Plant Archives Vol. 26, No. 1, 2026 pp. 2163-2170 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2026.v26.no.1.297

PRINCIPAL COMPONENT ANALYSIS OF MORPHOLOGICAL, GRAIN QUALITY AND NUTRACEUTICAL TRAITS IN A BIL POPULATION OF SWARNA × *ORYZA RUFIPOGON* IN RICE

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(Date of Receiving-11-02-2026; Date of Revision-02-03-2026; Date of Acceptance-18-04-2026)

ABSTRACT

Wild relatives of rice constitute an important reservoir of genetic diversity that can be utilized to enhance the agronomic performance and nutritional quality of cultivated rice. The present study evaluated a backcross inbred line (BIL) population derived from Swarna × *Oryza rufipogon* to assess phenotypic variability and trait relationships using principal component analysis (PCA). A total of 275 BILs along with four checks were evaluated during *Kharif*, 2024 at Agricultural Research Station, Bapatla and ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad, using an augmented block design. Traits related to phenology, yield components, grain quality and nutraceutical properties, including anthocyanin content, total phenol content and antioxidant activity were recorded. Principal component analysis revealed substantial phenotypic variability within the population. At Bapatla, the first two principal components explained 39.29% of total variation, while at Rajendranagar they explained 34.02% of total variation. Nutraceutical traits such as anthocyanin content, total phenols and antioxidant activity contributed strongly to the first principal component, whereas yield-related traits, including grains per panicle, panicle length and spikelet fertility contributed significantly to the second principal component. PCA biplot analysis revealed distinct clustering of traits and wide dispersion of genotypes, indicating the presence of considerable genetic variability generated through introgression from *Oryza rufipogon*. The results demonstrate the usefulness of principal component analysis for identifying major sources of phenotypic variation and promising genotypes for rice improvement programmes.

Key words : BIL population, *Oryza rufipogon*, PCA.

Introduction

Rice (*Oryza sativa* L.) is one of the most important staple crops worldwide and provides the primary source of calories for more than half of the global population. Sustained improvement in rice productivity is essential for ensuring food security in developing countries where rice constitutes the main dietary component (Khush, 2013). However, the genetic base of modern rice cultivars has narrowed due to extensive use of a limited number of elite parental lines during breeding programmes. This genetic bottleneck restricts the availability of novel alleles required for further improvement of complex traits such as yield stability, stress tolerance and grain nutritional quality (Tanksley and McCouch, 1997).

Wild relatives of rice represent valuable genetic resources that harbor diverse alleles absent in cultivated germplasm. Among them, *Oryza rufipogon*, the progenitor of cultivated rice, has been widely used as a donor for improving agronomic traits, disease resistance, and grain quality through introgression breeding (Brar and Khush, 1997). Introgression lines and backcross inbred populations derived from crosses between cultivated rice and wild species provide powerful genetic materials for studying trait variation and identifying favorable alleles contributed by wild relatives (Xiao *et al.*, 1998; Balakrishnan *et al.*, 2018).

In addition to improving yield, there is growing interest in enhancing the nutritional and functional quality of rice grains. Bioactive compounds such as anthocyanins, phenolic compounds and antioxidant metabolites have been reported to possess important health-promoting properties including anti-oxidative and anti-inflammatory effects (Goufo and Trindade, 2014). Wild rice species often contain higher concentrations of these compounds compared with cultivated varieties, making them promising donors for nutritional improvement.

Evaluation of complex breeding populations for multiple agronomic and biochemical traits generates multi-dimensional datasets that require advanced statistical tools for meaningful interpretation. Multivariate statistical techniques such as principal component analysis (PCA) are widely used to summarize phenotypic variability and identify the major traits contributing to diversity within germplasm collections (Jolliffe, 2002). PCA reduces the dimensionality of the dataset while retaining most of the variability, allowing visualization of relationships among traits and genotypes. Several studies have successfully applied PCA to characterize genetic diversity and trait associations in rice germplasm (Nachimuthu *et al.*, 2014; Oladosu *et al.*, 2018; Shrestha *et al.*, 2021).

Therefore, the present investigation was undertaken to evaluate a backcross inbred line population derived from Swarna × *Oryza rufipogon* for agro-morphological, grain quality and nutraceutical traits across two environments using principal component analysis, with the objective of identifying the major sources of phenotypic variation and potential genotypes for rice improvement programmes.

