PLANT SCIENCE TODAY
Vol 13(sp1): 1-13
https://doi.org/10.14719/pst.12264

eISSN 2348-1900

RESEARCH ARTICLE

Pre-breeding evaluation of rice genotypes for biofortification in Telangana region

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Received: 14 October 2025; Accepted: 08 February 2026; Available online: Version 1.0: 08 April 2026

Cite this article: Dubbala PDV, Narkhede GW, Thota S, Akshitha NY, Sharanabasav H, Subhashree D. Pre-breeding evaluation of rice genotypes for biofortification in Telangana region. Plant Science Today. 2026; 13(sp1): 1-13. https://doi.org/10.14719/pst.12264

Abstract

In India, rice (*Oryza sativa* L.) is the primary staple food consumed by a significant percentage of its population, but the polished grain of rice is low in essential micronutrients, including iron (Fe) and zinc (Zn), which contributes to micronutrient malnutrition in a huge percentage of the Indian population. The aim of the current research was to assess genetic variation, agronomic performance, grain quality traits and micronutrient content of famous rice genotypes grown in Telangana and Andhra Pradesh regions with the aim of finding an appropriate donor to practice genetic biofortification. Evaluation of 16 rice genotypes was done at the kharif season in 2024 under irrigated conditions and with a randomized block design in 3 replications. The analysis of variance, the parameters of genetic variability, correlation and path coefficient analysis and principal component analysis (PCA) were used to analyze 18 agronomic, quality and nutritional traits. The genetic variation of most traits was found to be highly significant ($p<0.01$). Both grain iron and zinc levels showed a moderate level of genotypic coefficient of variation (12.50 % and 14.05 %, respectively) with high heritability and genetic progression, which mean that it is highly genetically controlled. Iron and zinc contents had a strong positive relationship (r = 0.65), which implied that it would be feasible to improve simultaneously. Principal component analysis indicated that the four major components could account 82.2 % of the overall variation and the loading of iron and zinc was also high in the second major component. KNM 118 and Bhadrakali had the best iron (15.8 and 14.5 mg kg^{-1}) and zinc (28.3 and 27.0 mg kg^{-1}), whereas KNM 1638 and Varalu had better grain yield and productivity. The findings show that there is a genetic variability that can be exploited to biofortify rice with micronutrients in popular rice genotypes and indicate potential donor lines to generate high yield, iron and zinc-enriched rice varieties. These genotypes can also be used in targeted breeding programmes and confirmed by multi-location trials that can be used to enhance nutritional sustainability.

Keywords: biofortification; correlation; genetic variance; heritability; iron; *Oryza sativa*; phenotype; principal component analysis; zinc

Introduction

Rice (*Oryza sativa* L.) is a short-day, C_3 plant that is primarily cultivated in the agroecosystems of Asia. It belongs to the family Poaceae (Graminae) and is an annual, semi-aquatic, self-pollinating crop. There are 2 main domesticated species of rice: *O. sativa* (Asian rice) and *Oryza glaberrima* Steud. (African rice), both possessing the AA genome (2n = 24) (1). In India, rice plays a pivotal role in agriculture and food security, accounting for 20–25 % of total agricultural production and acting as a staple food for more than half of the population (2). Globally, rice provides a major proportion of daily caloric intake providing essential nutrients, carbohydrates, proteins, vitamins and minerals (3).

Despite its importance, polished rice grains tend to have low levels of these vital micronutrients which are further depleted during milling and processing resulting in insufficient dietary intakes compared to the recommended daily requirements of the human body which varies from 8 to 27 mg iron and 8 to 12 mg zinc among various populations (4, 5). Consequently, there is a high risk of iron and zinc nutritional deficiencies in those populations that largely rely on rice-based diets. It is studied that about 30 % of the world's population are Zn deficient and about 80 % are Fe deficient among which 30 % suffer from long term deficiency effects (6). In India, where rice is consumed by a high proportion of the population daily, micronutrient malnutrition is still a severe health concern.

Biofortification through plant breeding is a sustainable low-cost approach to solve hidden hunger by increasing the micronutrient level of staple crops without sacrificing the yield and acceptability to consumers (7, 8). The success of biofortification programs, however, relies on the presence of genetically diverse, locally adapted donor genotypes that can combine high micronutrient density with preferred agronomic as well as grain quality characteristics (9, 10). Some common and widely grown rice genotypes are desired by farmers and consumers in some parts of the country like Telangana and Andhra Pradesh because of the potential yield and quality of the grain, but the information about the iron and zinc content, genetic diversity and relations with yield and quality traits are limited.

In this regard, pre-breeding screening of popular rice genotypes is vital to determine promising donor lines to enhance a particular trait of enriching the nutritional value and yield. Simultaneously, evaluation of agronomic characteristics is important to be acceptable by the farmers and grain quality indicators like amylase content, gel consistency and protein content are crucial for consumer preference. Knowledge of the genetic variability, heritability and association of this yield, quality and nutritional traits contribute in developing effective methods of selection in biofortification based breeding programs.

Hence, the current study was carried out to compare 16 common rice genotypes that had been cultivated in Telangana and Andhra Pradesh for their agronomic performance, grain quality parameters and iron and zinc concentration. The objective of the study was also to examine the genetic variability, trait association and principal components to identify appropriate donor genotypes for the development of high yielding, iron and zinc enriched rice varieties that can grow in optimum local environment.

Materials and Methods

Experimental site

This research was conducted in the Instrumental Farm, School of Agriculture, SR University, Warangal. The work was carried out during the kharif season of 2024 from July to December in irrigated conditions.

Experimental material

For this research we used 16 popular and most promising lines produced and used in Andhra Pradesh and Telangana states of India. The rice genotypes used in this study are as mentioned in Table 1. The grains of the genotypes studied in the research with variability are shown in Fig. 1. The traits under study and their details are mentioned in Table 2. The experiment was laid in a randomized block design in 3 replications.

Standard agronomic practices recommended for irrigated rice cultivation of Telangana region were followed for the experiment in all plots. The seedlings were raised in a wet bed nursery and were transplanted to a well prepared and leveled main field 25 days after sowing. One seedling per hill was transplanted in the main field. Fertilizers and irrigation were provided as per regional recommendations to maintain a healthy crop. Pest, disease and weed management were carried according to necessity throughout the field.

Grain samples from each genotype were collected post-harvest, cleaned and were prepared for analysis. Iron and Zinc contents were analysed using X-ray fluorescence spectroscopy (XRF) at the Indian Institute of Rice Research (IIRR), Hyderabad. Standard non-destructive analytical and calibration procedures were performed to obtain the iron and zinc concentrations expressed in ppm (equivalent to mg/kg on a dry weight basis). Grain quality traits were estimated using laboratory procedures specific for each analysis. Amylose content was determined by using iodine colorimetric method expressing the results in percentage. Gel consistency was analysed by alkali digestion method and protein content was estimated using the Kjeldahl method.

Table 1. Genotypes used in the study and their parentage

Sl. No	Genotype	Another name	Parentage
1	BPT 5204	Samba Mahsuri	GEB 24/TN1/Mahsuri
2	Jai Sri Ram	-	HMT
3	RNR 15048	Telangana Sona	MTU 1010/JGL 3855
4	MTU 1001	Vijetha	MTU 5249/MTU 7014
5	KNM 118	Kunaram Sannalu	JGL 13595/MTU 1010
6	BPT 3291	Sona masuri	Sona/Mahsuri
7	Kavya	MTU 1121	BPT 5204/MTU DP 13
8	KNM 1638	KUNARAM VARI-02	JGL 11727/JGL 17004
9	MTU 1010	Cottondora Sannalu	Krishnaveni/IR64
10	Varalu	WGL-14377	WGL 20471/CR 5441/2
11	RNR 31479	Telangana sona	RNR 15048/Jai sri ram
12	Bhadrakali	WGL-3962	Phalguna/IR36
13	Erra Mallelu	WGL-20471	BC 5/W12708
14	Somnath	WGL- 347	-
15	Siddi	WGL 44	BPT 5204/ARC 5984- /Kavya
16	MTU 1064	Amara	PLA 1100/MTU 1010

Table 2. Observations under study

Sl. No.	Trait of Study	Trait Acronym	Units
1	Days to 50 % flowering	DTF	count
2	Plant height (cm)	PH	cm
3	Productive tillers per plant (no)	TPP	count
4	No. of panicles (no)	NOP	count
5	Panicle length (cm)	PL	cm
6	Grains per panicle (no)	GPP	count
7	1000-grain weight (g)	ThGW	gm
8	Grain yield per plant	GYPP	gm
9	Productivity (g)	PTY	gm
10	Seed length (mm)	SL	mm
11	Seed width (mm)	SW	mm
12	Seed thickness (mm)	ST	mm
13	Length/width ratio	LW	ratio
14	Amylose content	AMLS	%
15	Gel consistency	GLC	mm
16	Protein	Protein	%
17	Iron content	Fe	mg kg^{-1}
18	Zinc content	Zn	mg kg^{-1}

Fig. 1. Genotypes used in study.

Statistical analysis

Data collected for all the traits under study was analyzed using R Studio. Analysis of variance (ANOVA) was performed for randomized block design using the package agricolae. The significance of correlation coefficients calculated at n-2 degrees of freedom at two levels of probability, 5 % and 1 %. The estimated values compared with the tabulated values of Fisher and Yates (11). Path coefficient analysis, as suggested by Wright (12, 13) and further explained by Dewey and Lu (14), was employed to determine the direct and indirect contributions of various traits toward the total correlation coefficient with grain yield for significant variance and then was studied for correlation and heritability. Correlation and path coefficient analysis was done using lavaan package. Principal component analysis (PCA) was performed using FactoMineR and factorextra packages based on the correlation matrix to identify major contributing traits and patterns of genetic diversity among genotypes. All the principal components greater than 1 were significant.

Results and Discussion

The analysis of variance reported highly significant differences (p<0.01) among the rice genotypes for most of the traits studied, indicating the presence of substantial genetic variability in the experimental material (Table 3).

