observed yield is influenced by G, E and GEI, it is primarily the G and the GE interaction that are relevant when evaluating cultivar performance. Biplot analysis has emerged as a powerful statistical tool in plant breeding and agricultural research for visualizing these components (5). Traditional genotype-by-environment data often treats environmental variation as noise, limiting analysis to genotype main effects (5). To overcome this, the concept of the genotype (G) plus genotype × environment interaction (GGE) biplot which incorporates both G and GEI was introduced, along with various visualization techniques to address specific analytical challenges.

Genotype × environment interaction is particularly important for quantitative traits such as seed yield and protein content, as genotypes can perform differently under varying environmental conditions. Numerous studies have validated the GGE biplot methodology in MET analyses, confirming its utility for identifying stable and high-performing genotypes. In GGE biplot analysis, the first two symmetrically scaled principal components (PC1 and PC2) are obtained by applying singular value decomposition (SVD) to environment centered MET data. This graphical approach enables clear visualization of both genotype performance and GEI, aiding in the identification of mega-environments (MEs) and ideal genotypes (7).

The GGE biplot allows for simultaneous assessment of mean performance and stability across environments, offering insights into the representativeness and discriminative power of test locations. It facilitates the evaluation of whether a target environment consists of one or multiple mega-environments and whether GEI can be effectively utilized or minimized. This analytical framework provides answers to most genotype × environment (G × E) questions, making it a valuable decision-support tool for both short- and long-term breeding strategies.

One of the most informative features of a GGE biplot is its ability to display the “Which-Won-Where” pattern of a genotype by environment dataset. This pattern graphically identifies which genotype performs best in which environment by partitioning the biplot into sectors. Each vertex genotype represents the highest performer in the environments falling within its sector, thereby defining mega-environments and aiding in location-specific genotype selection.

The discriminativeness of an environment in GGE biplot analysis refers to its ability to differentiate among genotypes, i.e. how effectively it distinguishes the performance of different genotypes. It is represented by the length of the environment vector from the origin; longer vectors indicate greater discriminating ability. The representativeness of an environment reflects how closely it corresponds to the average or typical conditions of all test environments. In the GGE biplot, this is determined by the angle between the environment vector and the average environment axis (AEA); smaller angles denote more representative environments, while larger angles indicate less representative ones.

An environment that is both highly discriminating and strongly representative is considered ideal for genotype evaluation and selection, as it provides reliable information on both performance and stability. The GGE biplot also facilitates the identification of MEs, which are clusters of environments where a particular genotype consistently shows superior performance. Identifying these MEs allows breeders to recommend genotypes that are best suited to specific regions while developing more focused, efficient breeding and evaluation strategies.

The GGE biplot technique has been successfully utilized in a variety of crops, including durum wheat (8), barley (9), bambara groundnut (10) and sugarcane (11). Accordingly, GGE biplot analysis was employed in this study to identify high-performing, stable genotypes and suitable test environments for both grain yield and grain protein content.

Materials and Methods

This experiment included twelve nutrient-rich rice lines (Table 1). These comprised six newly developed elite nutrient rich genotypes selected through rigorous multi-stage breeding at Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur, Chhattisgarh, India, along with one elite genotype each from the International Rice Research Institute (IRRI), Philippines, and the National Rice Research Institute (NRRI), Cuttack, India. In addition, four rice lines were included as standard checks for yield, protein percentage, and micronutrient content.

The experimental material consisted of 12 nutritionally rich rice genotypes evaluated in a randomized complete block design (RCBD) with two replications across four diverse environments–Raipur (E1), Bilaspur (E2), Raigarh (E3) and Kawardha (E4)–during the kharif season of 2024–25 (Fig. 1). Each plot measured 2.3 × 3.0 m^2. This METs effectively captures substantial spatial variation in agro-climatic conditions while assessing genotypes for grain yield and protein content.

Table 1. List of nutrient-rich rice genotypes used in the study

Sl. no.	Code	Genotype	Source
1	G1	R-RHP-IC-214	IGKV, Raipur
2	G2	R-RHP-IC-216	
3	G3	R-RHP-IC-217	
4	G4	R-RHZ-RKC-224	
5	G5	R-RHZ-RKC-225	
6	G6	R-RHZ-CB-184	
7	G7	IRRI-147	IRRI, Philippines
8	G8	CR Dhhan-310	NRRI, Cuttack
9	G9	PROTEZIN	Protein check
10	G10	ZINCORICE-MS	Micronutrient check
11	G11	SWARNA	Yield check
12	G12	Chittimuthyalu	Micronutrient check

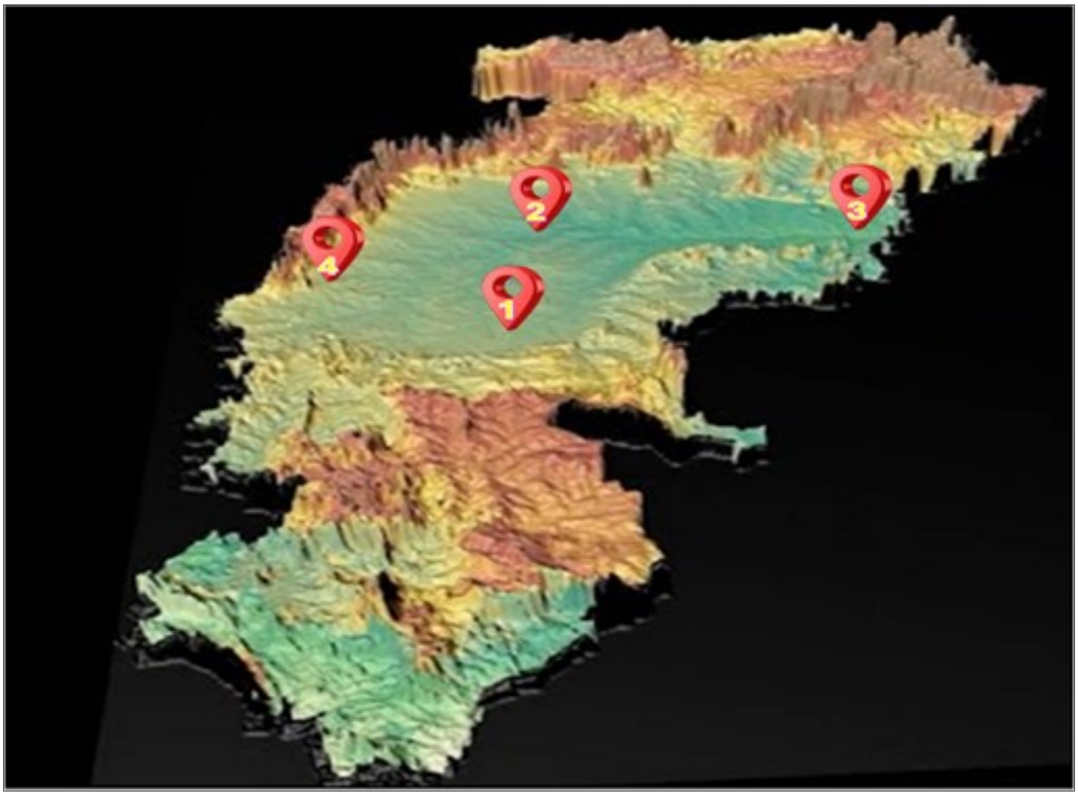


Fig. 1. Locations of the METs of 12 nutrient-rich rice lines in Chhattisgarh [Source: 3D MAP VIEW Chhattisgarh by CGPSCBABA (https://www.youtube.com/watch?v=wr78TSG2mRU)].

