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SHORT RESEARCH ARTICLE

Elucidation of rice (*Oryza sativa* L.) genotypes with high seed protein content using the GGE biplot approach

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Abstract

Rice has low protein content (6–7%), but its seed protein is superior due to better digestibility and hypoallergenic properties. Identification of rice genotypes with naturally rich and constant protein concentrations across environments is an important breeding goal for creating biofortified rice. In this context, the current study was conducted with 20 genotypes selected from the initial panel of 100 diverse genotypes based on their performance for seed protein content (SPC). The field trials were conducted, and the data were generated. GGE biplot analysis was utilized to estimate the stability of the genotypes across sites, offering insights into their performance and adaptability concerning protein content in grain. Significant effects of environment, genotypes, and genotype-environment interaction (GEI) were observed on SPCs. Both crossover and non-cross-over interactions were observed for the trait of interest. Kalinga III was identified as the most ideal genotype for SPC, along with CR Dhan 310 and Kalamahi as desirable genotypes, which can be recommended for breeding programs for developing biofortified rice cultivars to mitigate protein malnutrition.

Keywords: Hypoallergenic, seed protein content, biofortified rice, GGE biplot

Introduction

Globally, around 149 million children under five suffer from stunted growth, 45 million from wasting, and 38.9 million are overweight or obese (Katoch 2022). Undernutrition causes 45% of deaths in this age group, especially in countries like India. Protein-energy malnutrition (PEM) is a major concern in cereal-based diets. Plant-derived proteins offer a cost-effective, readily available alternative to animal proteins, with their effectiveness depending on extraction technology (Kumar et al. 2021). Rice grains typically contain 80%-85% starch, 4 to 10% protein, 1% lipid, and 10% moisture (Balindong et al. 2018). Although rice protein (6–7%) is lower than maize (10%) and wheat (12%-15%), it has the highest net protein utilization (Juliano 1992). Valued for its hypoallergenic properties and high nutritional quality, rice protein is used as a food supplement (Cao et al. 2009). Various studies have shown significant variation in seed protein content among rice genotypes. Gomez (1979) reported brown rice protein content of 4.3 to 18.2% (average 9.5%), while Kim et al. (2013) and Chattopadhyay et al. (2018) reported 4.01 to 14%. Genetic factors influence the protein content but are also significantly impacted by environmental conditions and soil factors like nitrogen (Cheang and Mohan Rao 1972). Analyzing genotype-by-environment (G × E) interactions helps identify stable genotypes and ideal test sites, making multi-location trials more effective and economical (Das et al. 2019; Bhattacharya et al. 2022; Chatterjee et al. 2023). The GGE biplot has become a popular tool for evaluating genotype-by-environment (GE) interactions, focusing on genotypic effects and their interactions rather than the direct influence of the environment (Yan et al. 2000). Researchers have used the GGE biplot to identify stable genotypes with high seed protein content in various crops (Subedi et al. 2021; Lee et al. 2023). The present investigation was aimed at determining

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Table 1. ANOVA for seed protein content (%) of 20 rice genotypes assessed at six locations during year 1 (2021) and year 2 (2022)

Source of variation	Df	Seed protein content (SPC)		
		Mean Sq	p-value	%contribution
Environment (E)	5	165.83	<0.0001	52.55
Genotype (G)	19	136.52	<0.0001	43.26
Genotype ×Environment (G× E)	95	13.21	<0.0001	4.19
Residuals	228	0.415		

* *p* <0.05, ** *p* <0.01

genotype × environment (G × E) interactions in selected rice genotypes having differential seed protein content and to identify the genotypes giving consistent performance across the environment.