Genetic variability

The genetic parameters of all the traits under study among the sixteen genotypes of rice are displayed in Table 4. Genotypic variance was highest for grains per panicle (1641.34), followed by days to 50 % flowering (139.75), plant height (124.03), grain yield per plant (109.51) and productivity (90.14) which implies large genetic differences among the genotypes for these traits. In general, the values of phenotypic variance were only slightly greater than the values of genotypic variance for most of the traits, which means that the impact of the environment on the expression of traits is relatively low.

Table 3. Analysis of variance for the 16 genotypes and 18 traits under study

Source of variation	Replication	Genotypes	Error
Df	2	49	98
Days to 50 % flowering	5.81	421.89**	2.63
Plant height (cm)	8.39	374.66**	2.57
Productive tillers per plant (no)	5.06	11.28*	1.58
No. of panicles (no)	3.15	6.01*	1.10
Panicle length (cm)	3.13	12.50*	0.99
Grains per panicle (no)	243.70	5006.80**	82.80
1000-grain weight (g)	2.94	34.75**	0.99
Productivity (g)	3.28	275.35**	4.93
SPAD Readings	3.03	27.44*	11.22
Seed length (mm)	0.32	2.26*	0.28
Seed width (mm)	0.03	0.35*	0.07
Length/width ratio	0.39	1.39*	0.58
Amylose content	1.11	17.42*	0.36
Gel consistency	1.30	151.25**	0.81
Total protein content	0.75	2.32*	0.22
Iron content	0.00	3.54*	0.00
Zinc content	0.00	28.24*	0.00
Grain yield per plant	69.83	357.02**	28.49

Table 4. Genetic variance studied among the 16 rice genotypes

Characters	Genotypic variance	Phenotypic variance	Genotypic coefficient of variance	Phenotypic coefficient of variance	Heritability (Broad sense)	Genetic advance	Genetic advance as percentage of mean
Days to 50 % flowering	139.75	142.39	13.03	13.15	0.98	24.13	26.59
Plant height (cm)	124.03	126.60	11.63	11.75	0.98	22.71	23.72
Productive tillers per plant (no)	3.23	4.82	15.08	18.41	0.67	3.03	25.45
No. of panicles (no)	1.64	2.74	12.06	15.60	0.60	2.04	19.21
Panicle length (cm)	3.84	4.83	8.28	9.29	0.79	3.60	15.21
Grains per panicle (no)	1641.34	1724.11	23.37	23.95	0.95	81.43	46.97
1000-grain weight (g)	11.25	12.24	15.32	15.98	0.92	6.63	30.27
Productivity (g)	90.14	95.07	16.23	16.66	0.95	19.04	32.55
SPAD Readings	5.41	16.63	4.74	8.30	0.33	2.73	5.56
Seed length (mm)	0.66	0.94	13.60	16.20	0.70	1.41	23.50
Seed width (mm)	0.09	0.17	17.34	23.38	0.55	0.46	26.48
Length/width ratio	0.27	0.85	14.37	25.62	0.31	0.60	16.61
Amylose content	5.69	6.05	11.15	11.50	0.94	4.76	22.27
Gel consistency	50.15	50.95	17.50	17.64	0.98	14.47	35.75
Total protein content	0.70	0.92	10.96	12.59	0.76	1.50	19.65
Iron content	1.18	1.18	12.50	12.50	1.00	2.24	25.74
Zinc content	9.41	9.41	14.05	14.05	1.00	6.32	28.93
Grain yield per plant	109.51	138.00	11.70	13.13	0.79	19.20	21.47

The highest genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were in grains per panicle (23.37 % and 23.95 respectively), followed by productivity (16.23 % and 16.66 respectively) and 1000-grain weight (15.32 % and 15.98 respectively). The genotypic and phenotypic coefficients of variation on iron (12.50 %) and zinc content (14.05) were moderate, indicating enough genetic variability for iron and zinc biofortification. The traits, Soil Plant Analysis Development (SPAD) readings and length to width ratio showed lower GCV in comparison to PCV value indicating larger environmental impact.

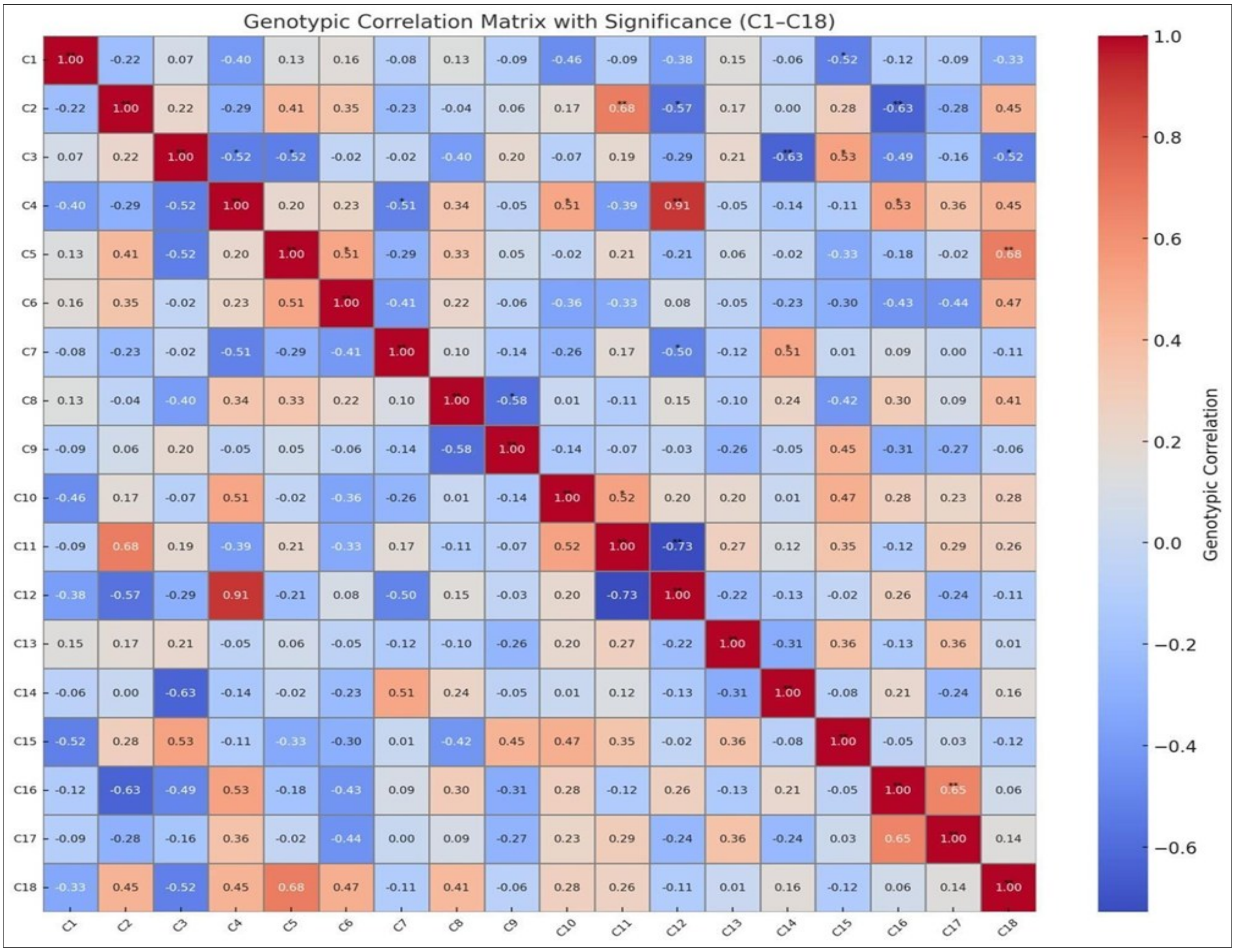
Most of the traits had high broad-sense heritability with iron and zinc content having the highest heritability (1.00), then followed by days to 50 % flowering (0.98), plant height (0.98), gel consistency (0.98), grains per panicle (0.95), productivity (0.95), amylose content (0.94) and 1000-grain weight (0.92). Grains per panicle (46.97 %), gel consistency (35.75 %), productivity (32.55 %), 1000-grain weight (30.27 %), zinc content (28.93 %) and iron content (25.74) were found to have high heritability and high genetic advance as a percentage of mean, coupled with high heritability indicating the presence of additive effect of genes and high scope for crop improvement through selection.

The high heritability and genetic advance of iron and zinc content as observed indicate that the genetic factors largely control the traits and thus can be effectively improved through conventional breeding methods (15–17). The high heritability and moderate to high genetic advance of micronutrient traits in rice have also been reported earlier supporting the possibility of genetic biofortification through selection-based breeding approaches (18).

Genotypic and phenotypic correlation

The genotypic and phenotypic correlations of all the traits under study are exhibited in Fig. 2 and 3. In this study, genotypic correlations were greater than their phenotypic correlations implying a strong genetic association between the traits and low environmental impact. Grain yield per plant had a very high positive genetic correlation with panicle length (0.68, $p<0.01$), grains per panicle and productivity indicating a high chance for yield enhancement, targeting these traits' improvement. In contrast, grain yield per plant showed a negative correlation with the number of productive tillers per plant (-0.52, $p<0.05$), showing that excessive tillering can cause a competitive effect resulting in reduced grain filling as reported by previous researchers (19). This negative correlation emphasises the value of balanced selection techniques instead of an inefficient increase in the number of tillers. The iron and zinc were positively correlated genotypically (0.65, $p<0.01$) proving that both micronutrients can be simultaneously improved through selection as reported earlier (20). On the other hand, both iron and zinc displayed negative correlation with the plant height (-0.63, $p<0.01$) indicating a possible conflict between vegetative growth and micronutrient accumulation. Similar negative correlation between micronutrient concentration and plant height have been reported in rice, which may be caused by dilution effects or differential assimilate partitioning (16, 21, 22). Among quality traits, seed width had a positive correlation (0.681, $p<0.01$) with plant height whereas length/width ratio had a strong positive correlation (0.9089, $p<0.01$) with the number of panicles but negative correlation with seed width (-0.7282, $p<0.01$). There was a negative correlation observed between gel consistency and productive tillers (-0.6311, $p<0.01$) suggesting that some aspects of cooking quality may be negatively impacted by increased tillering. Soil Plant Analysis Development readings and productivity (-0.584, $p<0.05$) showed a negative correlation between them, indicating that the chlorophyll content at the tillering stage may not impact grain yield directly in the given experimental conditions.