Materials and Methods

The experimental material consisted of a backcross inbred line population comprising 275 lines derived from the cross Swarna × *Oryza rufipogon* (Rao *et al.*, 2018). Four popular rice varieties, namely BPT-5204, DRR Dhan-45, BPT-2841 and Kalanamak, were included as checks for comparison. Field experiments were conducted during *Kharif*, 2024 at two locations: Agricultural Research Station, Bapatla and ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad. The experiments were laid out in an augmented block design with spacing of 20 × 15 cm between plants. Each entry was planted in two rows of two meters length. Standard agronomic practices were followed to raise a healthy crop.

Observations were recorded for several morphological traits including days to 50% flowering, productive tillers per plant, panicle length, total grains per panicle, spikelet fertility, test weight and grain yield per plant. In addition, grain quality and nutraceutical traits such as kernel length, kernel breadth, length-breadth ratio, amylose content, alkali spreading value, protein content, anthocyanin content, total phenol content and antioxidant activity were analyzed using standard laboratory procedures. Principal component analysis was performed using standardized data to identify the major components explaining phenotypic variation among the evaluated genotypes.

Results and Discussion

Principal component analysis of phenotypic variation

Principal component analysis (PCA) was performed to summarize the multidimensional variability present among agro-morphological, grain quality and nutraceutical traits in the backcross inbred line (BIL) population derived from the cross Swarna × *Oryza rufipogon*. The analysis revealed considerable phenotypic diversity among the evaluated genotypes across the two experimental environments, indicating the presence of substantial genetic variability generated through introgression from the wild progenitor species. Such wide phenotypic variation is commonly observed in interspecific breeding populations where recombination between cultivated and wild genomes produces diverse segregants (Brar and Khush, 1997; Tanksley and McCouch, 1997; Xiao *et al.*, 1998).

The eigenvalue distribution indicated that a limited number of principal components captured a large proportion of the total phenotypic variation (Table 1). At Bapatla, the first principal component (PC1) explained 22.12% of the total variance with an eigenvalue of 3.5386, followed by PC2 (17.18%) and PC3 (12.23%) with eigenvalues of 2.7485 and 1.9564, respectively. The cumulative variance explained by the first three components reached 51.52%, indicating that these components effectively summarized the major dimensions of phenotypic variability within the population. Similarly, at Rajendranagar, PC1 accounted for 21.76% of the variation, while PC2 and PC3 explained 12.26% and 11.24%, respectively, with the cumulative variance of the first three components reaching 45.27% (Table 1). The scree plot further confirmed that the first few principal components captured the majority of the variability, while

Table 1 : Eigenvalues, percentage variance and cumulative variance explained by principal components at Bapatla and Rajendranagar during *Kharif*, 2024.

Principal Component	Bapatla			Rajendranagar		
	Eigenvalue	Variance Explained (%)	Cumulative Variance (%)	Eigenvalue	Variance Explained (%)	Cumulative Variance (%)
PC1	3.5386	22.12	22.12	3.4823	21.76	21.76
PC2	2.7485	17.18	39.29	1.9613	12.26	34.02
PC3	1.9564	12.23	51.52	1.7991	11.24	45.27
PC4	1.4780	9.24	60.76	1.4608	9.13	54.40
PC5	1.1849	7.41	68.17	1.2798	8.00	62.39
PC6	1.0062	6.29	74.45	1.0764	6.73	69.12
PC7	0.9092	5.68	80.14	0.9917	6.20	75.32
PC8	0.7374	4.61	84.75	0.9860	6.16	81.48
PC9	0.7048	4.41	89.15	0.7323	4.58	86.06
PC10	0.5520	3.45	92.60	0.6556	4.10	90.16
PC11	0.4594	2.87	95.47	0.6392	3.99	94.15
PC12	0.2936	1.84	97.31	0.3472	2.17	96.32
PC13	0.1772	1.11	98.41	0.3312	2.07	98.39
PC14	0.1455	0.91	99.32	0.1606	1.00	99.40
PC15	0.0930	0.58	99.90	0.0923	0.58	99.97
PC16	0.0153	0.10	100.00	0.0044	0.03	100.00

Table 2 : Major trait loadings for the first two principal components at Bapatla and Rajendranagar during *Kharif*, 2024.