A total of 100 rice genotypes were initially evaluated for seed protein content during the 2019-2020 monsoon season at the Instructional Farm of Bidhan Chandra Krishi Viswavidyalaya (BCKV), Jaguli, West Bengal. Based on protein content, 20 cultivars (15 with high protein and 5 with low protein) were selected for multi-site, multi-year evaluation during the 2021 and 2022 monsoon seasons to study G × E interactions. The trial locations included the BCKV extended campus at Burdwan, the Regional Research Sub-Station (RRSS) at Chakdah, and the Instructional Farm of the Faculty of Agriculture, BCKV, Jaguli (Supplementary Table S1). Genotypes were transplanted with 20×15 cm spacing using 30-day-old seedlings. The recommended fertilizer dose and other package and practices were followed to raise a healthy crop. After harvesting, rice seeds were threshed, sun-dried to below 12% moisture, and stored in cloth bags to prevent contamination. Seed protein content (crude protein) was measured using the Micro-Kjeldahl method (Johri et al. 2000). Combined ANOVA using R Studio (R Development Core Team 2012) assessed the effects of genotype (G), environment (E), and their interaction (GEI). GGE biplot analysis, including AEC, «discriminating power vs. representativeness,» and «which-won-where» views, was used to evaluate genotype performance, stability and identify ideal environments.

GGE biplot analysis

Mean performance of 100 rice genotypes regarding agronomic as well as seed protein content is shown in Supplementary Table S2. The seed protein of 100 rice genotypes ranged from 3.69 to 12.60% with an average of 6.85%, whereas the grain yield/plant of the tested genotypes ranged from 5.53 to 21.39 g with an average value of 11.26 g. The combined ANOVA revealed highly significant effects ($p < 0.01$) of environment, genotypes, and genotype-environment interaction on seed protein content (Table 1). Environmental factors accounted for 52.55% of the variation, followed by genotypes (43.26%) and genotype-environment interactions (4.19%). Environmental influences have been recorded earlier in G × E interaction studies in several crops (Banik et al. 2024; Singh et al. 2024). The findings of the present study align with previous studies conducted earlier (Obua et al. 2021) and highlight that the environmental variation constituted the highest source of variability.

Kalinga III had the highest average SPC at 13.41%, while Bankut had the lowest at 4.07% (Table 2). Seed protein was highest in E3 (10.92%) and E4 (10.22%), and lowest in E6 (7.78%) and E2 (6.54%). The occurrence of crossover interaction (COI) was evident in certain genotypes as they exhibited fluctuating positions across different sites. However, some genotypes showed consistent performance, indicating the lack of crossover interaction (non-crossover) across different sites. Similar findings of both crossover and non-crossover interactions were also noted in this study, consistent with prior research (Naik et al. 2020; Tamang et al., 2022).

The AEC biplot showed the mean performance and stability of genotypes across multiple sites (Fig. 1). Principal components 1 and 2 explained 88.87% and 4.4% of the variability in seed protein content (SPC). Genotypes like Kalinga III (G10), Bindli (G4), and CR Dhan 310 (G8) were located towards the «AEC abscissa,» indicating higher SPC. Genotypes such as Bankut (G3) and Motibasant (G14) had lower SPC and were positioned to the left of the origin. The «AEC ordinate» reflected genotype stability, with shorter projections indicating better stability. Kalinga III (G10) was the most ideal, with high SPC and good stability. Genotypes CR Dhan 310 (G8) and Kalamahi (G9) were identified as desirable because they exhibited higher SPC and showed consistent performance, being positioned near the ideal genotype (Yan and Tinker 2006). Further, all the tested genotypes, as per their seed protein score, were classified into six primary clusters: Cluster I included 8 genotypes, Cluster II comprised of 3 genotypes, Cluster III contained 2 genotypes, Cluster IV consisted of 2 genotypes, Cluster V encompassed 4 genotypes, and Cluster VI included one genotype (Supplementary Fig. 2).

