

Variability for Grain and Nutritional Quality Parameters and Characterization of Genes for Amylose Content in Rice (*Oryza sativa* L.)

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Abstract

Rice, a vital global staple, holds significance for its role in addressing nutritional needs and poverty alleviation. This study evaluates the variability in grain quality parameters and micronutrient content among 24 rice genotypes from Maharashtra, India. Grain length, breadth, starch, amylose, amylopectin, iron, and zinc content were assessed. Significant variability was observed across all traits, with grain length ranging from 3.68 to 7.32mm, starch content from 69.87% to 80.11%, amylose content from 3.82-24.81% and amylopectin content from 46.40-76.29%. Intermediate amylose content genotypes were identified as promising for breeding programs targeting improved cooking quality. Furthermore, iron and zinc content varied significantly among genotypes ranging from 0.92-4.16 mg 100⁻¹ g, and 1.75-3.82 mg 100⁻¹ g, respectively, with several exceeding recommended thresholds. High genetic coefficients were noted for iron content, ratio of amylose-to-amylopectin, and length-breadth ratio, indicating potential for genotype selection in crop improvement programs. Additionally, high heritability and high genetic advance suggest the involvement of additive gene action in trait inheritance, supporting phenotypic selection. Molecular marker analysis facilitated the identification of genotypes suitable for improving amylose content and micronutrient levels in popular varieties like 'Indrayani'. This study underscores the significance of integrating molecular markers with traditional breeding methods for enhancing rice quality and nutritional value.

Key words : Amylose content; genetic variability; grain quality; grain micronutrient; characterization; marker-assisted selection.

Rice, known as the "Global Grain," serves as a fundamental dietary staple in over 100 nations, predominantly across Asia where it nourishes half of the population (Yugandhar et al., 2018). During the year 2022, with a global production of 776.46 million tonnes and occupying 165.04 million hectares of agricultural land, rice stands as the third most produced cereal, following maize and wheat. India, with an area of 46.4 million hectares under rice cultivation and a production of 196.24 million tonnes, ranks second only to China in rice production (<https://www.fao.org/faostat/en/#data/QCL/visualize> verified 12 May 2024).

As an economically vital food crop, rice plays a crucial role in addressing nutritional diversity and poverty alleviation. The physical quality

parameters of rice, including size, shape, and the ratio of length to breadth (L/B), are key determinants of its overall quality (Rita and Sarawgi, 2008). Additionally, starch composition, encompassing amylose and amylopectin content, significantly influence its cooking characteristics (Ahmed et al., 2007; Lisle et al., 2000).

Enhancement of grain and cooking quality of rice is central to most of the rice breeding programs and is driven by consumer acceptance and market demand. Amylose content emerges as a critical factor, deciding the texture of cooked rice. While low amylose content results in stickiness, intermediate levels yield fluffiness and tenderness, whereas high amylose content leads to dryness and firmness post-cooking. Moreover, the ratio of amylose-to-amylopectin influences

starch digestion rates and metabolic responses (Kaur et al., 2015).

Despite its significance, rice's micronutrient content is limited, potentially causing "hidden hunger" due to micronutrient malnutrition, particularly affecting vulnerable demographics such as children and women (Khush and Virk, 2000). Notably, iron and zinc are crucial trace minerals in rice grains, necessitating the identification of genotypes with elevated levels of these nutrients.

Therefore, this study was performed to evaluate the extent of variability in rice genotypes concerning grain quality parameters, including amylose, iron, and zinc content, focusing on landraces, lines, and varieties developed/identified by agricultural universities in Maharashtra State, India. Additionally, molecular marker analysis aims to identify promising donor genotypes for amylose content improvement, facilitating marker-assisted selection strategies, particularly targeting the popular 'Indrayani' variety being grown on around 70% of the rice growing area of western Maharashtra but characterized by lower amylose content.

Materials and methods

Material

Twenty-four rice genotypes comprising released varieties, promising lines, and landraces developed/identified by three Agricultural Universities in the Maharashtra State of India [MPKV, Rahuri (12); Dr. PDKV, Akola (3), and Dr. BSKKV, Dapoli (9)] and obtained from Agricultural Research Station, Radhanagari, Kolhapur District, Maharashtra were used in the present study (Table 1).