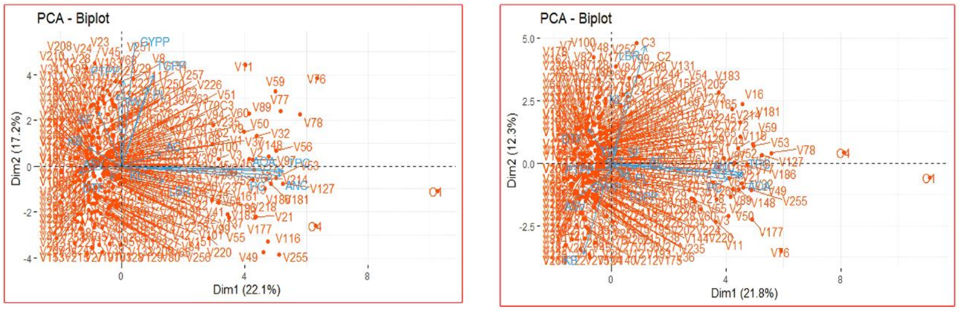
Trait	Bapatla		Rajendranagar	
	PC1 Loading	PC2 Loading	PC1 Loading	PC2 Loading
Days to 50% flowering (DTF)	0.035	-0.057	0.088	-0.197
Productive tillers per plant (PTPP)	-0.004	0.396	0.023	0.035
Panicle length (PL)	-0.079	0.366	-0.066	0.064
Total grains per panicle (TGPP)	-0.097	0.423	-0.070	0.094
Spikelet fertility (SF)	0.072	0.253	-0.044	-0.026
Test weight (TW)	0.012	0.335	0.028	-0.013
Grain yield per plant (GYPP)	-0.039	0.572	-0.044	0.081
Kernel length (KL)	-0.081	-0.009	-0.061	-0.416
Kernel breadth (KB)	0.103	0.087	0.110	0.528
L/B ratio	-0.129	-0.072	-0.128	-0.680
Amylose content (AC)	-0.119	0.061	-0.088	0.028
Alkali spreading value (ASV)	0.131	-0.028	0.000	0.046
Protein content (PC)	-0.454	-0.057	-0.443	0.094
Anthocyanin content (ANC)	-0.483	-0.044	-0.489	0.068
Total phenol content (TPC)	-0.490	-0.022	-0.501	0.054
Antioxidant activity (AOA)	-0.481	-0.017	-0.502	0.072

later components contributed progressively smaller proportions of variance (Fig. 3).

Similar magnitudes of variation explained by the first few principal components have been widely reported in rice diversity studies. For example, Nachimuthu *et al.* (2014) reported that the first three principal components explained nearly half of the total variation in a diverse rice panel. Oladosu *et al.* (2018) observed that PC1 contributed approximately 21.4% of total variability, while Christina *et al.* (2021) and Shrestha *et al.* (2021) reported comparable contributions of PC1 ranging between 20–25% in rice germplasm evaluated for yield and related traits. Similar findings were also reported by Duraiswamy *et al.* (2023) and Khatun *et al.* (2023) in rice breeding populations, confirming that PCA efficiently reduces dimensionality while retaining the majority of biologically meaningful information.

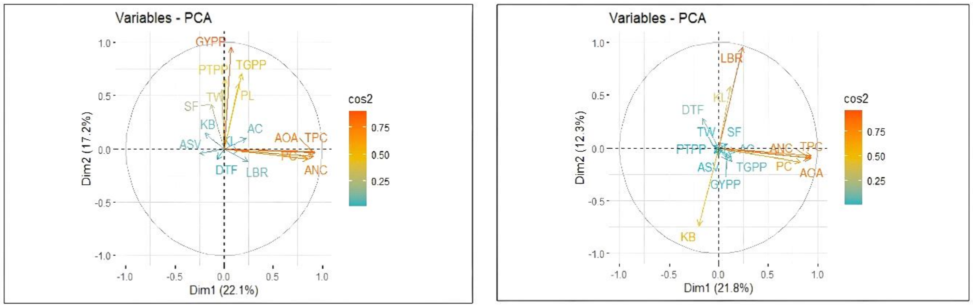
Trait contributions and biological interpretation of principal components

The loading pattern of individual traits provided important insights into the variables contributing most strongly to phenotypic diversity within the BIL population (Table 2). Nutraceutical traits including anthocyanin content, total phenol content, antioxidant activity and protein content exhibited high coefficient values in the first principal component.