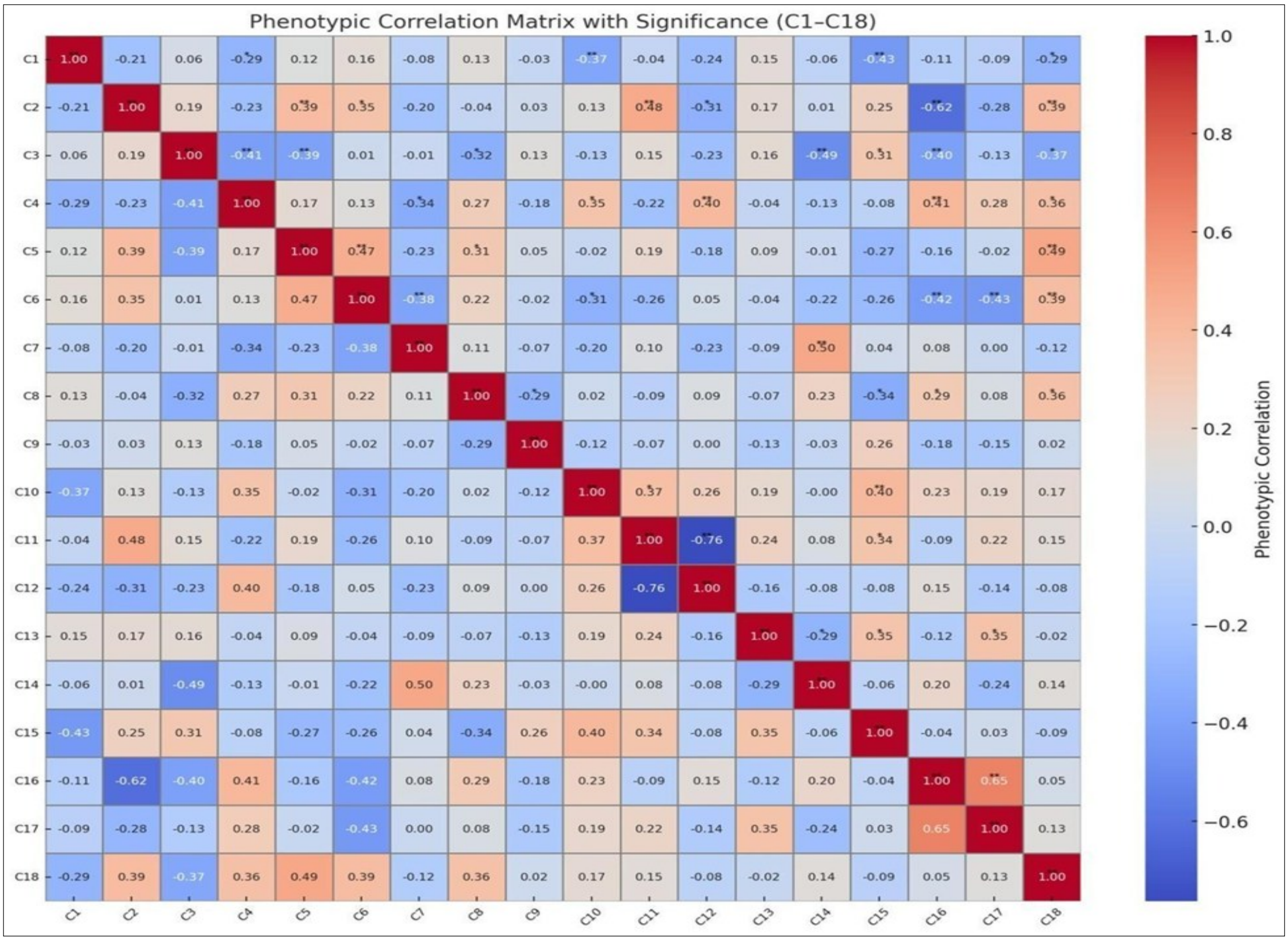
The phenotypic correlations were like genotypic correlations but to a lesser extent considering the moderate environmental influence. Panicle length, grains per panicle, plant height, productivity and number of panicles were positively correlated with grain yield per plant (0.4948, $p<0.01$; 0.386, $p<0.01$; 0.3938, $p<0.01$; 0.3552, $p<0.05$; 0.3619, $p<0.05$, respectively), whereas productive tillers (-0.367, $p<0.05$) exhibited a negative correlation. There was positive phenotypic correlation between iron and zinc content indicating a greater genetic component governing these traits reinforcing their suitability for biofortification-oriented breeding. In general, the correlation analysis indicates that selection for panicle length, grains per panicle and productivity would be effective for yield improvement and the positive association between iron and zinc content supports the feasibility of developing dual biofortified rice varieties.



Genotypic Correlation Matrix with Significance (C1–C18)

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18
C1	1.00	-0.22	0.07	-0.40	0.13	0.16	-0.08	0.13	-0.09	-0.46	-0.09	-0.38	0.15	-0.06	-0.52	-0.12	-0.09	-0.33
C2	-0.22	1.00	0.22	-0.29	0.41	0.35	-0.23	-0.04	0.06	0.17	0.68	-0.57	0.17	0.00	0.28	-0.63	-0.28	0.45
C3	0.07	0.22	1.00	-0.52	-0.52	-0.02	-0.02	-0.40	0.20	-0.07	0.19	-0.29	0.21	-0.63	0.53	-0.49	-0.16	-0.52
C4	-0.40	-0.29	-0.52	1.00	0.20	0.23	-0.51	0.34	-0.05	0.51	-0.39	0.91	-0.05	-0.14	-0.11	0.53	0.36	0.45
C5	0.13	0.41	-0.52	0.20	1.00	0.51	-0.29	0.33	0.05	-0.02	0.21	-0.21	0.06	-0.02	-0.33	-0.18	-0.02	0.68
C6	0.16	0.35	-0.02	0.23	0.51	1.00	-0.41	0.22	-0.06	-0.36	-0.33	0.08	-0.05	-0.23	-0.30	-0.43	-0.44	0.47
C7	-0.08	-0.23	-0.02	-0.51	-0.29	-0.41	1.00	0.10	-0.14	-0.26	0.17	-0.50	-0.12	0.51	0.01	0.09	0.00	-0.11
C8	0.13	-0.04	-0.40	0.34	0.33	0.22	0.10	1.00	-0.58	0.01	-0.11	0.15	-0.10	0.24	-0.42	0.30	0.09	0.41
C9	-0.09	0.06	0.20	-0.05	0.05	-0.06	-0.14	-0.58	1.00	-0.14	-0.07	-0.03	-0.26	-0.05	0.45	-0.31	-0.27	-0.06
C10	-0.46	0.17	-0.07	0.51	-0.02	-0.36	-0.26	0.01	-0.14	1.00	0.52	0.20	0.20	0.01	0.47	0.28	0.23	0.28
C11	-0.09	0.68	0.19	-0.39	0.21	-0.33	0.17	-0.11	-0.07	0.52	1.00	-0.73	0.27	0.12	0.35	-0.12	0.29	0.26
C12	-0.38	-0.57	-0.29	0.91	-0.21	0.08	-0.50	0.15	-0.03	0.20	-0.73	1.00	-0.22	-0.13	-0.02	0.26	-0.24	-0.11
C13	0.15	0.17	0.21	-0.05	0.06	-0.05	-0.12	-0.10	-0.26	0.20	0.27	-0.22	1.00	-0.31	0.36	-0.13	0.36	0.01
C14	-0.06	0.00	-0.63	-0.14	-0.02	-0.23	0.51	0.24	-0.05	0.01	0.12	-0.13	-0.31	1.00	-0.08	0.21	-0.24	0.16
C15	-0.52	0.28	0.53	-0.11	-0.33	-0.30	0.01	-0.42	0.45	0.47	0.35	-0.02	0.36	-0.08	1.00	-0.05	0.03	-0.12
C16	-0.12	-0.63	-0.49	0.53	-0.18	-0.43	0.09	0.30	-0.31	0.28	-0.12	0.26	-0.13	0.21	-0.05	1.00	0.65	0.06
C17	-0.09	-0.28	-0.16	0.36	-0.02	-0.44	0.00	0.09	-0.27	0.23	0.29	-0.24	0.36	-0.24	0.03	0.65	1.00	0.14
C18	-0.33	0.45	-0.52	0.45	0.68	0.47	-0.11	0.41	-0.06	0.28	0.26	-0.11	0.01	0.16	-0.12	0.06	0.14	1.00

Fig. 2. Genotypic correlation among the 18 traits under study.