Methods

The genotypes were characterized for grain

length (mm), grain breadth (mm), and length/breadth (L/B) ratio following standard procedures (IRRI, 1996). Similarly, starch content (Thimmaiah, 1999), amylose content (Thimmaiah, 1999), and amylopectin content (Thayumanavan and Sadasivam, 1984) were estimated. Based on amylose content, rice genotypes were grouped into waxy (1-2%), very low (2-9%), low (9-20%), intermediate (20-25%) and high (25-33%) as per Juliano (1972). Micronutrient elements, iron, and zinc content were estimated as per Zasoski and Burau (1977). The details are given below.

Estimation of grain length, breadth, and length-breadth (L/B) ratio : The length and breadth of rice grains were measured using a digital Vernier caliper, with five grains measured per genotype (IRRI, 1996). The L/B ratio was calculated by dividing the average length by the average breadth of the grains.

Estimation of starch content : Starch content was estimated by homogenizing a 0.1 g sample in 5 ml hot 80% ethanol to remove sugar, followed by centrifugation at 12000 rpm for 10 minutes followed by repeated washing till it did not give color with anthrone reagent and finally drying of the residue. 5.0 ml water and 6.5 ml 52% Perchloric acid was then added to the residue and the sample was refrigerated at 0°C for 20 min. The supernatant obtained by centrifuging at 12000 rpm for 10 minutes was saved, and anthrone reagent was added to determine starch content by measuring the color intensity at 630 nm. A graph of absorbance versus concentration of D-glucose was plotted and the glucose content was estimated using the standard graph. This was done by multiplying the value by a factor of 0.9 to arrive at the starch content.

Estimation of amylose content : Amylose content was estimated by transferring 100 mg powdered samples to a volumetric flask,

adding distilled ethanol and 1N NaOH, and leaving it overnight. After dilution, phenolphthalein indicator was added, followed by titration with 0.1N HCl until the pink color disappeared. Iodine reagent was then added, and absorbance was read at 590 nm to determine amylose content. The standard curve for amylose was prepared by taking 0.2, 0.4, 0.6, 0.8, and 1.0 ml of the standard amylose solution (Sigma Aldrich) against a blank for which was diluted by 1 ml of iodine reagent and the volume was made up to 50 ml with water and the absorbance was read at 590 nm. A graph of absorbance versus concentration of amylose was prepared.

Estimation of amylopectin and ratio of amylose-to-amylopectin : The amount of amylopectin was obtained by subtracting the amylose content from that of starch (Thayumanavan and Sadasivam, 1984). The ratio of amylose-to-amylopectin was calculated by dividing the average amylose content by the average amylopectin content of the grain.

Estimation of iron and zinc content : Iron and zinc content were estimated using the di-acid mixture method (Zasoski and Burau, 1977). Powdered samples were digested in an acid mixture of $\text{HNO}_3:\text{HClO}_4$ (9:4), and the resulting solution was filtered and analyzed using an Atomic Absorption Spectrophotometer. Concentration was expressed in $\text{mg } 100^{-1} \text{ g}$ against a standard curve.

Molecular marker analysis : Molecular marker analysis was performed using 20 SSR markers including 17 reported to be linked with gene/QTL for amylose content and three linked with aroma. DNA extraction, DNA amplification and analysis of the marker data as well as construction of the dendrogram were done as per Gavhane et al. (2019) and Mawalkar et al. (2024). Since the waxy gene is important in amylose synthesis because it encodes the enzyme granule bound starch synthase (GBSS)

and it is present on chromosome 6, majority of the markers were chosen from this chromosome. In addition, markers from other chromosomes where QTLs for amylose are reported were also used.

Statistical analysis : Mean values generated from three replications were analyzed using analysis of variance (ANOVA). Genetic parameters such as phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability in broad sense (h^2), and genetic advance as a percent of the mean (genetic gain) were calculated using statistical software INDOSTAT Services, Hyderabad, India.

Results and discussion

Considerable variation was observed among the 24 rice genotypes for all the studied traits. ANOVA indicated highly significant differences among genotypes for all characters, suggesting substantial variability within the studied material.