1. Raipur; 2. Bilaspur; 3. Raigarh; 4. Kawardha.

These testing sites were selected to represent a range of agro-ecological conditions within Chhattisgarh. The experimental sites are situated at 21°16′ N latitude and 82°55′ E longitude, with an average altitude of 292.98 m above mean sea level (amsl) and an annual average rainfall of 1132.19 mm. The soil type at the site ranges from red-yellow to clay loam, with a pH between 6.8 and 7.1 (Table 2). The single seedling was transplanted at a 15 × 20 cm distance. All the standard recommended cultural practices were followed. Fertilizers were applied as 100 kg N, 60 kg P_2O_5 and 40 kg K_2O ha^{-1}.

The performance of the cultivars was assessed using the parameters like grain yield and grain protein percentage. The grain yield per plot (kg) was recorded and converted to kg ha^{-1}. Grain protein content in milled rice was measured by the Micro-Kjeldahl method. Nitrogen content was calculated and multiplied by a conversion factor of 5.95 to estimate total protein (12).

Statistical analysis

The collected data were first subjected to pooled ANOVA, followed by additive main effects and multiplicative interaction (AMMI) analysis to identify stable genotypes across environments. Pooled ANOVA was conducted to assess the significance of G, E and G × E, which revealed highly significant effects for all three components, thereby justifying the application of AMMI analysis for stability evaluation. The AMMI model, which integrates traditional ANOVA with principal component analysis (PCA), was employed to dissect the GEI effectively.

Multiple comparisons were performed using RStudio (R version 4.5.0), with genotypes treated as fixed effects and environments considered random. Heatmaps for traits under study was calculated using package dplyr (version 1.1.4) (13) and ggplot2 (version 3.5.2) (14). The tidyr package (version 1.3.1) (15) was used to reshape and clean the data, facilitating subsequent analysis and visualization. The ggplot2 package (version 3.5.2) was used to generate GGE biplots (14). Analysis of variance was conducted using the agricolae package (version 1.3-7) (16). Multivariate stability analyses were conducted graphically using GGE and AMMI biplot models in R (version 4.5.0), with the metan package (version 1.19.0) (17) employed for modeling GEIs. These models, based on SVD, are widely used for visualizing interaction patterns and improving predictive accuracy in genotype evaluation. For a deeper understanding of genotype stability, AMMI-based stability indices were also calculated, including the AMMI Stability Value (ASV), the sum of the absolute values of the interaction principal component scores (SIPC) (18) and the Z-statistic (Za) (19). These stability parameters were computed using the AMMI stability package (version 0.1.4) (20) for both grain yield and grain protein content.

Results and Discussion

The present study is based on one year of multi-environment data,

Table 2. Details of environments used for field evaluation of nutrient -rich rice genotypes

Environment	Location	Season	Altitude (m amsl)	Latitude/Longitude	Average rainfall (mm)	Soil type	Soil pH
E1	Raipur	Wet season 2024	289.60	21° 16′ N / 81° 36′ E	1090.68	Red-yellow soil	7.1
E2	Bilaspur		292.30	22° 9′ N / 82° 12′ E	1056.00	Sandy clay loam	6.9
E3	Raigarh		237.00	21° 20′ N / 82° 55′ E	1232.70	Sandy loam	6.8
E4	Kawardha		353.00	22.02° N / 81.25° E	1149.36	Black soil	7.0

the inclusion of diverse and contrasting test environments, ensured that the genotypes were exposed to a broad range of climatic and edaphic conditions representative of the target production regions. This spatial heterogeneity serves as a valid surrogate for temporal variation, coupled with robust G × E interaction analysis using AMMI and GGE biplot models, ensures reliable identification of stable, high-performing genotypes and protein-rich lines.

Comparable approaches have been adopted in rice (5) and groundnut (21). Furthermore, single-year performance indicators such as yield relative to environment (year) maximum (YREM) possess adequate predictive power for multi-year performance, thereby supporting the scientific validity of single-season trials in preliminary genotype evaluation (22).

The distribution of mean yield and protein percentage across the four environments showed an approximately normal pattern with moderate variability among genotypes (Fig. 2, 3 and Table 3). Yield exhibited a wide range, reflecting adequate data dispersion to assess GEI, while protein percentage displayed a near-normal distribution. The continuous distribution of both traits and the lack of extreme outliers indicate that the dataset is suitable for AMMI and GGE biplot analyses to assess genotype performance and stability across environments.

Evaluating genotypes through METs is essential for identifying high-yielding and stable varieties, quantifying GEIs and selecting location-specific genotypes with specific adaptability. Pooled ANOVA conducted for each both traits revealed significant differences among the genotypes for grain yield and protein content, indicating substantial genetic variability within the test material.

The performance of genotypes for yield and protein percentage across environments (Fig. 3, Table 3), provides a clear visual comparison of genotype performance and environmental representativeness. Grain yield ranged from 1351.9 kg ha^{-1} (G12) in E1 to 7432.5 kg ha^{-1} (G4) in E3. Similarly, grain protein content varied from 6.5 % (G5) in E1 to 11 % (G3) in E2. These wide-ranging differences across locations reflect the influence of environmental factors such as temperature, rainfall and other agro-climatic conditions on genotype performance.

The variation in genotypic response is likely attributed to differential gene expression triggered by environmental conditions. Pooled means across environments further emphasized these differences, with grain yield ranging from 2016.3 kg ha^{-1} (G12) to 5838.9 kg ha^{-1} (G9) and protein content ranging from 8.2 % (G2) to 9.7 % (G3). Differential performance of genotypes of rice in relation to different environments has been estimated by earlier group of scientists (23–25).

Combined ANOVA was conducted to partition and quantify the main and interaction effects among genotypes, environments and their interactions (Table 4). The mean sum of squares revealed that the main effect of genotypes, the environmental effect and the GEI showed significant influence on both traits. For grain yield, the environment accounted for the largest portion of the total variation (56.64 %) followed by GEI (40.60 %) and genotypic effect (2.77 %). In the case of grain protein percentage, the GEI contributed the most (50.70 %), followed by the environment (48.89 %), while the genotypic effect was minimal (0.30 %). These results highlight the predominant influence of GEI over the genotypic effect; hence, the application of multivariate methods such as AMMI and GGE biplot analyses is justified for effectively capturing interaction patterns and identifying genotypes with stable performance. Similar findings on the predominance of GEI over genotypic effects have been reported (6, 25).

In the present study, significant GEI was clearly illustrated through AMMI analysis, where the interaction component was successfully partitioned into three principal components. The first two interaction principal components (IPCA1 and IPCA2) captured the majority of the GEI effects among the twelve nutritionally rich rice genotypes evaluated across four environments. Furthermore, the G + GE variation explained by the GGE biplot was 43.37 % for grain yield and 51 % for grain protein content, indicating a substantial contribution of both genotype and GEI to the total variation in these traits. These results are consistent with previous reports (6, 25), confirming the suitability of the AMMI model for analyzing GEI in rice.

Relationships among test environments

Biplot analysis enables simultaneous graphical representation of both genotypes and environments on the same axes, allowing for the visualization of their interrelationships. In an AMMI biplot, displacement along the horizontal axis represents differences in the mean (additive) effects of genotypes or environments, while displacement along the vertical axis reflects variation due to GEI. Genotypes that cluster together exhibit similar adaptive responses across environments, whereas closely grouped environments tend to exert similar influences on genotype performance. A genotype with an interaction principal component axis (IPCA) score close to zero is considered to have minimal interaction with the environment and, therefore, is regarded as stable.