The GGE biplot illustrates the relationship between test environments using environment vectors. Acute angles between environment vectors indicate a positive correlation, and throughout the year, all six sites exhibited positive correlations. In the GGE biplot approach, an 'ideal'

Table 2. The mean seed protein content (%) of 20 rice genotypes varied at six locations during the years 2021 and 2022

Genotype Code	Genotypes	E1	E2	E3	E4	E5	E6	Mean value	LSD
		Seed protein content (%)							
G1	Altaluti	4.2	3.48	7.12	5.37	3.22	3.84	4.54	n
G2	Balam	7.89	8.82	11.24	11.26	7.08	6.55	8.81	l
G3	Bankut	5.57	1.89	6.4	4.42	3.64	2.47	4.07	p
G4	Bindli	12.36	13.68	13.05	13.24	12.93	12.81	13.01	b
G5	Burma Black	10.7	9.44	12.51	10.64	7.81	7.46	9.76	f
G6	Chakhao	12.22	10.78	11.82	13.56	12.07	12.84	12.22	c
G7	Chamarmoni	9.69	7.82	12.09	10.62	7.86	6.84	9.15	ij
G8	CR Dhan 310	11.81	9.58	12.3	12.56	11.15	10.75	11.36	d
G9	Kalamahi	10.95	7.61	10.75	13.07	8.09	10.4	10.14	e
G10	Kalinga III	14.1	11.18	13.9	14.05	13.88	13.37	13.41	a
G11	Kalonuniya	10.06	4.76	13.13	10.56	8.65	9.81	9.5	gh
G12	Laljeera	7.95	5.62	12.3	11.8	8.72	8.11	9.08	k
G13	Mahasugandhi	12.71	5.94	11.5	11.53	8.41	6.81	9.48	h
G14	Motibasant	7.77	2.08	7.49	7.56	2.65	3.56	5.19	m
G15	Normal Joha	9.77	5.84	11.64	11.19	7.4	9.37	9.2	i
G16	Pakuri	4.42	2.49	7.05	4.58	4.27	3.46	4.38	o
G17	Radhatilak	9.37	5.36	11.78	11.27	9.77	9.86	9.57	g
G18	Sarbati	5.37	2.49	7.76	5.16	2.99	3.25	4.5	n
G19	Sitabhog	9.62	5.6	12.48	10.78	8.64	7.43	9.09	jk
G20	Talmari	9.33	6.28	12.05	11.17	7.47	6.69	8.83	l
Mean		9.29	6.54	10.92	10.22	7.84	7.78		
Range	Minimum value	4.2	1.89	6.4	4.42	2.65	2.47		
	Maximum	14.1	13.68	13.9	14.05	13.88	13.37		
LSD(5%)		1.07	0.90	1.21	0.95	1.04	1.17		
CV%		7.07	8.29	6.68	5.64	8.02	9.09		

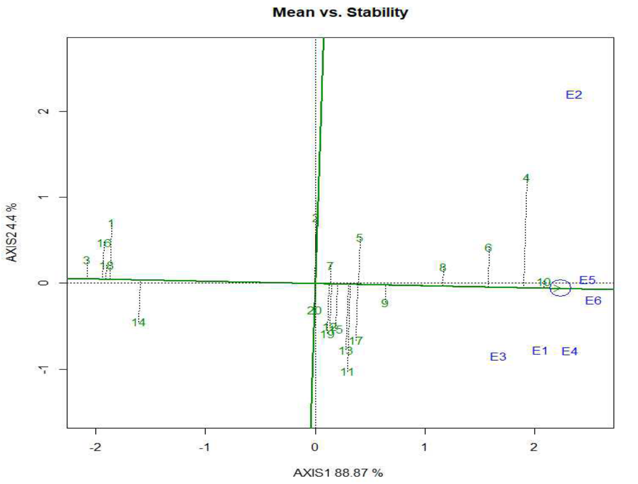

Fig. 1. Mean vs. stability view of the GGE biplot of 20 genotypes for seed protein content across 6 testing locations.