Grain length and breadth : Grain length and breadth exhibited wide variation among genotypes (Table 1). Grain length ranged from 3.68 mm (Tilsha) to 7.32 mm (Bhogavati), with an average of 5.71 mm, while grain breadth varied from 1.57 mm (PDKV Tilak) to 2.77 mm (KJT 5), with an average of 1.95 mm. The length-to-breadth (L/B) ratio ranged from 1.46 (Tilsha) to 4.26 (Bhogavati), with an average of 3.03.

Grain dimensions play a crucial role in the marketing and commercial classification of rice. Various standards exist worldwide for classifying rice based on grain size and shape. For example, in the European Union and India, different classifications are applied based on grain length and L/B ratio. In India, grains are classified into long slender (length 6.0 mm and above, L/B ratio 3.0 and above), long bold (length 6.0 mm and above, L/B ratio less than 3.0), medium

slender (length less than 6 mm, L/B ratio 2.5 to 3.0), short slender (length less than 6 mm and L/B ratio 3 and above) and short bold (length less than 6 mm, L/B ratio less than 3) types (Ramaiah, 1969). In our study, based on these criteria, the genotypes were categorized into long slender (8), medium slender (3), short slender (8), and short bold types (5).

Quality composition of rice grain : Starch, comprising amylose and amylopectin, is the primary energy source in rice grains. Starch content varied significantly among genotypes, ranging from 69.87% (PDKV Tilak) to 80.11% (Nilbhat), with an average of 72.60% (Table 2). Amylose content ranged from 3.82% to 24.81%, with an average of 20.92%. In the

Table 1. Details of rice genotypes used along with their pedigree and grain quality parameters

Genotype	Pedigree	Source grain length (mm)	Grain length (mm)	Grain breadth (mm)	L/B ratio	Grain type
Indrayani	Ambemohar 157 x IR 8	MPKV, Rahuri	6.46	1.77	3.68	Long slender
Pawana	Pusa 33 x IR 28	MPKV, Rahuri	6.56	1.95	3.38	Long slender
Phule Samrudhi	Indrayani x Sonsali	MPKV, Rahuri	6.63	1.86	3.57	Long slender
Bhogavati	Selection from Basmati Composite	MPKV, Rahuri	7.32	1.73	4.26	Long slender
Phule Radha	TN 1 x Kolamba 540	MPKV, Rahuri	5.31	1.62	3.28	Short slender
Phule Maval	Pawana x Indrayani	MPKV, Rahuri	5.75	2.58	2.24	Short Bold
Kundalika	RTN 24 x IET 3228	MPKV, Rahuri	5.51	1.74	3.17	Short slender
Ambemohar 157	Local selection from Maval (Pune district)	MPKV, Rahuri	4.37	2.19	2.00	Short bold
LK 248	Local collection from Nashik (MS)	MPKV, Rahuri	5.17	1.81	2.86	Medium slender
Tilsha	Landrace	Local collection from Nashik district	3.68	2.53	1.46	Short bold
KJT 2	RP6-17 x RP4-14	Dr. BSKKV, Dapoli	6.38	1.85	3.45	Long slender
Nilbhat	Landrace	Local collection from Nashik district	5.64	2.22	2.53	Medium slender
KJT 3	IR-36 x KJT 35-3	Dr. BSKKV, Dapoli	5.52	2.25	2.47	Short bold
KJT 5	Selection from BR-827-35-3-1-1-1-R	Dr. BSKKV, Dapoli	5.97	2.77	2.16	Short bold
KJT 6	Heera x KJT- 184	Dr. BSKKV, Dapoli	5.23	1.59	3.29	Short slender
KJT 7	Patel 3 x KJT 9-333	Dr. BSKKV, Dapoli	6.31	1.83	3.45	Long slender
KJT 184	TN-1 x Kolamba-540	Dr. BSKKV, Dapoli	5.59	1.85	3.03	Short slender
RTN 5	Zinia-63 x IR-64	Dr. BSKKV, Dapoli	5.84	1.81	3.23	Short slender
RTN 73-1	RTN 23-1 x Karjat 87-2	Dr. BSKKV, Dapoli	5.40	2.14	2.74	Medium slender
PKV Khamang	SYE-35-5 x BMT-70	Dr. PDKV, Akola	5.57	1.86	3.04	Short slender
PKV HMT	Selection from HMT Sona	Dr. PDKV, Akola	5.16	1.60	3.23	Short slender
PDKV Tilak	Daya x SYE-63-2003	Dr. PDKV, Akola	5.27	1.57	3.35	Short slender
RTN Purple	Local selection from Ratnagiri district	Dr. BSKKV, Dapoli	6.23	1.79	3.49	Long slender
Super Pawana	Mutant from Pawana	MPKV, Rahuri	6.18	1.84	3.37	Long slender
Mean	5.71	1.95	3.03			
Range	3.68-7.32	1.57-2.77	1.46-4.26			
S.E.	0.126	0.072	0.146			
CD at 5%	0.359	0.204	0.415			
CD at 1%	0.480	0.272	0.554			