The environment-vector view of the GGE biplot is presented in Fig. 4. This biplot is based on an environment centered (centering = 2), unscaled (scaling = 0) and environment-metric preserving (SVP = 2) G × E matrix, with axes drawn to scale, as per the default settings of the GGE biplot software. The biplots accounted for 93 % of the total variation in grain yield and 80.08 % in protein content, effectively capturing the patterns of G + GE interaction. These visualizations support the identification of genotypes suitable for specific environments and reinforce genotype selection strategies. The findings are consistent with previous reports (26, 27). Similar trends in protein content have also been documented in chickpea (28).

The lines extending from the biplot origin to each test environment are known as environment vectors. The cosine of the angle between any two vectors approximates the correlation between those environments. For grain yield, all environment pairs showed positive correlations as indicated by acute angles between their vectors, except for E2–E3 pair, which exhibited a right angle, suggesting no correlation. In contrast, for protein content, all environments were positively correlated except for E1–E3 pair, which were uncorrelated due to their orthogonal vector relationship. A positive correlation between environments implies that genotypes perform similarly across those locations and a more consistent ranking of genotypes. This reflects a relatively lower level of G × E interaction and supports reliable genotype selection across positively correlated sites.

The distance between vectors reflects the dissimilarity in their ability to discriminate among genotypes. For yield, the environments appeared to cluster into two groups: E1, E2 and E4 formed one group, while E3 stood apart as a distinct environment.

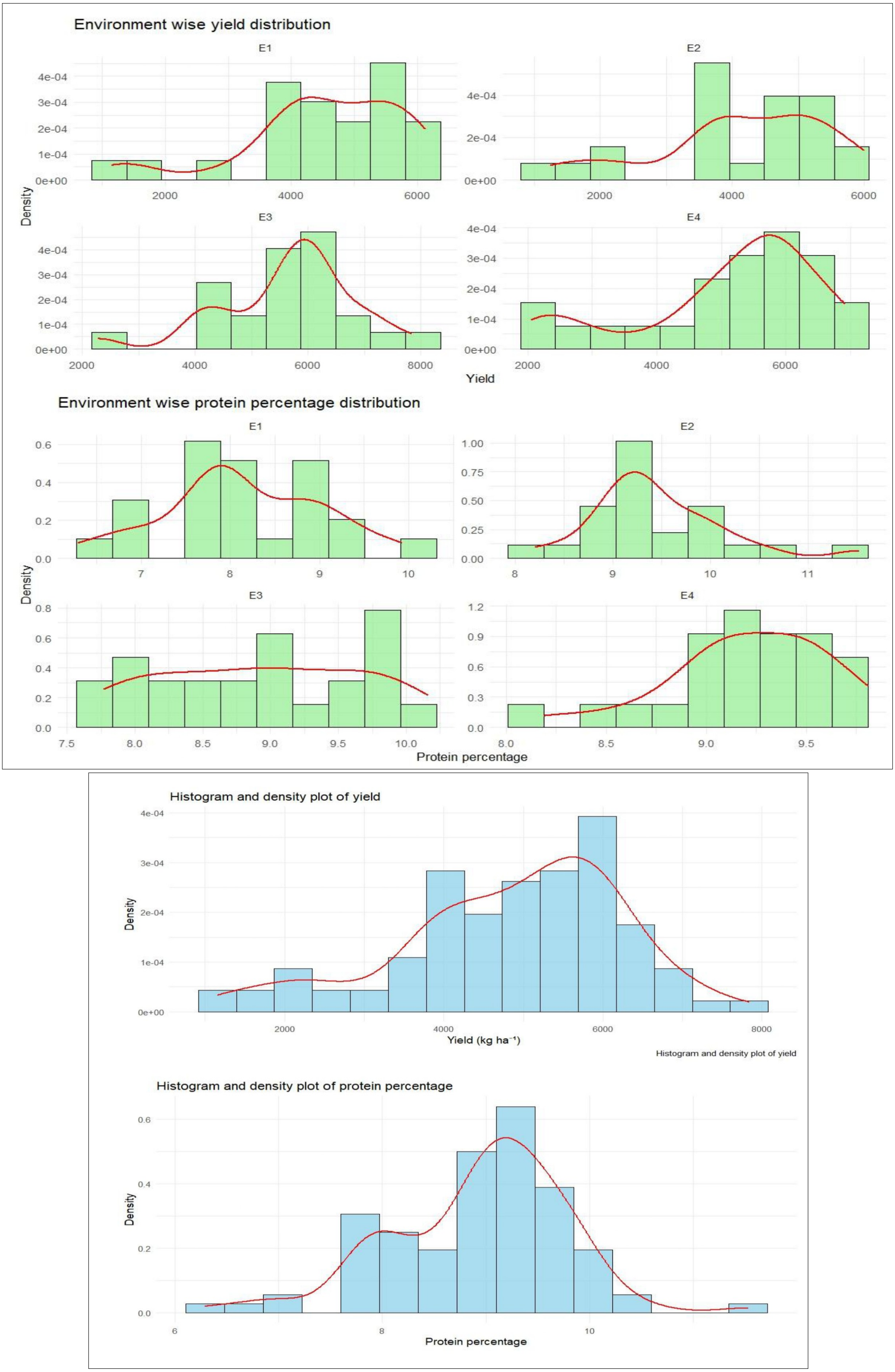
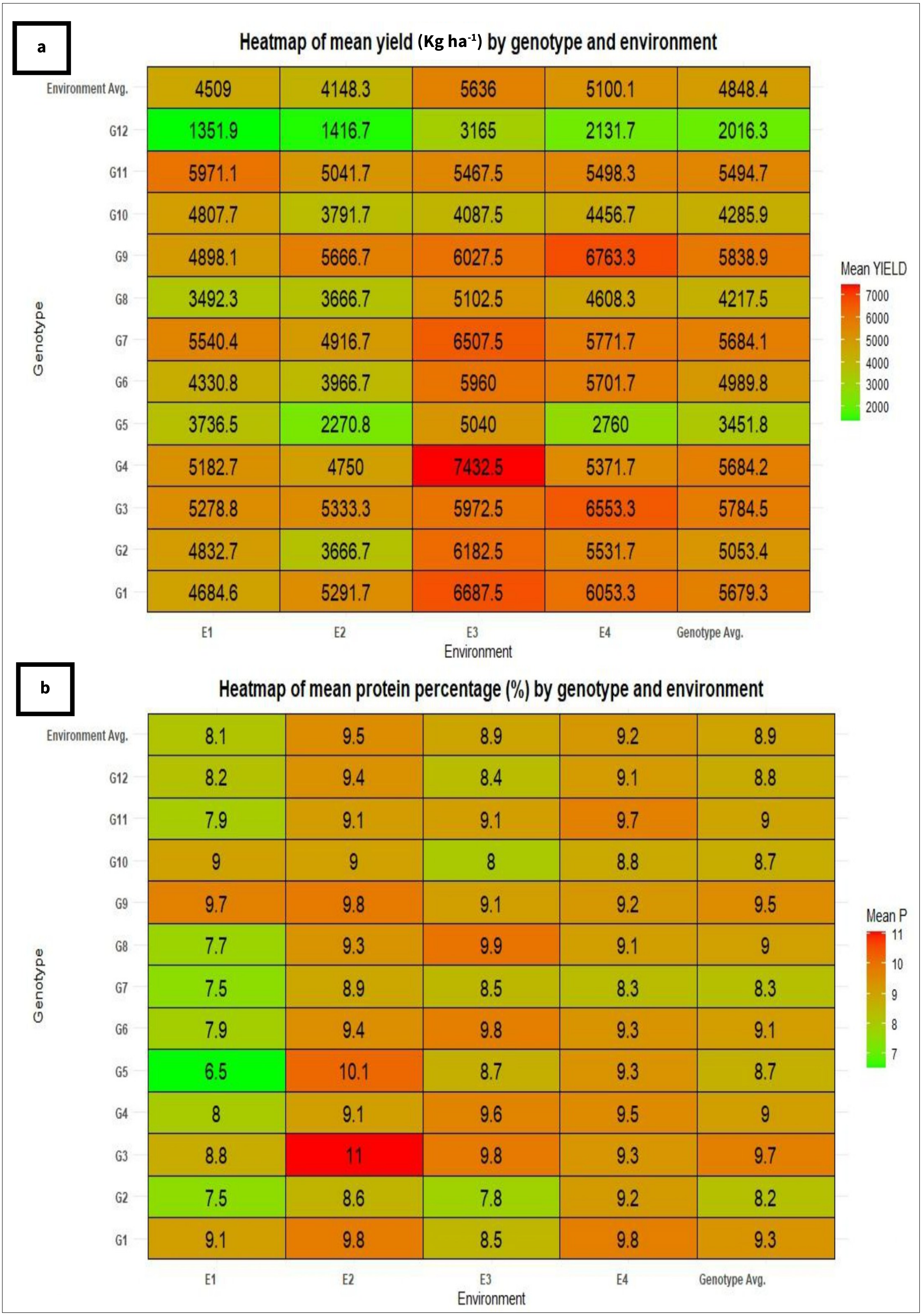