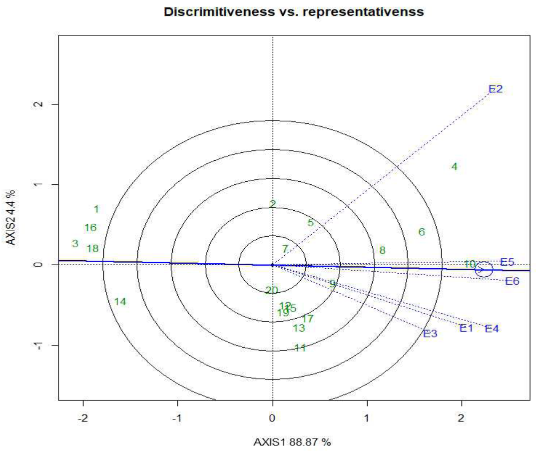

Fig. 2. Discriminative vs representativeness view of testing location based on GGE biplot of 20 rice genotypes regarding seed protein content.

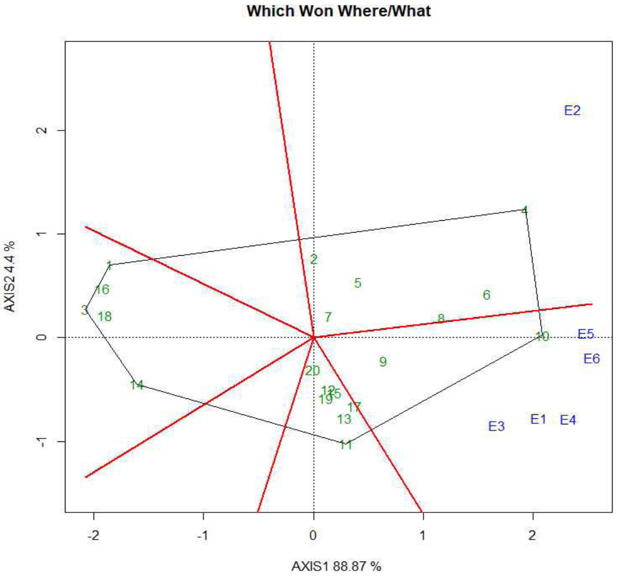

Fig. 3. The «Which-Won-Where» View of the GGE Biplot depicts the performance of 20 rice genotypes across 6 testing locations regarding seed protein content

test environment is chosen based on its ability to effectively discriminate between genotypes, its representativeness, and a high desirability index (Xu et al. 2014). The «discrimination power» of a test environment is indicated by the length of its vector, with longer vectors showing better ability to differentiate genotypes. E2 (Chakdah 2021) had the longest vector, followed by E6 (Jaguli 2022), making them the most discriminating environments (Fig. 2). The «representativeness» of an environment is determined by the angle with the AEC, with E5 (Chakdah 2022) and E6 (Jaguli 2022) being the most representative. E6 was identified as the best environment for both discrimination power and representativeness. The «desirability index» combines these factors, and E6 (Jaguli 2022) had the highest index (3.96), making it the ideal site for evaluating genotypes with general adaptability (Supplementary Table S3).

The ‘which-won-where’ graph is a 2-D polygonal representation used to identify mega-environments and genotypes with specific adaptability. Genotypes at the vertices of the polygon show either the highest or lowest performance across environments. In this study, genotypes such as Altaluti (G1), Bankut (G3), Motibasant (G14), Kalonuniya (G11), Kalinga III (G10), and Bindli (G4) were positioned at the vertices (Fig. 3). Mega Environment 1 (ME 1), represented by E2 (Chakdah 2021), had Bindli (G4) as the winning genotype. Mega Environment II (ME II), including E1, E3, E4, E5, and E6, had Kalinga III (G10) as the winning genotype.