MPKV, Mahatma Phule Krishi Vidyapeeth, Rahuri; Dr. PDKV, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola; Dr. BSKKV, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli
SE, standard error, CD, critical difference

present study, genotypes were classified into three categories based on amylose content: very low (Nilbhat), low (Indrayani, Pawana, and IGP 13-12-19), and intermediate (remaining 20 genotypes). Amylopectin content ranged from 46.40% to 76.29%, with a mean of 51.68%. The ratio of amylose to amylopectin varied widely among genotypes, from 0.05 to 0.53. The amount of amylose determines how sticky, tender, and soft the rice grain will be after cooking and the amount of amylopectin

determines the cooking quality and texture. Intermediate amylose content is preferred by consumers due to desirable cooking qualities and such rice upon cooking become fluffy, soft, moist, and tender when cooled.

The popular rice variety Indrayani, cultivated extensively in Maharashtra, exhibits low amylose content (~17%), resulting in stickiness after cooking. There's a growing demand to enhance its amylose content. Our study identified high-

Table 2. Details of grain and nutritional quality parameters of the rice genotypes

Genotype	Starch (%)	Amylose (%)	Amylopectin (%)	Amylose/Amylopectin ratio	Iron (mg 100 ⁻¹ g)	Zinc (mg 100 ⁻¹ g)
Indrayani	73.52	17.16	56.36	0.31	1.16	2.16
Pawana	76.05	19.02	57.02	0.33	1.23	2.40
Phule Samrudhi	72.60	21.32	51.29	0.42	1.75	2.42
Bhogavati	73.41	24.38	49.02	0.50	2.03	2.82
Phule Radha	71.42	21.64	49.78	0.45	1.49	2.81
Phule Maval	71.20	24.81	46.40	0.53	1.58	2.96
Kundalika	74.35	23.93	50.42	0.48	0.98	2.32
Ambemohar 157	70.18	21.98	48.20	0.45	2.08	1.91
LK 248	71.10	24.33	46.77	0.52	1.29	2.29
Tilsha	70.06	20.84	49.23	0.43	0.92	2.34
KJT 2	71.70	23.98	47.71	0.51	2.27	2.08
Nilbhat	80.11	3.82	76.29	0.05	1.88	2.11
KJT 3	71.02	20.25	50.77	0.40	1.42	2.48
KJT 5	71.12	22.17	48.95	0.45	1.29	3.02
KJT 6	72.37	21.26	51.11	0.42	1.25	2.79
KJT 7	72.53	22.20	50.34	0.44	2.14	1.98
KJT 184	72.62	21.93	50.69	0.43	1.54	2.85
RTN 5	72.56	21.34	51.22	0.42	1.08	2.35
RTN 73-1	72.56	23.10	49.46	0.47	1.33	1.75
PKV Khamang	73.33	22.12	51.22	0.43	1.58	3.01
PKV HMT	70.67	22.49	48.18	0.47	1.24	3.07
PDKV Tilak	69.87	21.21	48.66	0.44	3.02	2.78
RTN Purple	75.68	20.84	54.85	0.38	4.16	3.82
IGP 13-12-19	72.42	16.12	56.30	0.29	1.48	2.58
Mean	72.60	20.93	51.68	0.42	1.67	2.55
Range	69.87-80.11	3.82-24.81	46.40-76.29	0.05-0.53	0.92-4.16	1.75-3.82
S.E.	0.713	1.249	1.447	0.038	0.072	0.034
CD at 5%	2.03	3.55	4.12	0.10	0.20	0.09
CD at 1%	2.71	4.74	5.50	0.14	0.27	0.13

SE, standard error. CD, critical difference

yielding genotypes with intermediate amylose content suitable for breeding programs aimed at improving Indrayani's cooking quality.