Fig. 2. Distribution pattern of yield and protein content.



	E1	E2	E3	E4	Genotype Avg.
Environment Avg.	4509	4148.3	5636	5100.1	4848.4
G12	1351.9	1416.7	3165	2131.7	2016.3
G11	5971.1	5041.7	5467.5	5498.3	5494.7
G10	4807.7	3791.7	4087.5	4456.7	4285.9
G9	4898.1	5666.7	6027.5	6763.3	5838.9
G8	3492.3	3666.7	5102.5	4608.3	4217.5
G7	5540.4	4916.7	6507.5	5771.7	5684.1
G6	4330.8	3966.7	5960	5701.7	4989.8
G5	3736.5	2270.8	5040	2760	3451.8
G4	5182.7	4750	7432.5	5371.7	5684.2
G3	5278.8	5333.3	5972.5	6553.3	5784.5
G2	4832.7	3666.7	6182.5	5531.7	5053.4
G1	4684.6	5291.7	6687.5	6053.3	5679.3

	E1	E2	E3	E4	Genotype Avg.
Environment Avg.	8.1	9.5	8.9	9.2	8.9
G12	8.2	9.4	8.4	9.1	8.8
G11	7.9	9.1	9.1	9.7	9
G10	9	9	8	8.8	8.7
G9	9.7	9.8	9.1	9.2	9.5
G8	7.7	9.3	9.9	9.1	9
G7	7.5	8.9	8.5	8.3	8.3
G6	7.9	9.4	9.8	9.3	9.1
G5	6.5	10.1	8.7	9.3	8.7
G4	8	9.1	9.6	9.5	9
G3	8.8	11	9.8	9.3	9.7
G2	7.5	8.6	7.8	9.2	8.2
G1	9.1	9.8	8.5	9.8	9.3

Fig. 3. The variations in (a) yield and (b) protein percentage of 12 nutrient-rich rice lines across four environments during kharif 2024-25 cropping season.

Table 3. Mean and stability statistics values of grain yield and protein percentage for nutrient-rich rice genotypes across four tested environments

Genotype	Yield								Protein percentage							
	E1	E2	E3	E4	Mean yield	ASV	SIPC	ZA	E1	E2	E3	E4	Mean protein %	ASV	SIPC	ZA
G1	4684.62 ± 738.46	5291.66 ± 708.33	6687.5 ± 507.5	6053.34 ± 176.66	5679.28	0.10	15.51	0.13	9.07 ± 0.23	9.8 ± 0.09	8.51 ± 0.21	9.75 ± 0.06	9.28	0.53	1.03	0.27
G2	4832.69 ± 594.23	3666.66 ± 166.66	6182.5 ± 227.5	5531.66 ± 711.66	5053.38	8.87	9.73	0.10	7.5 ± 0.46	8.57 ± 0.35	7.78 ± 0.02	9.15 ± 0.07	8.25	0.16	0.91	0.17
G3	5278.84 ± 490.38	5333.34 ± 166.66	5972.5 ± 362.5	6553.34 ± 343.34	5784.50	16.53	21.11	0.22	8.75 ± 0.02	11.04 ± 0.49	9.85 ± 0.06	9.27 ± 0.08	9.73	0.18	1.32	0.25
G4	5182.69 ± 286.54	4750 ± 250	7432.5 ± 407.5	5371.67 ± 215	5684.22	22.67	26.14	0.27	7.96 ± 0.11	9.07 ± 0.17	9.62 ± 0.14	9.5 ± 0.08	9.03	0.29	0.99	0.22
G5	3736.54 ± 55.77	2270.84 ± 104.16	5040 ± 450	2760 ± 236.67	3451.84	25.64	41.95	0.41	6.51 ± 0.22	10.14 ± 0.11	8.67 ± 0.13	9.31 ± 0.2	8.66	0.81	1.76	0.45
G6	4330.77 ± 61.54	3966.66 ± 33.34	5960 ± 115	5701.67 ± 135	4989.78	1.20	12.11	0.11	7.86 ± 0.21	9.41 ± 0.05	9.75 ± 0.16	9.3 ± 0.11	9.08	0.41	0.85	0.22
G7	5540.38 ± 594.24	4916.66 ± 166.66	6507.5 ± 442.5	5771.67 ± 5	5684.06	2.66	7.77	0.07	7.46 ± 0.47	8.87 ± 0.3	8.48 ± 0.58	8.34 ± 0.16	8.29	0.08	0.37	0.07
G8	3492.31 ± 550	3666.66 ± 83.34	5102.5 ± 432.5	4608.34 ± 111.66	4217.45	0.94	10.37	0.09	7.72 ± 0.08	9.29 ± 0	9.94 ± 0.22	9.07 ± 0.06	9.00	0.51	1.23	0.30
G9	4898.08 ± 809.61	5666.66 ± 166.66	6027.5 ± 157.5	6763.34 ± 146.66	5838.89	20.22	34.93	0.34	9.7 ± 0.23	9.84 ± 0.05	9.06 ± 0.04	9.22 ± 0.28	9.46	0.69	1.19	0.33
G10	4807.69 ± 480.77	3791.66 ± 41.66	4087.5 ± 47.5	4456.67 ± 570	4285.88	16.78	39.64	0.37	8.98 ± 0.02	9.02 ± 0.08	8.02 ± 0.01	8.84 ± 0.12	8.72	0.79	0.83	0.29
G11	5971.15 ± 94.23	5041.67 ± 125	5467.5 ± 227.5	5498.33 ± 205	5494.66	12.78	35.19	0.33	7.93 ± 0.08	9.11 ± 0.09	9.09 ± 0.09	9.68 ± 0.13	8.95	0.14	0.78	0.15
G12	1351.92 ± 198.08	1416.66 ± 166.66	3165 ± 890	2131.66 ± 78.34	2016.31	6.30	13.60	0.13	8.23 ± 0.41	9.36 ± 0.03	8.37 ± 0.04	9.14 ± 0.3	8.77	0.25	0.53	0.14

Table 4. Combined analysis of variance for grain yield and protein percentage in nutrient-rich rice genotypes