Phenotypic Correlation Matrix with Significance (C1–C18)

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18
C1	1.00	-0.21	0.06	-0.29	0.12	0.16	-0.08	0.13	-0.03	-0.37	-0.04	-0.24	0.15	-0.06	-0.43	-0.11	-0.09	-0.29
C2	-0.21	1.00	0.19	-0.23	0.39	0.35	-0.20	-0.04	0.03	0.13	0.48	-0.31	0.17	0.01	0.25	-0.62	-0.28	0.39
C3	0.06	0.19	1.00	-0.41	-0.39	0.01	-0.01	-0.32	0.13	-0.13	0.15	-0.23	0.16	-0.49	0.31	-0.40	-0.13	-0.37
C4	-0.29	-0.23	-0.41	1.00	0.17	0.13	-0.34	0.27	-0.18	0.35	-0.22	0.40	-0.04	-0.13	-0.08	0.41	0.28	0.36
C5	0.12	0.39	-0.39	0.17	1.00	0.47	-0.23	0.31	0.05	-0.02	0.19	-0.18	0.09	-0.01	-0.27	-0.16	-0.02	0.49
C6	0.16	0.35	0.01	0.13	0.47	1.00	-0.38	0.22	-0.02	-0.31	-0.26	0.05	-0.04	-0.22	-0.26	-0.42	-0.43	0.39
C7	-0.08	-0.20	-0.01	-0.34	-0.23	-0.38	1.00	0.11	-0.07	-0.20	0.10	-0.23	-0.09	0.50	0.04	0.08	0.00	-0.12
C8	0.13	-0.04	-0.32	0.27	0.31	0.22	0.11	1.00	-0.29	0.02	-0.09	0.09	-0.07	0.23	-0.34	0.29	0.08	0.36
C9	-0.03	0.03	0.13	-0.18	0.05	-0.02	-0.07	-0.29	1.00	-0.12	-0.07	0.00	-0.13	-0.03	0.26	-0.18	-0.15	0.02
C10	-0.37	0.13	-0.13	0.35	-0.02	-0.31	-0.20	0.02	-0.12	1.00	0.37	0.26	0.19	-0.00	0.40	0.23	0.19	0.17
C11	-0.04	0.48	0.15	-0.22	0.19	-0.26	0.10	-0.09	-0.07	0.37	1.00	-0.76	0.24	0.08	0.34	-0.09	0.22	0.15
C12	-0.24	-0.31	-0.23	0.40	-0.18	0.05	-0.23	0.09	0.00	0.26	-0.76	1.00	-0.16	-0.08	-0.08	0.15	-0.14	-0.08
C13	0.15	0.17	0.16	-0.04	0.09	-0.04	-0.09	-0.07	-0.13	0.19	0.24	-0.16	1.00	-0.29	0.35	-0.12	0.35	-0.02
C14	-0.06	0.01	-0.49	-0.13	-0.01	-0.22	0.50	0.23	-0.03	-0.00	0.08	-0.08	-0.29	1.00	-0.06	0.20	-0.24	0.14
C15	-0.43	0.25	0.31	-0.08	-0.27	-0.26	0.04	-0.34	0.26	0.40	0.34	-0.08	0.35	-0.06	1.00	-0.04	0.03	-0.09
C16	-0.11	-0.62	-0.40	0.41	-0.16	-0.42	0.08	0.29	-0.18	0.23	-0.09	0.15	-0.12	0.20	-0.04	1.00	0.65	0.05
C17	-0.09	-0.28	-0.13	0.28	-0.02	-0.43	0.00	0.08	-0.15	0.19	0.22	-0.14	0.35	-0.24	0.03	0.65	1.00	0.13
C18	-0.29	0.39	-0.37	0.36	0.49	0.39	-0.12	0.36	0.02	0.17	0.15	-0.08	-0.02	0.14	-0.09	0.05	0.13	1.00

Fig. 3. Phenotypic correlation among the 18 traits under study.

Path coefficient analysis

Path coefficient analysis was carried out to partition the genotypic correlation coefficients into direct and indirect effects of traits on grain yield per plant thus providing a clearer understanding of the importance of individual traits in achieving the yield (Fig. 4 –7). This analysis assists in identifying traits that can have a direct contribution to yield improvement and those whose impact is indirect through other traits. Among all the traits under study, the direct positive impact of productivity was the highest in grain yield per plant (0.8647), followed by grains per panicle (0.6042), panicle length (0.3743), 1000-grain weight (0.1491) and seed length (0.1359). The high and significant level of correlation between productivity and grain yield is attributed to its strong direct influence and indicates it to be the most effective selection criterion in improving the yield under the current experimental conditions. The contribution of grains per panicle and the length of panicle were also significant in yield both directly and indirectly through productivity. On the other hand, length/width ratio showed the most significantly negative direct effect on grain yield (-0.8036) followed by seed width (-0.3686), days to 50 % flowering (-0.2877) and productive tiller per plant (-0.1837). This may possibly be due to inefficient assimilating partitioning and increased intra-plant competition. The positive impact of productivity was augmented by the indirect contribution through grains per panicle (0.1832) and panicle length (0.0862) but it was decreased by the negative effect of length/width ratio (-0.1883) and seed width (-0.0665). Grains per panicle had an indirect impact on the yield by increasing productivity (0.2623) and panicle length (0.2011). There was a positive correlation between panicle length and productivity and grains per panicle, reinforcing the role of these traits as key yield determinants. Micronutrients showed contrasting effects on grain yield. Iron exhibited a direct negative impact on yield (-0.1032) and indirectly through the plant height (-0.1025) and grains per panicle (-0.2533). Similarly, there was a direct negative influence of zinc (-0.0942) but a positive indirect influence through iron (0.0676). These results indicate that while iron and zinc are strongly associated with each other, their accumulation may be associated with trade-offs with certain yield-related traits, emphasising the need for balanced selection strategies in biofortification breeding.

The high negative direct effect of length/width ratio (-0.8036) was reinforced by seed width (-0.2813) but partially buffered by the positive indirect effect though number of panicles (0.2859). This indicates that grain shape traits can significantly affect yield performance and should be carefully considered during selection. In general, the path coefficient analysis revealed that the most significant characteristics that affect the grain yield per plant are productivity, grains per panicle and panicle length due to the strong direct and indirect effects as reported in rice by previous researchers (23). Even though the direct influence of micronutrient characteristics on yield was not positive, their high level of heritability and correlation with each other indicates that the simultaneous increase in yield and micronutrient composition is possible with the help of reasonable parental selection and effective breeding plans (24–28).

Principal component analysis

The principal component analysis (PCA) was conducted to determine the pattern of genetic diversity among the sixteen rice genotypes in terms of eighteen agronomic, quality and nutritional traits (Fig. 8–10). The analysis results showed that the first 4 PCs with eigenvalues of more than one cumulatively accounted for 82.2 % of the overall variation, which demonstrated that the first 4 PCs were sufficient in explaining the multivariate structure of the data (Table 5).

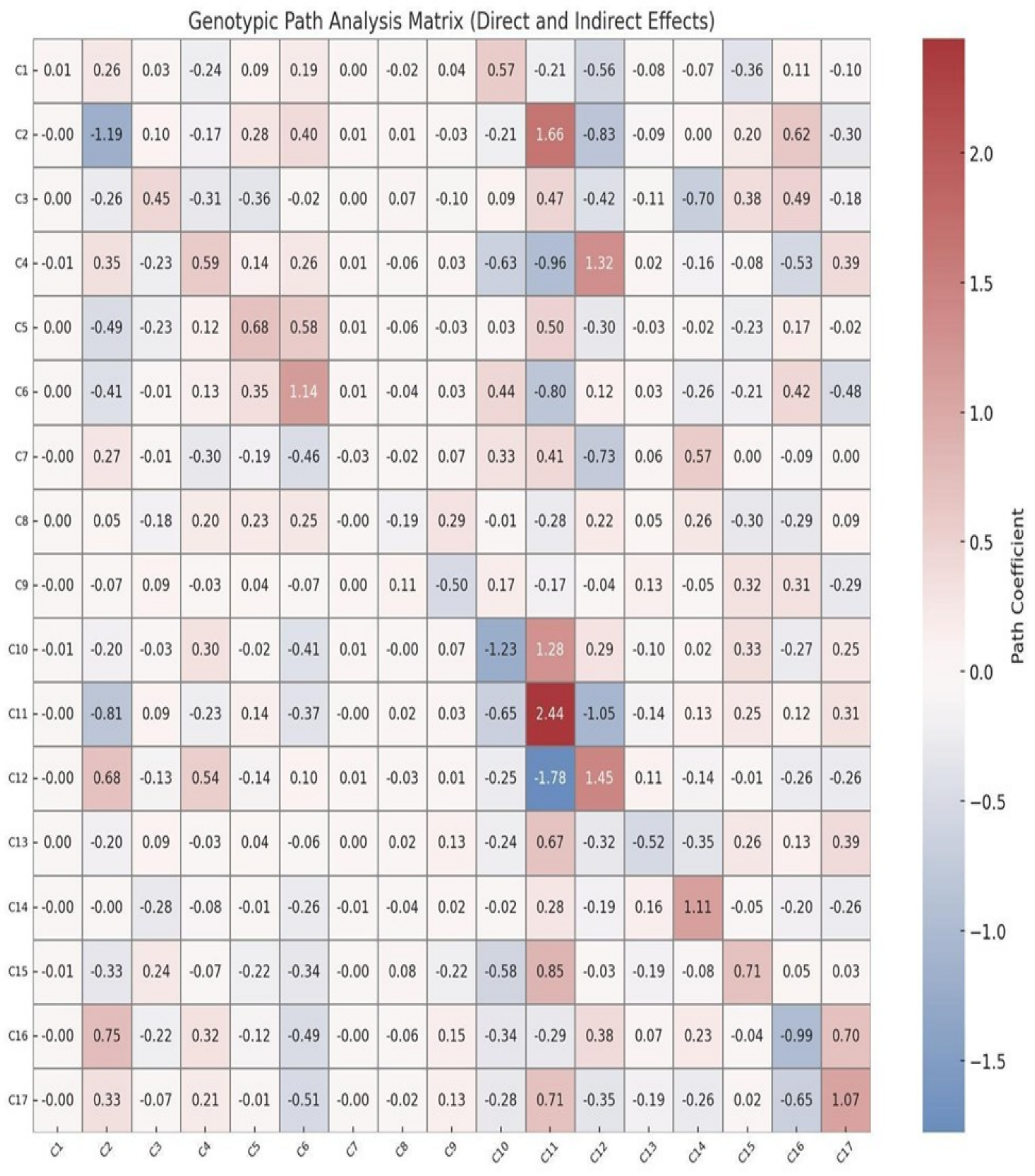
The first principal component (PC1) with an eigenvalue of 6.5 explained 36.1 % of the total variation and was largely related to yield-related traits. Productivity (0.88), grains per panicle (0.82), grain yield per plant (0.78), panicle length (0.68) and 1000-grain weight (0.40) had high positive loadings (Table 6). This is an indication that PC1 is a yield component axis, which summarises the contribution of the significant yield determining traits. The second principal component (PC2) described 23.3 % of the total variation and its eigenvalue was 4.2 and was predominantly related to micronutrient traits. Iron (0.84) and zinc (0.88) showed positive loadings while negative loadings were observed in plant height (-0.62) and grains per panicle (-0.58). It is an indication of a biofortification axis and reflects a possible trade-off between the accumulation of micronutrients and some yield related or vegetative traits. The third principal component (PC3), which has an eigenvalue of 2.8, explained 15.6 % of the total variation and it was defined by grain quality characteristics, such as gel consistency (0.78), amylose content (0.72) and protein content (0.35). This component reflects variation related cooking and nutritional qualities that are significant in consumer preference. The fourth principal component (PC4) explained 7.2 % of the total variation (eigenvalue = 1.3) and was largely influenced by the grain shape parameters. Seed length (0.62) and length-to-width ratio (0.58) had positive loadings, whereas seed width (-0.68) had a negative loading, which demonstrated variation in the morphology of the grains among the genotypes. The scree plot further confirms the contributions of the first four principal components to the total variation (Fig. 11).