(a) Bapatla **(b) Rajendranagar**

Fig. 1 : PCA biplot showing the distribution of genotypes and trait vectors based on the first two principal components (PC1 and PC2) in the Swarna × *Oryza rufipogon* BIL population at (a) Bapatla and (b) Rajendranagar.



(a) Bapatla **(b) Rajendranagar**

Fig. 2 : Correlation circle plot of variables showing the contribution and association of agro-morphological, grain quality and nutraceutical traits with the first two principal components at (a) Bapatla and (b) Rajendranagar.

At Bapatla, PC1 showed strong negative values for total phenol content (–0.490), anthocyanin content (–0.483), antioxidant activity (–0.481) and protein content (–0.454) (Table 2). Contribution analysis further confirmed the dominance of these traits in PC1, where total phenol content contributed 24.02%, followed by anthocyanin content (23.32%), antioxidant activity (23.18%) and protein content (20.58%) (Table 3). Similarly, at Rajendranagar, the largest contributions to PC1 were observed from antioxidant activity (25.18%), total phenol content (25.06%), anthocyanin content (23.88%) and protein content (19.66%) (Table 3). These results clearly indicate that variation in grain biochemical composition constituted the primary axis of phenotypic differentiation among the evaluated genotypes.

The strong association among anthocyanins, phenolic compounds and antioxidant activity is biologically expected because these compounds are synthesized through interconnected flavonoid and phenylpropanoid metabolic pathways responsible for antioxidant defense mechanisms in plants (Goufo and Trindade, 2014). Wild rice species often accumulate higher concentrations of such bioactive metabolites compared with cultivated varieties, which explains the large variability observed in nutraceutical traits within introgression populations derived from wild relatives (Brar and Khush, 1997; Xiao *et al*., 1998).

The second principal component (PC2) was mainly influenced by yield-related and grain morphology traits. At Bapatla, the largest contribution to PC2 was observed from grain yield per plant (32.77%), followed by total grains per panicle (17.85%), productive tillers per plant (15.66%), panicle length (13.40%) and test weight (11.25%) (Table 3). These traits also exhibited relatively high loading values in PC2, including 0.572 for grain yield per plant, 0.423 for total grains per panicle, 0.396 for productive tillers per plant, and 0.366 for panicle length (Table 2), indicating that PC2 represented variation associated with yield potential and its component traits. At Rajendranagar, PC2 was largely influenced by grain morphology traits, where length–breadth ratio contributed 46.32%, followed by kernel breadth (27.92%) and kernel length (17.29%) (Table 3). These traits also recorded high loading values in PC2, including “0.680 for length–breadth ratio, 0.528 for kernel breadth, and “0.416 for kernel length (Table 2). Similar observations have been reported by Asante *et al*. (2019), Devasena *et al*. (2023) and Sharif *et al*. (2023), who reported that grain size and shape traits often form independent principal components in multivariate analyses of rice germplasm.

Trait associations revealed by PCA biplot

The PCA biplot (Fig. 1) provided a graphical representation of the relationships among traits and the

Table 3 : Contribution (%) of traits to the first two principal components at Bapatla and Rajendranagar during *Kharif*, 2024.

Trait	Bapatla		Rajendranagar	
	Contribution to PC1 (%)	Contribution to PC2 (%)	Contribution to PC1 (%)	Contribution to PC2 (%)
Days to 50% flowering (DTF)	0.13	0.33	0.77	3.90
Productive tillers per plant (PTPP)	0.00	15.66	0.05	0.13
Panicle length (PL)	0.62	13.40	0.44	0.43
Total grains per panicle (TGPP)	0.94	17.85	0.49	0.93
Spikelet fertility (SF)	0.51	6.41	0.19	0.07
Test weight (TW)	0.01	11.25	0.08	0.02
Grain yield per plant (GYPP)	0.15	32.77	0.19	0.65
Kernel length (KL)	0.65	0.01	0.38	17.29
Kernel breadth (KB)	1.07	0.76	1.20	27.92
L/B ratio	1.67	0.52	1.64	46.32
Amylose content (AC)	1.42	0.37	0.77	0.08
Alkali spreading value (ASV)	1.71	0.08	0.00	0.22
Protein content (PC)	20.58	0.32	19.66	0.92
Anthocyanin content (ANC)	23.32	0.19	23.88	0.48
Total phenol content (TPC)	24.02	0.05	25.06	0.30
Antioxidant activity (AOA)	23.18	0.03	25.18	0.52