Source of variation	Df	Yield					Protein percentage				Proportion of Variation
		Sum Sq	Mean Sq	*F* value	Pr(>*F*)	Proportion of variation	Sum Sq	Mean Sq	*F* Value	Pr(>*F*)	
ENV	3	3.09E + 07	10313232	27.3	4.00E - 03	-	23.645	7.8816	204.989	7.81E - 05	-
REP(ENV)	4	1.51E + 06	377823	1.37	2.59E - 01	-	0.154	0.0384	0.409	8.01E - 01	-
GEN	11	1.21E + 08	10989016	39.89	3.06E - 19	-	16.773	1.5248	16.229	4.51E - 12	-
GEN:ENV	33	2.22E + 07	672057	2.44	2.99E - 03	-	24.532	0.7434	7.912	4.60E - 10	-
PC1	13	1.18E + 07	905461	3.29	1.50E - 03	53.1	14.482	1.114	11.86	0.00E + 00	59
PC2	11	8.54E + 06	776085	2.82	7.10E - 03	38.5	5.52	0.5018	5.34	0.00E + 00	22.5
PC3	9	1.87E + 06	207773	0.75	6.62E - 01	8.4	4.53	0.5033	5.36	1.00E - 04	8.4
Residuals	44	1.21E + 07	275517	-	-	-	4.134	0.094	-	-	-
Total	128	2.10E + 08	1639130	-	-	-	93.769	0.7326	-	-	-

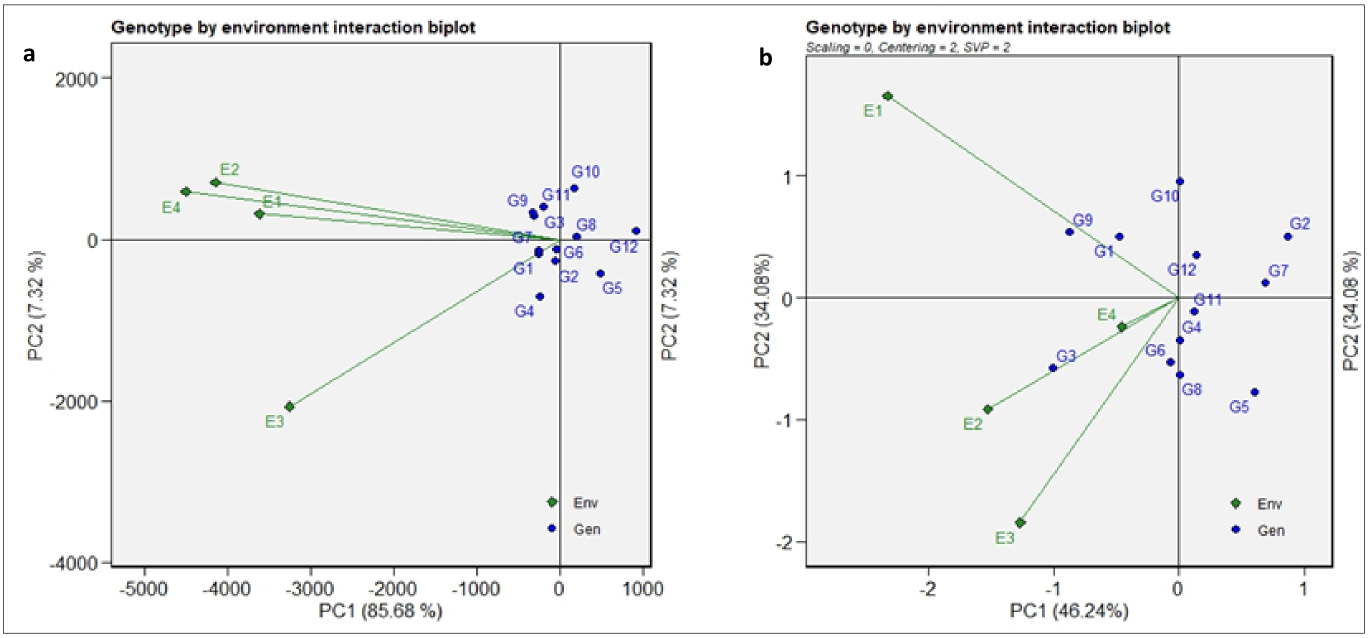


Fig. 4. Environment-vector view of the GGE biplot showing similarities among test environments under four locations for (a) yield and (b) protein percentage.

In the case of protein percentage, E2, E3 and E4 formed a cluster, with E1 behaving independently. The close association among certain environments suggests redundancy in the information they provide regarding genotype performance (24, 27). These findings are in agreement with previous results (29–31).

Discriminating ability and representativeness of test environments

In this study the environment vector view of the GGE biplot (Fig. 5), highlights the AEA, which extends from the biplot origin through the average position of all test environments. This graphical representation facilitates the assessment of the relationships among environments, as well as the discriminating ability and representativeness of each test location (7, 11).

The length of an environment vector reflects the standard deviation of genotypic performance within that environment and serves as an indicator of its discriminating ability. Longer vectors indicate environments that effectively differentiate among genotypes (32). Environments with small angles to the AEA are considered more representative of the overall target environment. Ideally, test environments should be both highly discriminating and representative, as these are most effective for selecting broadly adapted genotypes.

Environments that are discriminating but not representative can still be useful for identifying specifically adapted genotypes, particularly if the target environments can be subdivided into distinct MEs. Within a single mega-environment framework, such environments though non-representative yet highly discriminating be useful for identifying and eliminating unstable genotypes. Conversely, non-discriminating environments represented by very short vectors offer limited value, as they provide minimal information on genotype differences and are therefore less suitable for selection.

The concentric circles in the biplot assist in visually comparing the vector lengths, further illustrating the discriminating capacity of each environment. Based on the analysis, E3 was identified as the most discriminating environment for yield, while

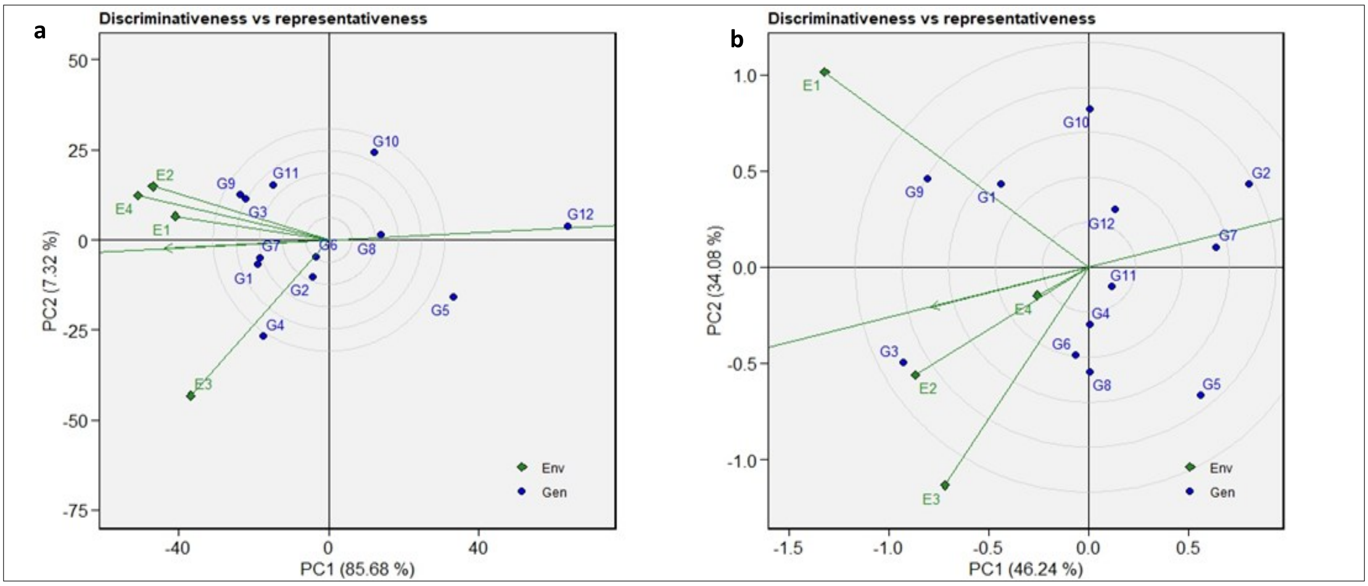


Fig. 5. Discrimination and representativeness view of the GGE biplot showing the discriminating ability and representativeness of the four test environments for (a) yield and (b) protein percentage.