Micronutrient content : Iron and zinc content varied considerably among genotypes. Iron content ranged from 0.92 to 4.16 mg 100⁻¹ g, with RTN Purple exhibiting the highest and Tilsha the lowest iron content. Zinc content ranged from 1.75 to 3.82 mg 100⁻¹ g, with RTN Purple showing the highest and RTN 73-1 the lowest zinc content (Table 2). Thirteen genotypes had iron content more than the recommended threshold (14.5 ppm = 1.45 mg 100⁻¹ g) (Johnson et al., 2011), while eight genotypes had zinc content more than the recommended threshold (28 ppm = 2.8 mg 100⁻¹ g) (<http://www.harvestplus.org/content/zinc-rice-india>). Genotype RTN Purple had significantly higher values for both, iron and zinc and also had better amylose content suggesting its superiority for nutritional as well as cooking quality.

Genetic variability parameters for grain quality traits : Genotypic and phenotypic coefficients of variation were computed to assess variability in the studied traits (Table 3). Phenotypic variation was largely determined by

genotype, indicating the potential for phenotypic selection in trait enhancement. High genetic coefficients were observed for several traits, suggesting ample genetic variability exploitable in breeding programs.

The characters expressing high heritability (b.s.) are least influenced by environmental factors and selection for the character is easy. Heritability coupled with genetic advance gives a better estimate than heritability alone in determining the selection gain for the trait. High heritability coupled with high genetic advance was recorded for grain dimensions, amylose content, amylopectin content, ratio of amylose-to-amylopectin, iron, and zinc content. This indicates the predominance of additive gene actions, supporting the efficacy of phenotypic selection. Similar findings were reported in earlier studies (Babu et al., 2012; Bollinedi et al., 2020; Rathi et al., 2010). Estimation of the genotypic correlation coefficient is of paramount importance and is more stable to the plant breeders because it brings about the simultaneous genetic advancement of many characteristic traits or a simple complex trait that is a genetically correlated response to selection. In the present study, genotypic correlation coefficients revealed significant associations

Table 3. Genetic variability parameters for grain quality of rice genotypes

Character	Range	Mean ± S.E.	Geno- typic varia- nce	Pheno- typic varia- nce	GCV (%)	PCV (%)	Herita- bility (b.s.) (%)	GA	GA as % of mean
Grain length	3.68-7.32	5.71±0.126	0.568	0.616	13.197	13.740	92.20	1.491	26.110
Grain breadth	1.57-2.77	1.95±0.072	0.100	0.115	16.248	17.450	86.96	0.607	31.166
L/B ratio	1.46-4.26	3.03±0.146	0.368	0.432	20.007	21.671	85.18	1.153	38.050
Starch content	70.06-80.11	72.60±0.713	4.603	6.128	2.955	3.410	75.11	3.830	5.276
Amylose content	3.82-24.81	20.93±1.249	16.086	20.764	19.166	21.775	77.47	7.272	34.752
Amylopectin content	46.40-76.29	51.68±1.447	33.723	40.002	11.238	12.239	84.30	10.984	21.255
Ratio of amylose-to-amylopectin	0.05-0.53	0.42±0.038	0.008	0.013	21.947	26.895	61.54	0.154	36.894
Iron content	0.92-4.16	1.67±0.072	0.504	0.519	42.402	43.045	97.11	1.440	86.044
Zinc content	1.75-3.82	2.55±0.034	0.216	0.220	18.270	18.415	98.18	0.951	37.342

SE, standard error, GCV, genotypic coefficient of variation; PCV, genotypic coefficient of variation, GA, genetic advance

among traits, aiding in trait-based selection strategies.

Molecular marker analysis and identification of cross combination of genotypes for improvement of amylose content :

Out of the 20 markers used, 15 showed successful amplification in the studied genotypes. This indicates the suitability of these markers for genotypic analysis as well as characterization of gene/QTL for amylose content in rice genotypes. The range of 2 to 6 alleles per marker, with an average of 3.67 alleles per marker, suggests substantial allelic variation within the studied genotypes (Table 4). This diversity provides a rich genetic pool for breeding programs to select desirable traits. The polymorphic information content PIC values ranged from 0.076 (RM510) to 0.592 (RM3), with an average PIC of 0.309. Similar trend was observed for heterozygosity values, further confirming the genetic diversity among the studied genotypes. Out of 15 polymorphic markers studied, nine of them amplified unique alleles specific to different rice genotypes. This suggests that these markers have potential utility in characterizing and distinguishing between different rice varieties. This information can be crucial in breeding programs, conservation efforts, and in utilizing these genotypes in the marker-assisted selection program. Although only two markers exhibited high PIC values (>0.5), the overall PIC values indicate moderate levels of polymorphism, which is essential for marker-assisted selection.