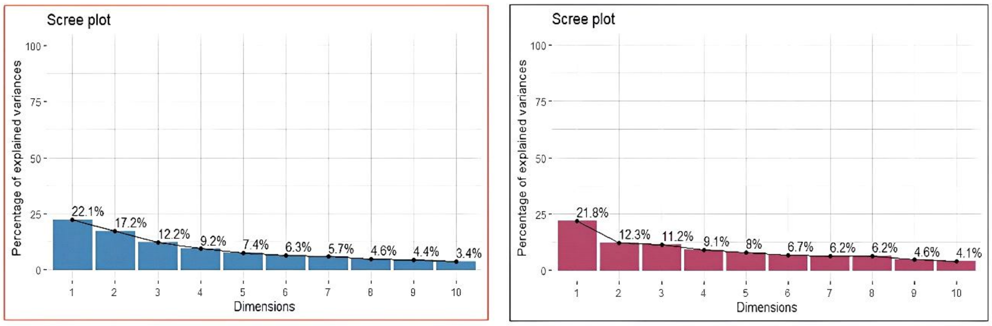
Table 4 : Top contributing traits to the first two principal components across environments.

Principal Component	Bapatla – Top Contributing Traits	Contribution (%)	Rajendranagar – Top Contributing Traits	Contribution (%)
PC1	Total phenol content (TPC)	24.02	Antioxidant activity (AOA)	25.18
	Anthocyanin content (ANC)	23.32	Total phenol content (TPC)	25.06
	Antioxidant activity (AOA)	23.18	Anthocyanin content (ANC)	23.88
	Protein content (PC)	20.58	Protein content (PC)	19.66
PC2	Grain yield per plant (GYPP)	32.77	L/B ratio	46.32
	Total grains per panicle (TGPP)	17.85	Kernel breadth (KB)	27.92
	Productive tillers per plant (PTPP)	15.66	Kernel length (KL)	17.29
	Panicle length (PL)	13.40	Days to 50% flowering (DTF)	3.90

distribution of genotypes based on the first two principal components. Traits located close to each other in the biplot indicate strong positive correlations, whereas traits located in opposite directions indicate negative associations. In the present study, nutraceutical traits including anthocyanin content, total phenol content and antioxidant activity were closely grouped in the biplot, forming a distinct cluster (Fig. 1). This clustering pattern indicates strong positive correlations among these biochemical traits. Yield-related traits such as grain yield per plant, total grains per panicle and productive tillers per plant also formed a separate cluster, reflecting their collective contribution to plant productivity. Grain quality traits including kernel length, kernel breadth and length-breadth ratio were positioned in a different region of the biplot, indicating that grain morphology represents a separate dimension of phenotypic variation within the population. Similar clustering patterns have been reported in previous studies evaluating phenotypic diversity in rice germplasm. For example, Oladosu *et al.* (2018), Duraiswamy *et al.* (2023) and Sai Venkat *et al.* (2024) observed distinct clusters representing yield components, grain quality traits and biochemical attributes in PCA analyses of rice genotypes.

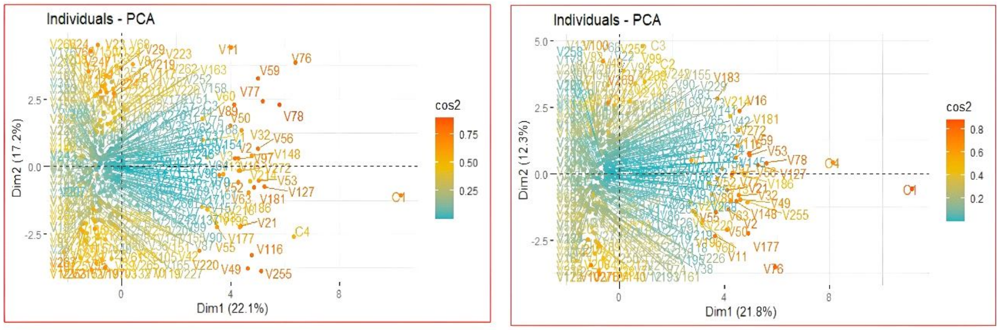
Correlation circle and variable contributions