E1 and E3 were the most discriminating for protein percentage. In contrast, E1 (for yield) and E4 (for protein percentage) were found to be the least discriminating environments. Such environments are less informative and may not be suitable for future METs aimed at identifying superior or stable genotypes.

The angle between a test environment's vector and the AEA reflects its representativeness; a smaller angle indicates a higher degree of representativeness of the overall target environment (7). Based on this criterion, E1 and E4 were the most representative environments for yield and protein percentage respectively, whereas E3 (for yield) and E1 (for protein percentage) were identified as the least representative. Environments that are both highly discriminating and representative are ideal for identifying generally adapted genotypes. In this study, E4 was both discriminating and representative for yield, while E2 fulfilled this role for protein content; thus, both environments are valuable locations for genotype selection under broad adaptation. In contrast, E3 (for yield) and E1 and E3 (for protein percentage) were found to be discriminating but not representative. This suggests their potential value in selecting genotypes with specific adaptability, particularly in diverse or stratified environments. Previous studies have also reported environments that are both highly discriminating and representative for yield in biofortified rice and maize (30, 33).

Ideal test environments for selecting generally adapted genotypes

Within a single ME, an ideal test location should be both highly informative (discriminating) and closely aligned with the average environment. In Fig. 6, the "ideal test environment" is located at the centre of the first concentric circle of the GGE biplot and environments closer to this point are considered more desirable. This position corresponds to the positive end of the Average Environment Axis (AEA), indicating the most representative environment. Its distance from the biplot origin equals the length of the longest environmental vector, reflecting the highest discriminatory ability among the environments.

Based on GGE biplot analysis, E4 was closest to the ideal environment and is therefore considered the most suitable for selecting genotypes with broad adaptation for yield, whereas E3 was the least suitable. For protein percentage, E2 emerged as the best environment, while E1 was the least effective for genotype selection. Ideal environments for protein and other quality traits in sorghum and *Vigna* spp. have been reported (34, 35).

Mean performance and stability of the genotypes

Biplot analysis serves as a vital statistical tool for evaluating genotypic performance across diverse environments. The Average Environment Coordination (AEC) facilitates the ranking of genotypes based on both mean performance and stability. The single-arrowed line is the AEC abscissa (or AEA), indicating higher mean performance, whereas the "AEC ordinate," denoted by a line perpendicular to the AEC abscissa and passing through the origin of the biplot, represented genotype stability. Moreover, the stability of the genotype could be accessed through the length of the projection in both directions from the AEC abscissa, that is, the AEC ordinate. Thus, if the genotype had greater projection from the AEC abscissa, it would be less stable (36).

The relationship between mean performance and stability for grain yield and protein percentage is presented in Fig. 7. For grain yield, genotype G9 exhibited the highest mean performance, followed by G3, G7 and G1, while G12 recorded the lowest yield. Genotypes G4, G10 and G5, positioned farthest from the AEC ordinate, were identified as the least stable, while G8, G7 and G1 exhibited greater stability. These results are consistent with previous studies (26, 32, 37).

The highest mean protein content was recorded in G3, followed by G9 and G1, while G2 showed the lowest. Genotypes G5 and G10 were the most unstable, as indicated by their distance from the AEC ordinate, G11 and G7 emerged as the most stable performers, being closest to the AEC. These findings are in line with previous reports in chickpea (28) and rice (31, 38).

Genotype ranking: identification of ideal genotypes

The genotype ranking biplot serves as a powerful visualization tool for identifying superior genotypes based on both mean performance and stability across multiple environments. In GGE biplot analysis, ideal genotypes are those that combine high

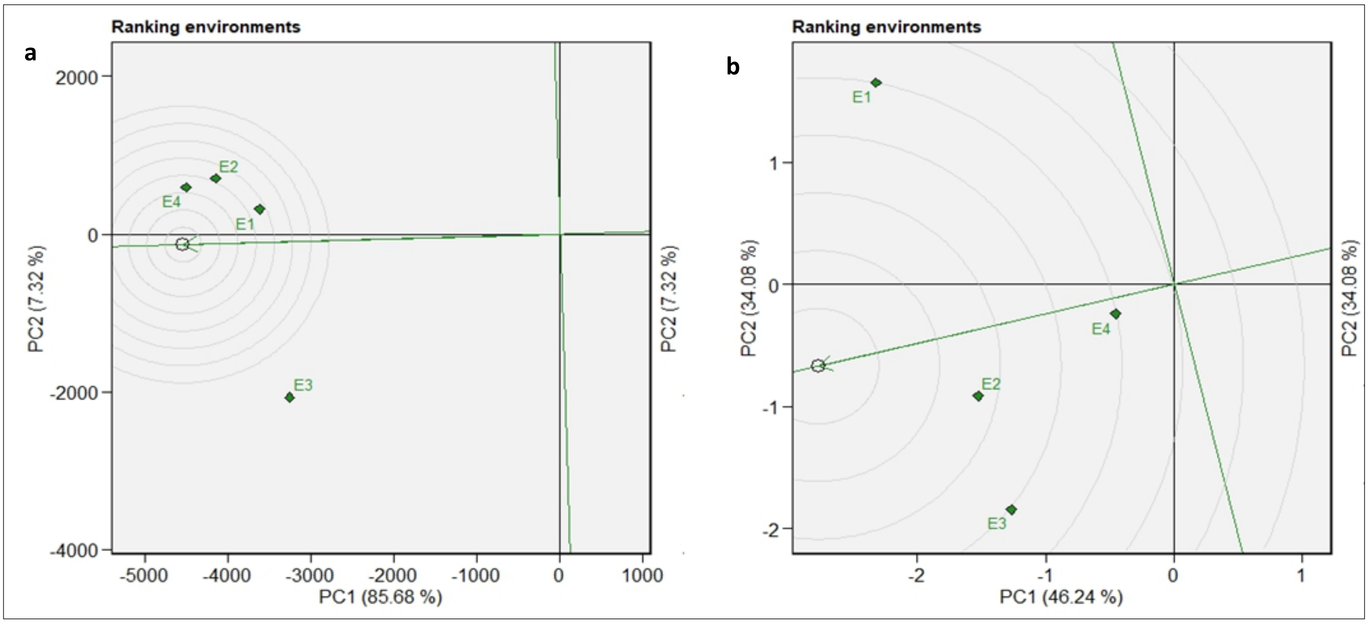


Fig. 6. GGE biplot "environment ranking" pattern for environment comparison with the ideal environment showed the G+G×E interaction effect of 12 nutrient-rich rice genotypes under four locations for (a) yield and (b) protein percentage.