In this study, all test locations were divided into two unique mega-environments, revealing top-performing genotypes and indicating crossover-type G × E interactions. This highlighted the need for breeding programs to focus on specific adaptability. Detecting mega-environments effectively aids in evaluating genotypes and test environments, suggesting optimal deployment strategies for specific genotypes within each location (Yan and Tinker, 2006). Therefore, this research study offers significant new insight into the considerable variability in SPC in rice and highlights the significant role of genotype-environment interaction on trait expression. The ideal genotypes identified, such as Kalinga III, hold promise for the development of rice varieties rich in protein content.

Supplementary materials

Supplementary Tables S1 to S3 and Supplementary Fig. 1 are provided and can be accessed at www.isgpb.org.

Authors’ contribution

Conceptualization of research (AR, AD); Designing of the experiments (AD, SSA); Contribution of experimental materials (AR, AD); Execution of field/lab experiments and data collection (AK, SSA, JD, KP); Analysis of data and interpretation (SSA, KP, SSD); Preparation of the manuscript (AK, AD, JD, AR).

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Supplementary Table S1. An overview of the test environment in India

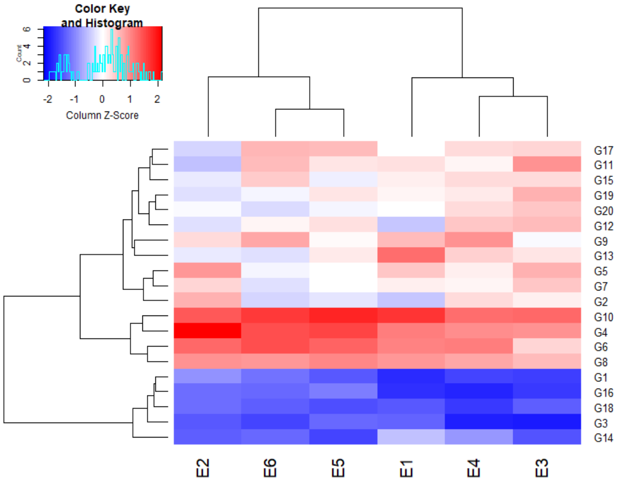
Testing sites	Year	Latitude	Longitude	Rainfall (mm)	Temp (°C)		Relative humidity	
					Min	Max	Min	Max
E1(Burdwan)	2021	23º27´ N	87º93´ E	45.86	23.6	30.3	62.8	91.5
E2(Chakdah)	2021	23º08´ N	88º53´ E	40.98	21.1	26.2	69.8	90.7
E3 (Jaguli)	2021	22º93´N	88º55´ E	39.47	21	28.7	70.0	93.8
E4 (Burdwan)	2022	23º27´N	87º93´ E	32.38	22.5	31.4	71.8	98.6
E5 (Chakdah)	2022	23º08´ N	88º53´ E	25.1	21.0	31.0	72.4	96.6
E6(Jaguli)	2022	22º93´ N	88º55´ E	26.59	21.2	31.1	75.0	95.9

Supplementary Table S2. Descriptive statistics regarding seed protein content in 100 rice genotypes

Parameter	Mean	Range		GCV	PCV	Heritability	GA as % over Mean
		Min	Max				
Seed protein content (%)	6.85	3.69	12.60	13.49	13.61	92.32	27.61
Grain Length/breadth	3.21	1.95	5.59	19.96	21.62	85.17	38.00
Test weight(g)	17.94	10.6	28.08	24.27	24.50	94.07	49.57
Grain yield/plant(g)	11.26	5.53	21.39	28.79	29.83	81.14	47.33

Supplementary Table S3. Evaluation parameters for standardized test sites

Environment	Discriminating power	Representativeness	Desirability index
E1	4.03	0.93	3.73
E2	4.13	0.90	3.70
E3	3.97	0.92	3.66
E4	4.07	0.90	3.67
E5	4.02	0.95	3.83
E6	4.10	0.97	3.96



Supplementary Fig. 1. Hierarchical cluster analysis showing the relationship between 20 tested genotypes for seed protein in 6 testing locations