Table 5. Eigen vectors and eigen values of the principal components of 16 traits of rice (*Oryza sativa*)

	Eigen values	Variance percent	Cumulative variance percent
Dim.1	3.58	19.89	19.9
Dim.2	3.02	16.76	36.7
Dim.3	2.84	15.75	52.4
Dim.4	2.42	13.44	65.8
Dim.5	1.86	10.35	76.2
Dim.6	1.11	6.19	82.4
Dim.7	0.83	4.58	87.0
Dim.8	0.73	4.07	91.0
Dim.9	0.56	3.09	94.1
Dim.10	0.38	2.14	96.3
Dim.11	0.29	1.59	97.9
Dim.12	0.24	1.32	99.2
Dim.13	0.13	0.73	99.9
Dim.14	0.01	0.08	100.0
Dim.15	0.00	0.02	100.0
Dim.16	0.00	0.00	100.0

Table 6. Principal component contributions for all 16 traits in rice (*Oryza sativa*)

	Dim 1	Dim 2	Dim 3	Dim 4	Dim 5	Dim 6
1	0.06	0.41	3.87	3.85	25.57	6.26
2	5.33	23.99	23.27	0.22	4.72	10.52
3	0.98	2.84	9.62	14.71	0.22	5.57
4	32.99	0.50	12.35	1.36	3.19	1.72
5	7.36	4.36	11.67	3.55	2.14	1.47
6	11.93	0.24	0.21	1.17	0.11	24.63
7	0.01	0.29	1.41	1.92	30.84	0.29
8	0.01	13.89	0.04	27.67	9.20	4.27
9	3.46	2.79	1.46	0.75	0.16	0.48
10	8.95	11.30	15.08	2.55	2.36	0.19
11	15.81	1.02	4.28	1.45	4.97	18.67
12	0.03	0.20	2.44	0.82	7.45	0.78
13	3.40	0.09	6.62	7.54	0.23	0.42
14	0.03	6.00	0.08	15.51	0.68	1.75
15	2.81	4.22	1.01	4.81	0.20	13.70
16	0.60	21.62	0.34	5.86	1.73	3.03



Genotypic Path Analysis Matrix (Direct and Indirect Effects)

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17
C1	0.01	0.26	0.03	-0.24	0.09	0.19	0.00	-0.02	0.04	0.57	-0.21	-0.56	-0.08	-0.07	-0.36	0.11	-0.10
C2	-0.00	-1.19	0.10	-0.17	0.28	0.40	0.01	0.01	-0.03	-0.21	1.66	-0.83	-0.09	0.00	0.20	0.62	-0.30
C3	0.00	-0.26	0.45	-0.31	-0.36	-0.02	0.00	0.07	-0.10	0.09	0.47	-0.42	-0.11	-0.70	0.38	0.49	-0.18
C4	-0.01	0.35	-0.23	0.59	0.14	0.26	0.01	-0.06	0.03	-0.63	-0.96	1.32	0.02	-0.16	-0.08	-0.53	0.39
C5	0.00	-0.49	-0.23	0.12	0.68	0.58	0.01	-0.06	-0.03	0.03	0.50	-0.30	-0.03	-0.02	-0.23	0.17	-0.02
C6	0.00	-0.41	-0.01	0.13	0.35	1.14	0.01	-0.04	0.03	0.44	-0.80	0.12	0.03	-0.26	-0.21	0.42	-0.48
C7	-0.00	0.27	-0.01	-0.30	-0.19	-0.46	-0.03	-0.02	0.07	0.33	0.41	-0.73	0.06	0.57	0.00	-0.09	0.00
C8	0.00	0.05	-0.18	0.20	0.23	0.25	-0.00	-0.19	0.29	-0.01	-0.28	0.22	0.05	0.26	-0.30	-0.29	0.09
C9	-0.00	-0.07	0.09	-0.03	0.04	-0.07	0.00	0.11	-0.50	0.17	-0.17	-0.04	0.13	-0.05	0.32	0.31	-0.29
C10	-0.01	-0.20	-0.03	0.30	-0.02	-0.41	0.01	-0.00	0.07	-1.23	1.28	0.29	-0.10	0.02	0.33	-0.27	0.25
C11	-0.00	-0.81	0.09	-0.23	0.14	-0.37	-0.00	0.02	0.03	-0.65	2.44	-1.05	-0.14	0.13	0.25	0.12	0.31
C12	-0.00	0.68	-0.13	0.54	-0.14	0.10	0.01	-0.03	0.01	-0.25	-1.78	1.45	0.11	-0.14	-0.01	-0.26	-0.26
C13	0.00	-0.20	0.09	-0.03	0.04	-0.06	0.00	0.02	0.13	-0.24	0.67	-0.32	-0.52	-0.35	0.26	0.13	0.39
C14	-0.00	-0.00	-0.28	-0.08	-0.01	-0.26	-0.01	-0.04	0.02	-0.02	0.28	-0.19	0.16	1.11	-0.05	-0.20	-0.26
C15	-0.01	-0.33	0.24	-0.07	-0.22	-0.34	-0.00	0.08	-0.22	-0.58	0.85	-0.03	-0.19	-0.08	0.71	0.05	0.03
C16	-0.00	0.75	-0.22	0.32	-0.12	-0.49	-0.00	-0.06	0.15	-0.34	-0.29	0.38	0.07	0.23	-0.04	-0.99	0.70
C17	-0.00	0.33	-0.07	0.21	-0.01	-0.51	-0.00	-0.02	0.13	-0.28	0.71	-0.35	-0.19	-0.26	0.02	-0.65	1.07

Fig. 4. Genotypic path coefficient for the traits under study.