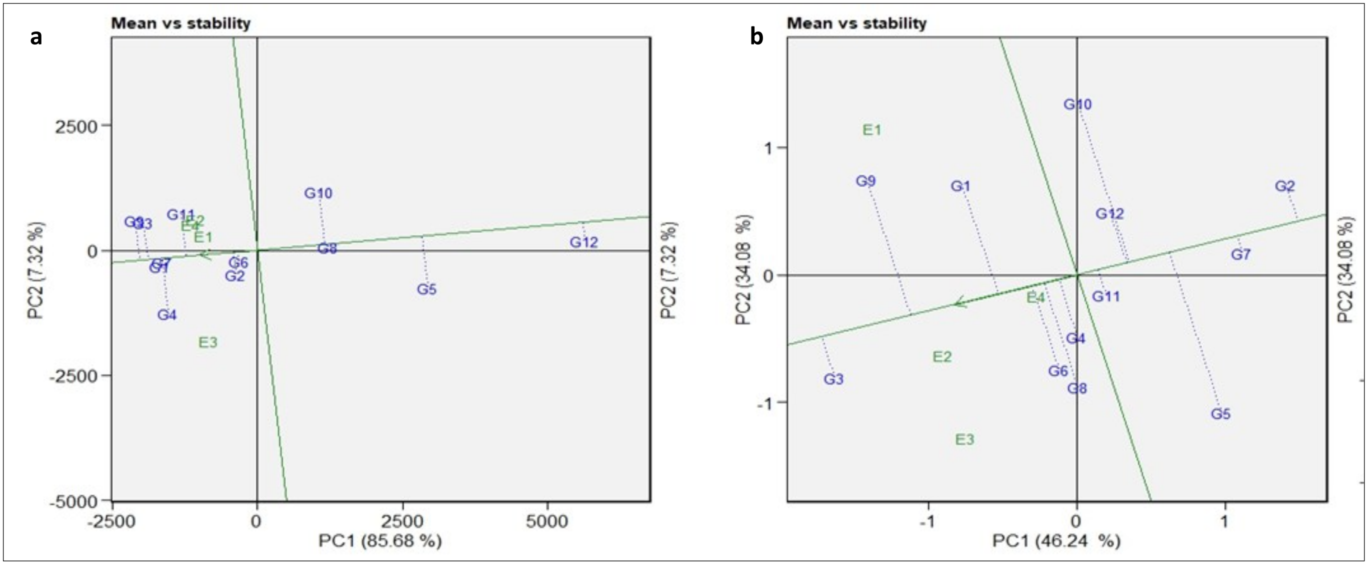


Fig. 7. GGE biplot showing the mean vs. stability ranking of 12 nutrient-rich rice genotypes across four locations for (a) grain yield and (b) protein percentage.

average performance with high stability (7). These genotypes are typically located near the arrowhead on AEC axis and fall within or close to the innermost concentric circle in the biplot. When no genotype lies within this ideal circle, those closest to it are considered most desirable genotypes (11, 26, 32). In the present study G7 and G1 were identified as the most desirable genotypes for grain yield, whereas G3 and G9 emerged as ideal genotypes for protein percentage, based on their proximity to the ideal point in the corresponding biplot (Fig. 8).

These genotypes exhibited both high mean performance and stability, making them suitable candidates for broad adaptation. The importance of selecting genotypes with this dual advantage has also been emphasized in earlier studies (26, 27). Genotypes located farther from the AEC axis exhibited greater variability in performance across environments, indicating lower stability and potential environment-specific adaptation. Conversely, genotypes closer to the AEC axis showed more consistent performance, reflecting better adaptability and higher stability.

In the current analysis, G7 and G8 were identified as the most stable genotypes for yield, while G7 and G11 were the most stable for protein percentage, as evidenced by their minimal deviation from the AEC ordinate. These findings reinforce the utility of GGE biplot analysis in identifying high-performing, stable genotypes suitable for multi-environment cultivation.

Selection of superior genotypes based on additive main effects and multiplicative interaction stability analysis

A set of AMMI-derived stability statistics including the ASV, SIPC and the absolute value of the relative contribution of IPCAs to the interaction (Za) was calculated for both grain yield and protein percentage (Table 3). According to these indices, lower values indicate greater stability of genotypes (11).

For protein percentage, genotypes G7, G11 and G2 were identified as stable according to ASV, while SIPC indicated the highest stability for G7 and G12. Based on the Za value, G7, G11 and G12 emerged as stable genotypes. Regarding grain yield, ASV identified G1, G8 and G6 as stable, whereas both SIPC and Za indicated G7, G8 and G2 as stable genotypes.

Considering all three-stability metrics together, G8 and G2 were identified as stable for yield, while G7 showed stability for

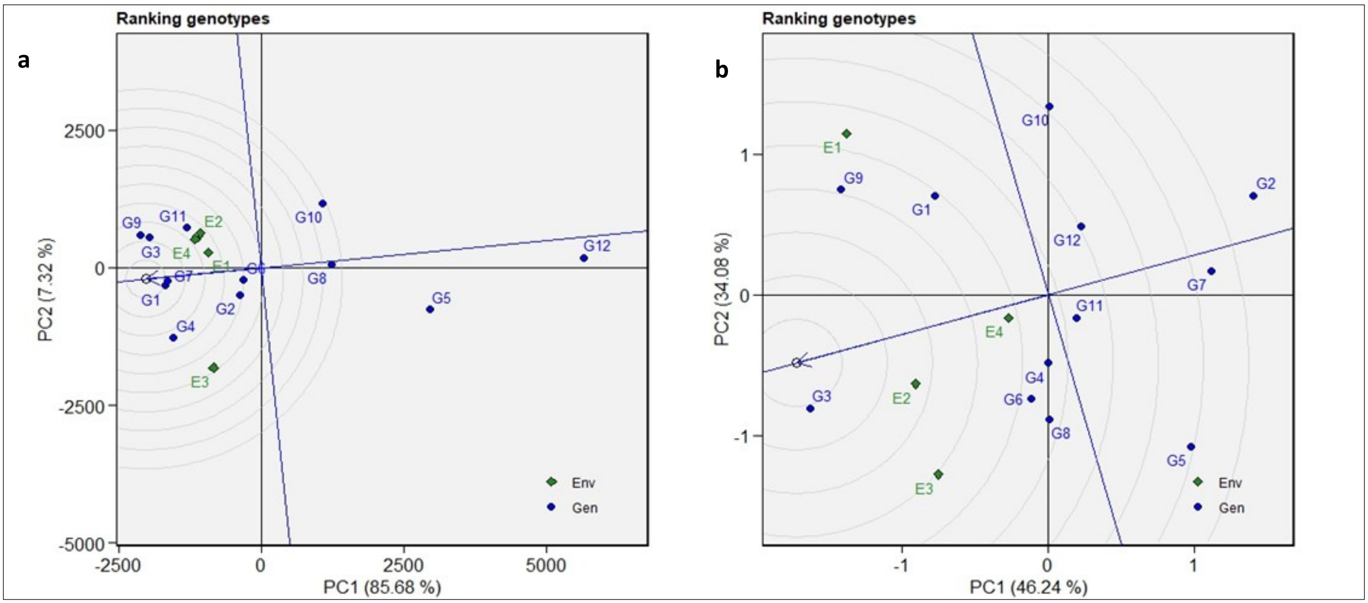


Fig. 8. GGE biplot “ranking genotypes” view for genotype comparison with ideal genotype, showing G + G × E interaction effects of 12 nutri-rich rice genotypes under four locations for (a) yield and (b) protein percentage.

protein content. These findings were consistent with the GGE biplot results. Notably, G7 consistently exhibited above-average performance for both traits and was also identified as highly stable across all environments, highlighting its potential as a superior genotype for multi-location cultivation.

These results suggest that the AMMI-based stability indices largely agreed in identifying stable genotypes, indicating that the use of either a single index or a combination of these parameters is sufficient for effective genotype selection. Comparable findings have been reported in sugarcane (11), barley (39), chickpea (40) and lentil (41).