The correlation circle plot (Fig. 2) further illustrated the relationships among variables and their contributions to the principal components. Traits positioned near the circumference of the circle exert stronger influence on



(a) Bapatla **(b) Rajendranagar**

Fig. 3 : Scree plot illustrating eigenvalues and percentage of variance explained by principal components for agro-morphological, grain quality and nutraceutical traits at (a) Bapatla and (b) Rajendranagar.



(a) Bapatla **(b) Rajendranagar**

Fig. 4 : PCA scatter plot of individuals (genotypes) showing the distribution of the Swarna × *Oryza rufipogon* BIL population along the first two principal components (PC1 and PC2) at (a) Bapatla and (b) Rajendranagar.

the principal components, whereas traits located closer to the origin contribute less to the overall variability. In this study, nutraceutical traits such as anthocyanin content, total phenol content and antioxidant activity were positioned near the outer region of the circle along the PC1 axis, confirming their strong contribution to the first principal component (Fig. 2). Conversely, yield-related attributes including grain yield per plant, total grains per panicle and productive tillers per plant were positioned along the PC2 axis, indicating their major contribution to the second dimension of phenotypic variation.

Distribution of genotypes and implications for breeding

The PCA scatter plot (Fig. 4) revealed a wide distribution of genotypes across the principal component axes, indicating substantial phenotypic variability within the BIL population. Such wide dispersion is commonly observed in populations derived from interspecific hybridization, where recombination between cultivated and wild genomes generates novel allelic combinations and transgressive segregants (Tanksley and McCouch, 1997; Xiao *et al.*, 1998; Balakrishnan *et al.*, 2016). Introgression from wild rice species such as *Oryza rufipogon* has been widely recognized as an effective strategy for broadening the genetic base of cultivated rice and introducing beneficial alleles associated with yield improvement, stress tolerance and enhanced grain quality (Brar and Khush, 1997; Yadavalli *et al.*, 2022; Gowthami *et al.*, 2024; Magudeeswari *et al.*, 2024; Sridhar *et al.*, 2025). The broad phenotypic variability observed in the present study therefore reflects the successful incorporation of genetic diversity from the wild progenitor into the cultivated background.

From a breeding perspective, genotypes positioned along the PC2 axis associated with grain yield per plant (32.77%), total grains per panicle (17.85%) and productive tillers per plant (15.66%) may serve as promising donor lines for improving yield potential in rice breeding programmes. Similarly, genotypes associated with higher values of anthocyanin content, phenolic compounds and antioxidant activity, which collectively contributed more than 90% of the variation in PC1, represent valuable genetic resources for developing nutritionally enhanced rice varieties.

Conclusion

The backcross inbred line population derived from *Swarna* × *Oryza rufipogon* exhibited substantial phenotypic variability for agro-morphological, grain quality and nutraceutical traits across the two experimental environments. Principal component analysis revealed that nutraceutical traits such as anthocyanin content, total phenol content, antioxidant activity and protein content constituted the primary source of phenotypic variation, while yield component traits including grain yield per plant, total grains per panicle and productive tillers per plant represented the second major dimension of variability. The wide dispersion of genotypes observed in the PCA scatter plot indicates that the introgression of alleles from the wild progenitor has generated considerable genetic diversity within the BIL population.

From a breeding perspective, genotypes located in the positive quadrant of the PC2 axis with high values for grain yield, panicle length and grains per panicle may serve as potential donor lines for improving yield potential in rice breeding programmes. Similarly, genotypes positioned along the PC1 axis associated with higher anthocyanin, phenol and antioxidant content represent valuable genetic resources for developing nutritionally enriched rice varieties. Therefore, the identified BIL lines provide promising opportunities for selecting genotypes combining high productivity with enhanced nutraceutical quality, which is increasingly important for developing next-generation rice varieties with improved health benefits.

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