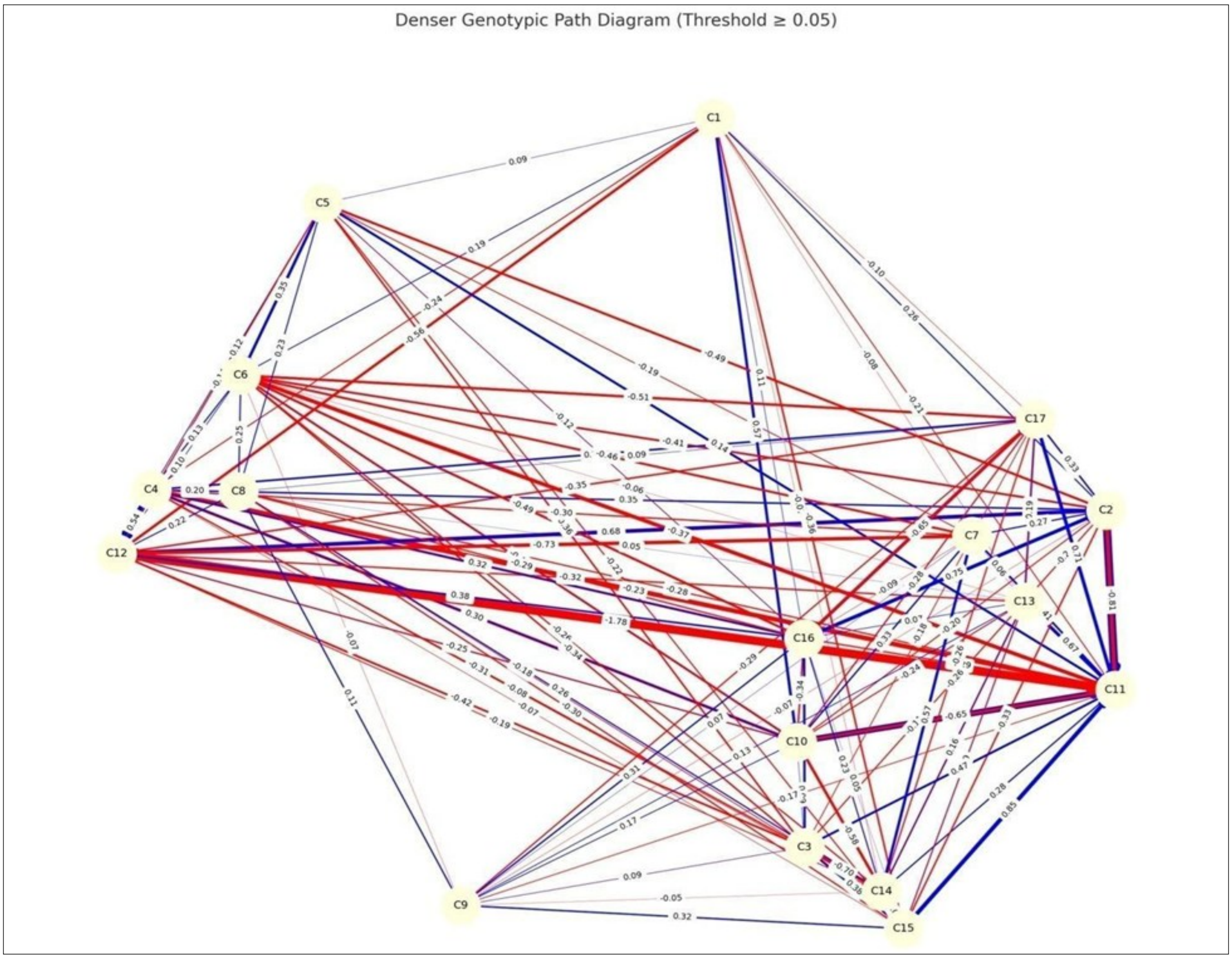
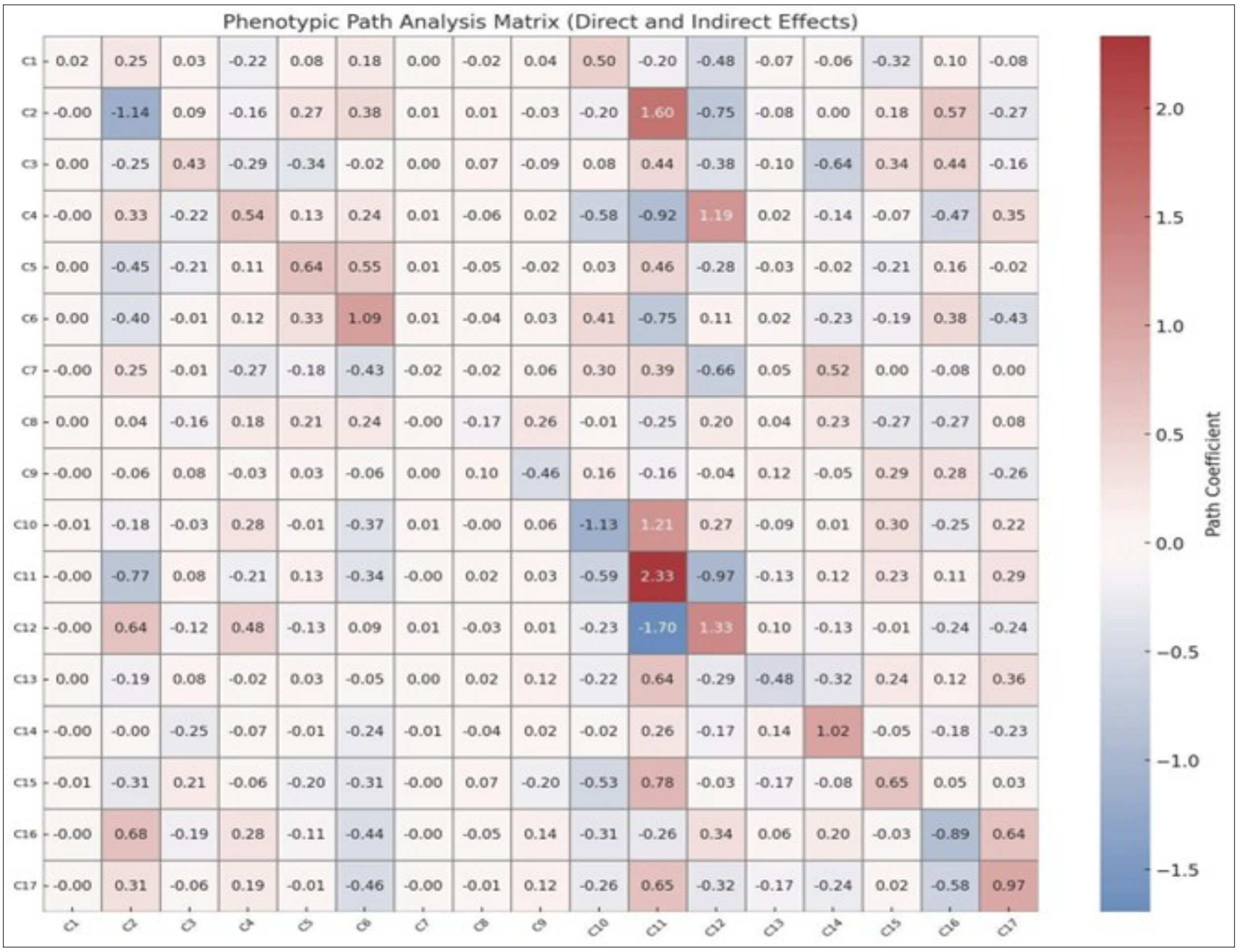


Fig. 5. Genotypic path coefficient diagram for the traits under study.



	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17
C1	0.02	0.25	0.03	-0.22	0.08	0.18	0.00	-0.02	0.04	0.50	-0.20	-0.48	-0.07	-0.06	-0.32	0.10	-0.08
C2	-0.00	-1.14	0.09	-0.16	0.27	0.38	0.01	0.01	-0.03	-0.20	1.60	-0.75	-0.08	0.00	0.18	0.57	-0.27
C3	0.00	-0.25	0.43	-0.29	-0.34	-0.02	0.00	0.07	-0.09	0.08	0.44	-0.38	-0.10	-0.64	0.34	0.44	-0.16
C4	-0.00	0.33	-0.22	0.54	0.13	0.24	0.01	-0.06	0.02	-0.58	-0.92	1.19	0.02	-0.14	-0.07	-0.47	0.35
C5	0.00	-0.45	-0.21	0.11	0.64	0.55	0.01	-0.05	-0.02	0.03	0.46	-0.28	-0.03	-0.02	-0.21	0.16	-0.02
C6	0.00	-0.40	-0.01	0.12	0.33	1.09	0.01	-0.04	0.03	0.41	-0.75	0.11	0.02	-0.23	-0.19	0.38	-0.43
C7	-0.00	0.25	-0.01	-0.27	-0.18	-0.43	-0.02	-0.02	0.06	0.30	0.39	-0.66	0.05	0.52	0.00	-0.08	0.00
C8	0.00	0.04	-0.16	0.18	0.21	0.24	-0.00	-0.17	0.26	-0.01	-0.25	0.20	0.04	0.23	-0.27	-0.27	0.08
C9	-0.00	-0.06	0.08	-0.03	0.03	-0.06	0.00	0.10	-0.46	0.16	-0.16	-0.04	0.12	-0.05	0.29	0.28	-0.26
C10	-0.01	-0.18	-0.03	0.28	-0.01	-0.37	0.01	-0.00	0.06	-1.13	1.21	0.27	-0.09	0.01	0.30	-0.25	0.22
C11	-0.00	-0.77	0.08	-0.21	0.13	-0.34	-0.00	0.02	0.03	-0.59	2.33	-0.97	-0.13	0.12	0.23	0.11	0.29
C12	-0.00	0.64	-0.12	0.48	-0.13	0.09	0.01	-0.03	0.01	-0.23	-1.70	1.33	0.10	-0.13	-0.01	-0.24	-0.24
C13	0.00	-0.19	0.08	-0.02	0.03	-0.05	0.00	0.02	0.12	-0.22	0.64	-0.29	-0.48	-0.32	0.24	0.12	0.36
C14	-0.00	-0.00	-0.25	-0.07	-0.01	-0.24	-0.01	-0.04	0.02	-0.02	0.26	-0.17	0.14	1.02	-0.05	-0.18	-0.23
C15	-0.01	-0.31	0.21	-0.06	-0.20	-0.31	-0.00	0.07	-0.20	-0.53	0.78	-0.03	-0.17	-0.08	0.65	0.05	0.03
C16	-0.00	0.68	-0.19	0.28	-0.11	-0.44	-0.00	-0.05	0.14	-0.31	-0.26	0.34	0.06	0.20	-0.03	-0.89	0.64
C17	-0.00	0.31	-0.06	0.19	-0.01	-0.46	-0.00	-0.01	0.12	-0.26	0.65	-0.32	-0.17	-0.24	0.02	-0.58	0.97

Fig. 6. Phenotypic path coefficient for the traits under study.