Biplot analysis for interpreting multivariate data in multi environment trials

By visualizing the "which-won-where" pattern, the GGE biplot provides insights into critical aspects of GEI, including crossover interaction, specific adaptation and regional differentiation. This approach supports targeted breeding by allowing researchers to match genotypes with the environments in which they perform best, thereby enhancing selection efficiency and exploiting specific adaptation. In this method, a polygon is constructed by connecting the genotypes farthest from the biplot origin. These outermost genotypes, located at the vertices of the polygon, are considered the "winning genotypes" in one or more environments as they exhibit either superior or inferior performance, while the remaining genotypes enclosed within the polygon do not show superior performance in any environment (7). This is visually represented in Fig. 9, which highlights the environmental sectors and their respective top-performing genotypes. The GGE biplot analysis effectively partitioned the testing environments into distinct MEs. For grain yield, the four environments clustered into two sectors: E1, E2 and E4 grouped in one sector, while E3 formed a separate sector. For protein content, E1 occupied one sector, with the remaining environments grouped in another.

For grain yield, G4, G5, G12, G10 and G9 occupied the vertex positions of polygon, whereas for protein content, the vertex genotypes included G9, G2, G10, G5 and G3. The perpendicular lines in the biplot represent equality lines, which aid in the visual comparison of performance between neighbouring genotypes. Within each sector, the genotype at the vertex is considered the winning genotype (7). This study demonstrates the utility of GGE biplot in identifying both stable and high-performing nutrient rich rice genotypes across diverse locations.

Genotype G9 emerged as the top performer for yield in environments E2 and E4, while G4 performed best in E3. For protein content, G9 was the leading genotype in E1, whereas G5 showed the poorest performance in the same environment. In E2, G3 ranked highest for protein, while G2 recorded the lowest performance.

The equality lines in the GGE biplot further clarified genotype performance across environments. For yield, the comparison between G9 and G4 revealed that G9 outperformed G4 in E2 and E4, while G4 had an advantage in E3. Similarly, the equality lines between G9 and G10 indicated G9's superior performance across all environments. Genotype G11, situated along the line connecting G9 and G10, consistently ranked between them, implying a yield performance order of G9 > G11 > G10 across all test environments. For protein content, the equality lines between G9 and G3 indicated G9's superiority in E1, while G3 excelled in E2, E3 and E4. In E1 and E3, G9 consistently outperformed G5.

Genotypes located at the vertices of the polygon are generally less stable but may be ideal for specific environments, whereas those positioned closer to the origin tend to be more stable across environments (7, 23). The obtained results align with previous studies (26, 42–46), which observed similar patterns, reinforcing the consistency and reliability of the current findings.

In parallel, the AMMI model has been widely used to analyze METs, effectively partitioning genotype and environment effects while minimizing error associated with GEI. It is also crucial in addressing the impacts of climate change and has the potential to significantly increase rice production in the future (45). Such applications have been demonstrated in previous studies (47, 48) and can be utilized in breeding programs to enhance rice yield and

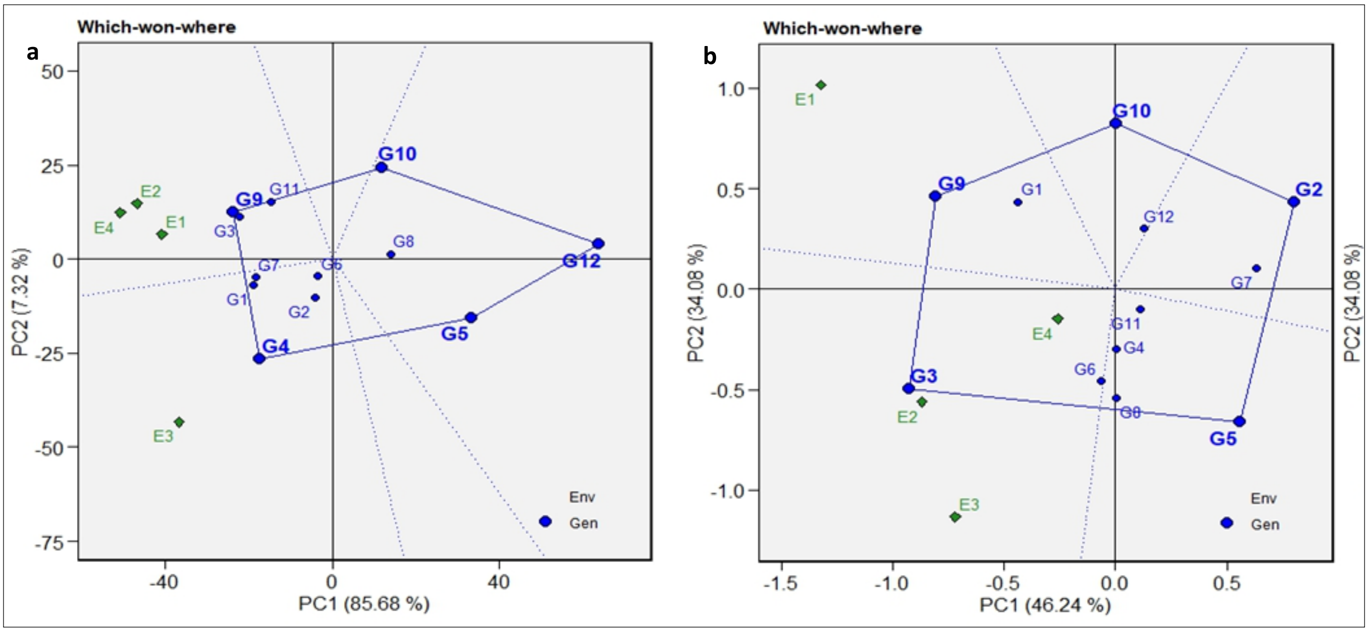


Fig. 9. "Which-won-where" pattern of the GGE biplot polygon view showing the genotype (G) + G × E interaction effects of 12 nutrient-rich rice genotypes under four locations for (a) yield and (b) protein percentage.

grain quality attributes. The utility of GGE biplots in identifying high-performing genotypes and suitable testing locations in coordinated variety testing programs has also been documented in rice (6, 49). Similar findings have been reported in sugarcane (11) and in Bambara groundnut accessions (50).

Conclusion

In the current study, the first two principal components (PC1 and PC2) of the GGE biplot jointly explained 91.60 % of the total variation in grain yield and 81.50 % of the variation in grain protein content. The analysis of "discriminativeness vs. representativeness" of environments revealed that E3 was the most discriminating and E1 the least discriminating for grain yield, whereas for protein percentage, E1 and E3 were the most discriminating and E4 the least. For genotype selection, E1 and E4 emerged as the most representative environments for grain yield and protein percentage respectively. Positive correlations were observed among most environment pairs for both traits, except between E2 and E3 for yield and E1 and E3 for protein percentage. Among the tested environments, E3 recorded the highest grain yield, whereas E2 showed the highest protein content. Genotype G9 was the top performer for yield in most environments, while G3 excelled in protein content across the majority of environments. In addition, high mean yield coupled with strong stability was observed in G7 and G1. For protein content, G11 and G7 were the most stable, though G3 had the highest protein percentage. For grain yield, genotypes G8 and G2 were identified as the most stable. Environment-wise winners for grain yield included G9 in E1, E2, E4 and G4 in E3, whereas for protein content, genotypes G9, G3, G8 and G1 emerged as the best-performing in E1, E2, E3 and E4 respectively. Overall, this study highlights the utility of MET and GGE bi-plot analyses in evaluating genotype performance and identifying stable, high-performing genotypes. Notably, genotype G9 for grain yield and G3 for protein percentage emerged as promising candidates for future breeding programs focused on high yield and nutritional quality through protein bio-fortification.

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Authors' contributions

All authors contributed to the conceptualization and design of the study. Data collection, material preparation and data analysis were carried out by HS and VP. The initial draft of the manuscript was written by HS, VP and SR. HS, GC and SKB critically reviewed the manuscript for intellectual content and overall quality. All authors read and approved the final version of the manuscript.

Compliance with ethical standards

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

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT in order to reframe the sentence. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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