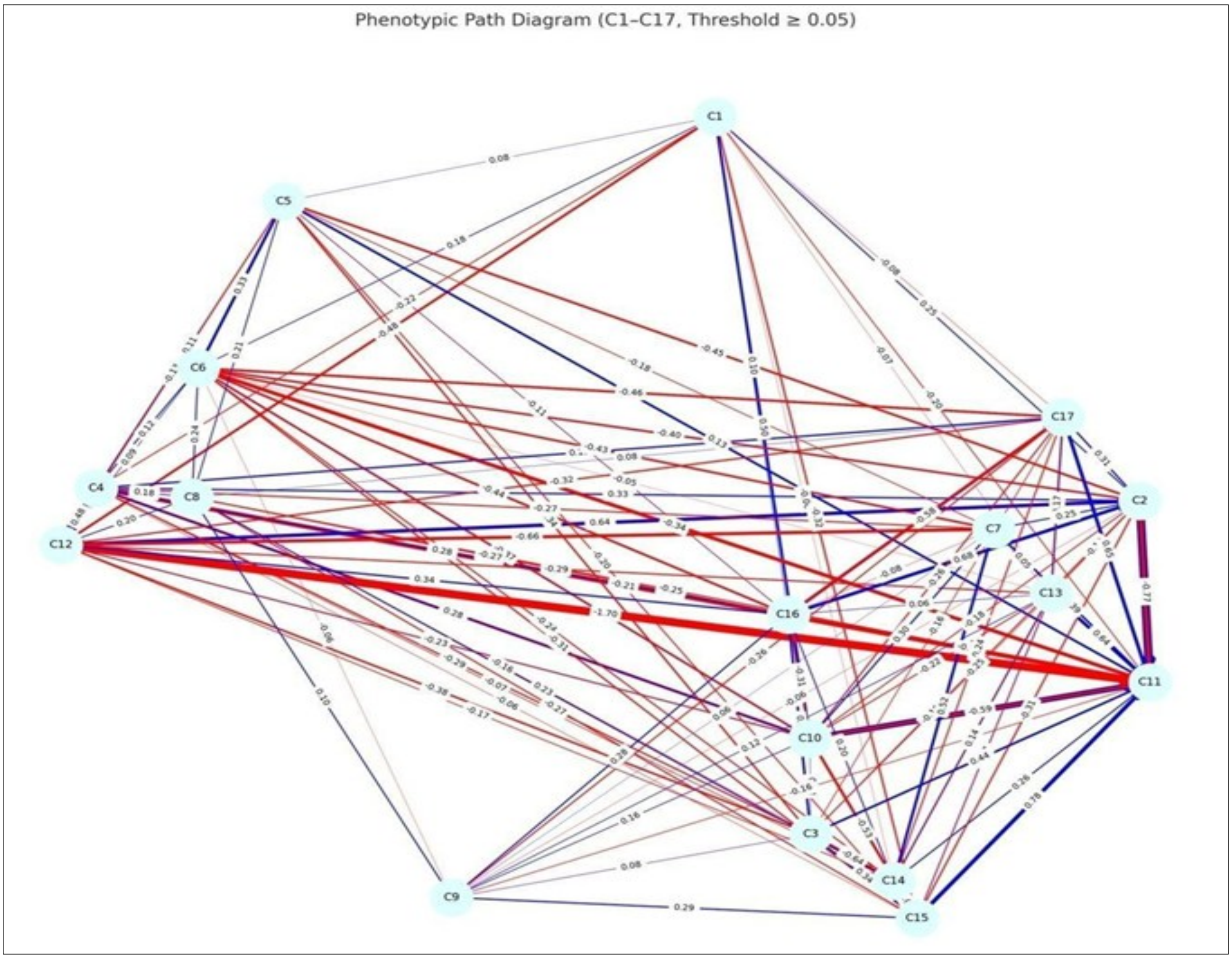


Fig. 7. Phenotypic path coefficient diagram for the traits under study.

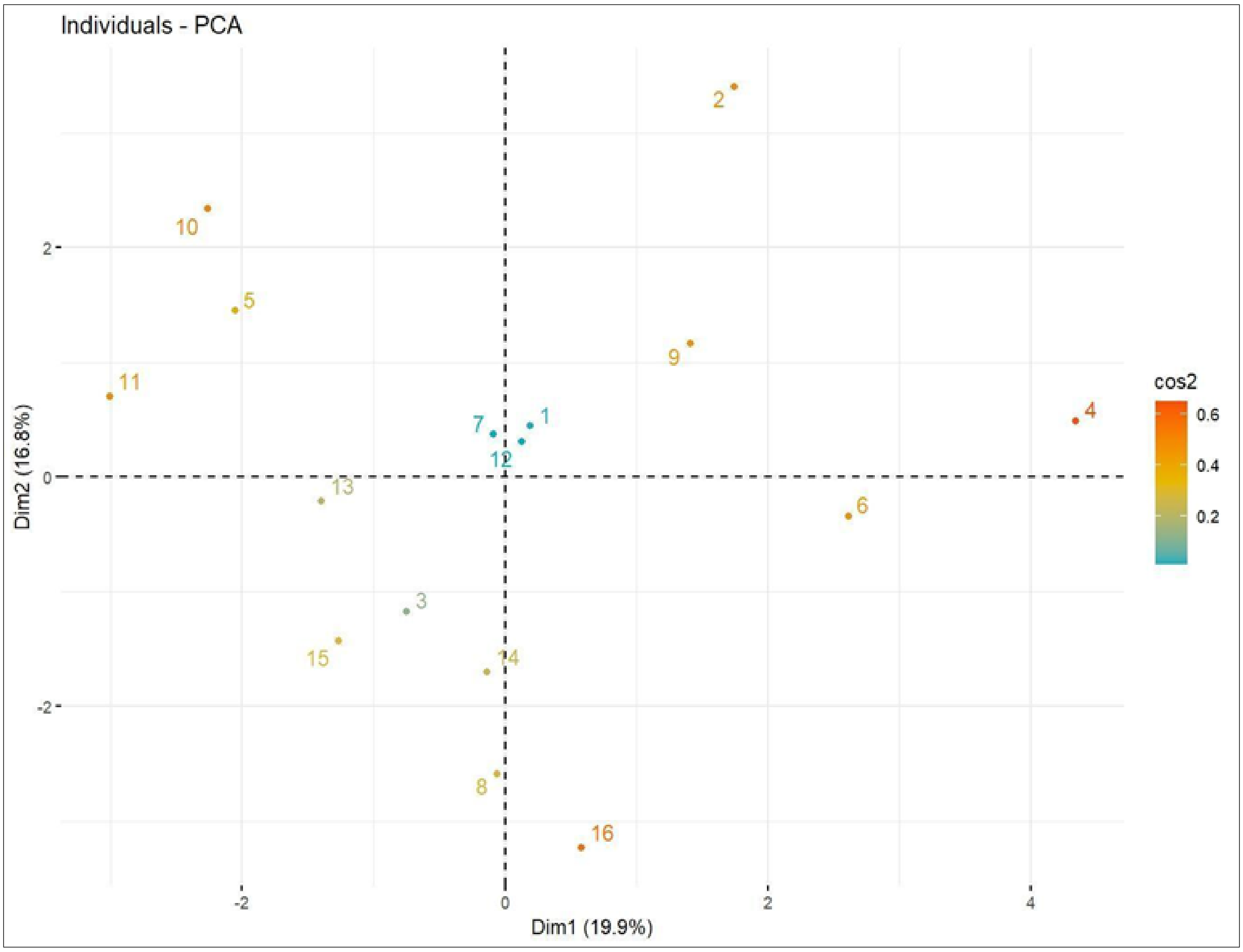


Fig. 8. Principal component analysis biplot showing distribution of genotypes across first two principal components.

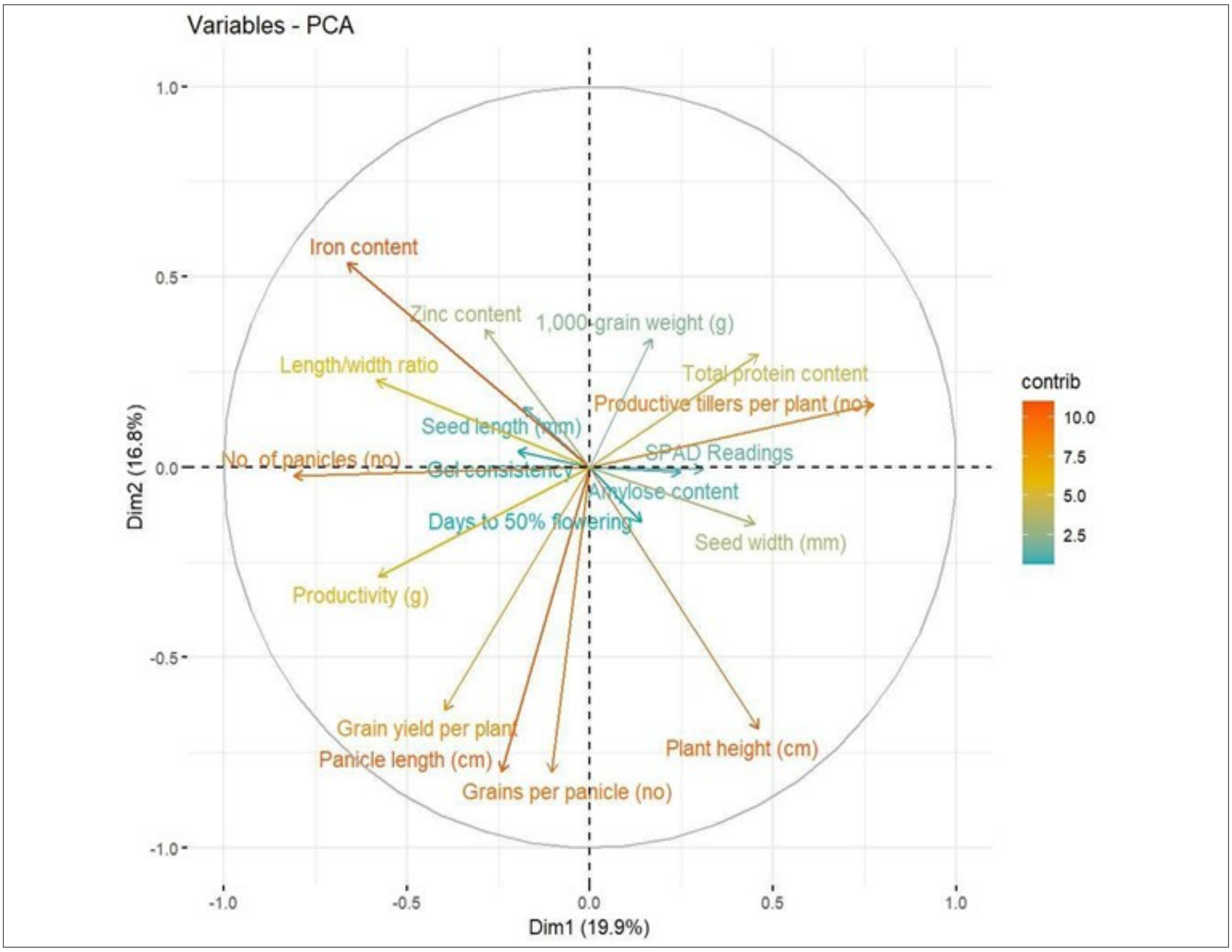


Fig. 9. Principal component analysis biplot showing variation among traits.

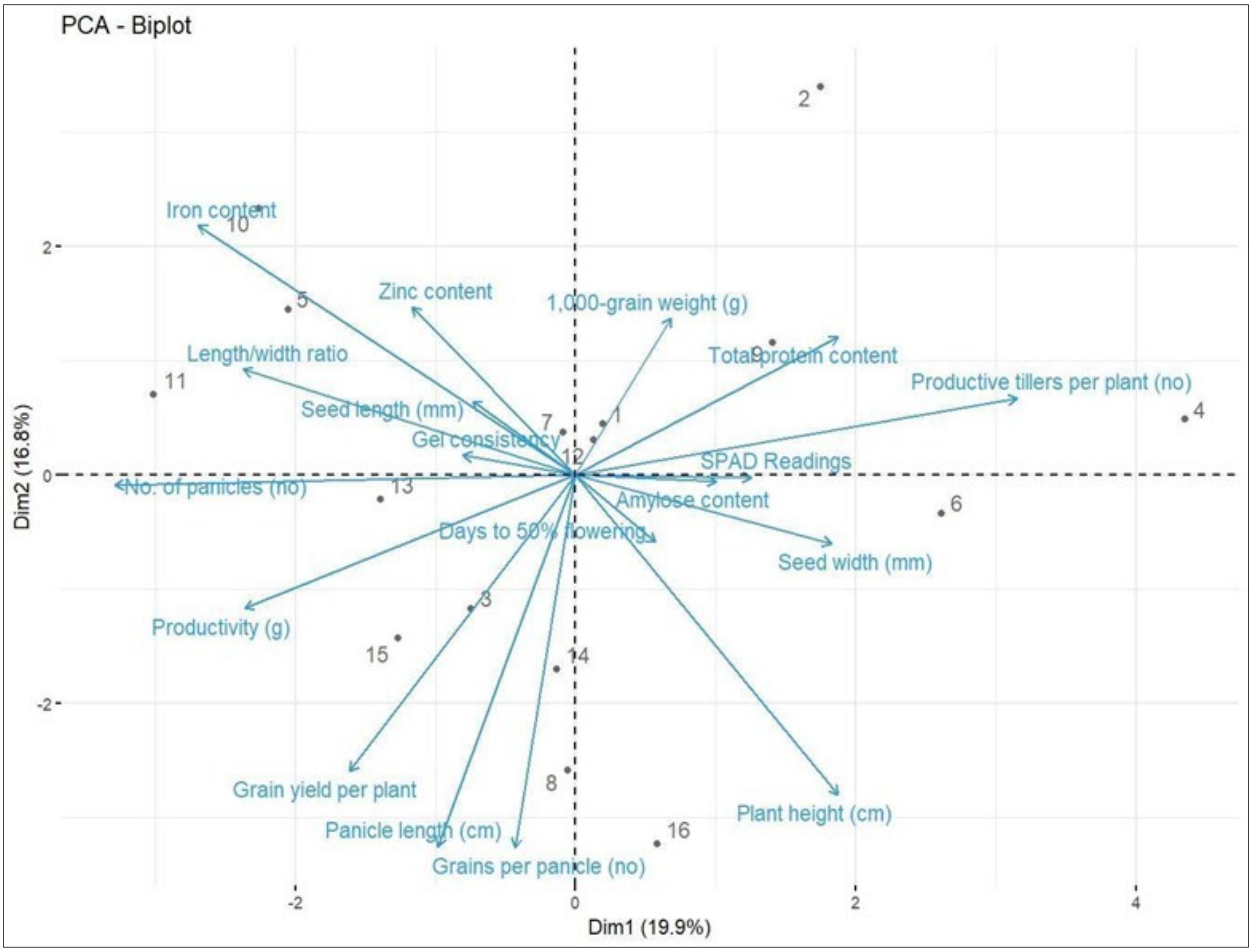


Fig. 10. Genotype by trait biplot showing distribution of genotypes across first two principal components.

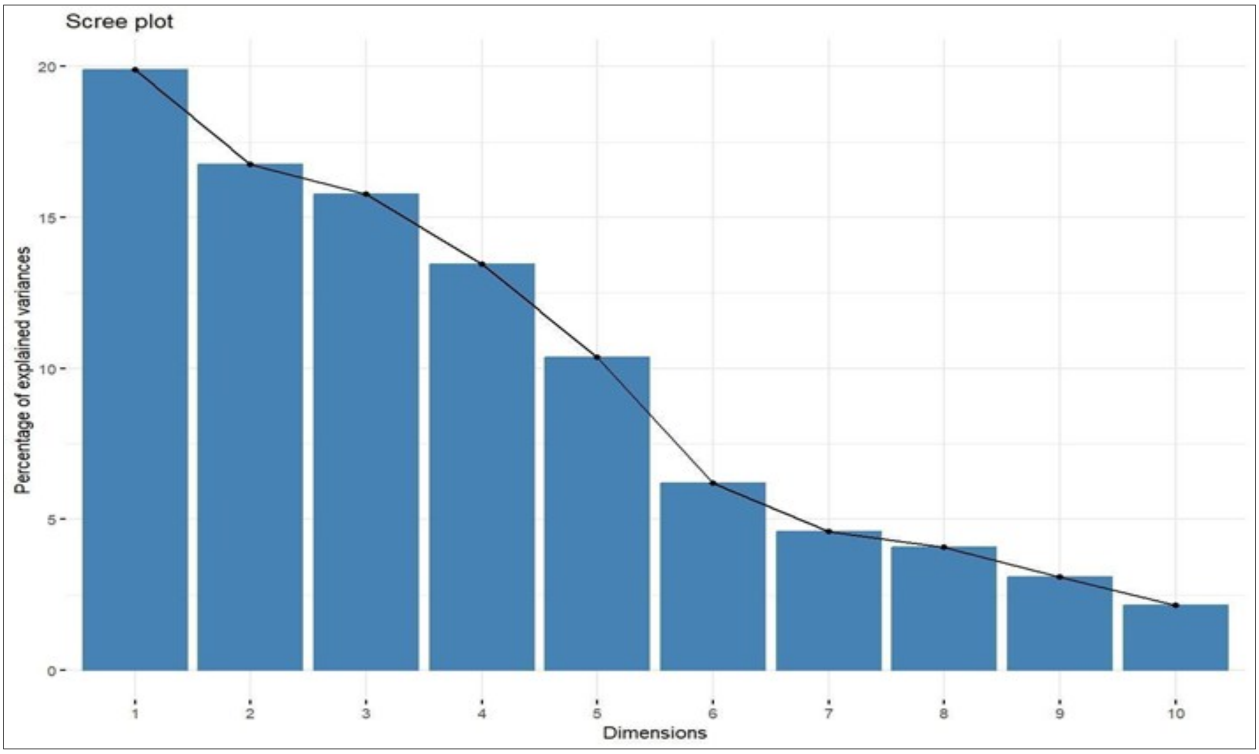


Fig. 11. Scree plot representing percent explained variance.

The organization of the yield traits, the micronutrient traits, quality traits and grain shape traits into specific principal components implies that the trait groups are controlled by partially independent genetic factors. The separation between the micronutrient characteristics and the yield related traits contributes to the idea of simultaneous enhancement through suitable selection strategies. In addition, the fact that the major traits like grain yield, iron and zinc levels are highly heritable shows that the use of PCA in selection can be successfully utilised in biofortification-based rice breeding programs.

The analysis of the PCA biplot clearly shows that genotypes were grouped according to their trait profiles. The genotypes KNM 118 and Bhadrakali were also found to be closer to the vectors that portrayed the amount of iron and zinc content, which denoted their possible use as micronutrient-enriched donor lines. KNM 1638 and Varalu, on the contrary, grouped around the vectors of yield and productivity, indicating their superior performance in the areas of yield. This opposite but complementary placement of these genotypes implies their possible usefulness in breeding schemes, which are directed at the combination of high yield with higher micronutrient levels.

Conclusion

The current research revealed that there is a high level of genetic variation among the sixteen rice genotypes in the agronomic, grain quality and micronutrient traits and this can be exploited for crop improvement. High genotypic and phenotypic coefficients of variation, which are associated with high heritability and genetic advance for important traits like grains per panicle, productivity, grain yield per plant, iron and zinc content, indicate that they are largely influenced by additive gene action and can be successfully enhanced by selection-based breeding methods. Correlation and path coefficient analysis indicated that productivity, grains per panicle and panicle length are the most significant traits influencing grain yield per plant because of their strong direct and indirect effects. The negative direct impact of excessive tillering and some grain shape characteristics on yield highlights the essence of well-balanced plant architecture in yield enhancement programs. Strong positive correlation between iron and zinc levels indicates the possibility to improve the two micronutrients simultaneously, but with a careful balancing of traits for biofortification due to the negative direct impacts on yield. These findings were further confirmed by principal component analysis where yield-related, micronutrient, grain quality traits and grain shape parameters were significantly split into distinct factors that accounted for a large proportion of the total variation. The partial genetic independence among these trait groups suggests that simultaneous improvement of yield and micronutrient content is achievable through appropriate selection strategies. According to the multivariate analysis and total trait performance, KNM 118 and Bhadrakali were identified as promising donor genotypes in terms of high levels of iron and zinc content whereas KNM 1638 and Varalu showed better yield and productivity. The complementary performance of these genotypes shows their possible value in breeding schemes to produce high yielding, iron and zinc fortified rice genotypes that can be adapted to the Telangana and Andhra Pradesh regions. Further studies are needed to validate these findings through multi location and multi season studies. Incorporating molecular markers and examining micronutrient bioavailability will enhance breeding efficiency and nutrition. These integrated strategies will improve the production of nutritionally fortified rice varieties with better yield and quality contributing to sustainable food and nutritional security.

Acknowledgements

The authors express their sincere gratitude to Indian Institute of Rice Research, Hyderabad, for providing the facilities and technical support for the analysis of iron and zinc content in rice grains.

Authors' contributions

DPDV conducted the experiment, recorded observations and analyzed the data. TS conceptualised and supervised the research. NGW assisted in conducting the experiment and contributed to data analysis and guidance. ANY, SH and SD helped in editing, summarising and revising the manuscript and approved the final version. All authors read and approved the final manuscript.

Compliance with ethical standards

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

Ethical issues: None

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Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

Publisher information: Plant Science Today is published by HORIZON e-Publishing Group with support from Empirion Publishers Private Limited, Thiruvananthapuram, India.