The markers could effectively group the genotypes into two broad clusters, with one cluster containing the majority (22) of the genotypes and the other comprising only two genotypes (Fig. 1). Further, both these clusters were divided into two sub-clusters. This clustering pattern likely reflects the genetic relatedness among the studied genotypes. The study also suggests that while the molecular

markers linked with QTL/gene for amylose content could group genotypes effectively, the molecular diversity observed was less compared to trait-based diversity. This highlights the importance of integrating both trait-based and molecular marker information can facilitate more efficient and targeted crop improvement strategies (Zafar and Jianlong, 2023).

Markers, Wx, W2R, RM3, RM541, and RM217 linked with QTL/gene for amylose

Table 4. Details of amplification using different markers

Marker	Alleles amplified	Product size (bp)	PIC	Unique alleles	Heterozygosity
RM586	6	195-235	0.502	3	0.527
RM190	5	160-190	0.288	4	0.298
RM402	2	140-145	0.194	0	0.218
RM7434	4	125-140	0.337	1	0.357
RM340	3	150-160	0.272	0	0.291
Wx	3	120-130	0.363	0	0.402
RM42	2	180-185	0.079	1	0.083
RM421	5	265-300	0.288	4	0.298
RM510	2	122-130	0.076	1	0.079
RM3	5	150-180	0.592	0	0.627
RM217	3	150-180	0.322	0	0.350
W2R	4	245-275	0.382	2	0.419
RM85	5	100-125	0.299	4	0.310
RM282	3	135-145	0.264	1	0.288
RM541	3	185-200	0.370	0	0.406
Total alleles	55	-	-	21	-
Average	3.67	-	0.309	-	-

PIC, polymorphism information content

Table 5. Polymorphic markers between Indrayani and high amylose containing rice genotypes and the product size in base pair

Markers	Indrayani	Bhogavati	Phule Radha	LK-248	RTN Purple
Wx	130	120	120	120	120
W2R	275	250	250	250	250
RM3	180	170	180	170	180
RM541	190	200	190	190	190
RM217	180	180	180	170	170

content and mapped on chromosome 6, are particularly relevant for marker-assisted foreground selection and offer targeted opportunities for improving both grain quality and micronutrient content in varieties like Indrayani by crossing it with high yielding varieties Phule Radha, Bhogavati and RTN Purple having intermediate amylose content as well as higher iron and zinc content (Table 5).

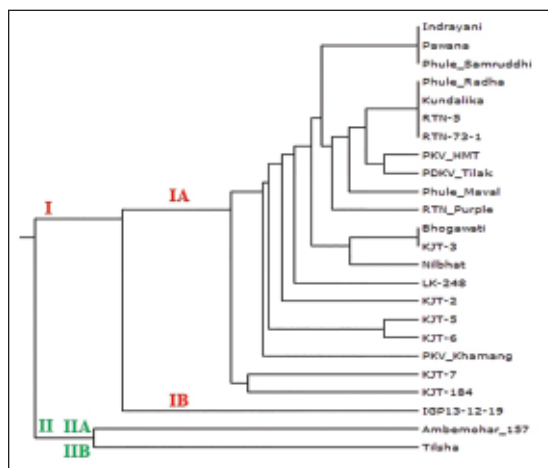


Fig. 1. Consensus tree showing clustering of rice genotypes using marker data.

Conclusion

The findings of this study are significant in identifying promising rice genotypes with intermediate amylose content and desirable levels of iron and zinc, which could contribute to the improvement of the popular variety Indrayani. By surpassing the recommended thresholds of 14.5 ppm for iron in 13 genotypes and 28 ppm for zinc in eight genotypes, with 6 genotypes being common, this study highlights the potential for these genotypes to address the nutritional deficiencies associated with iron and zinc in rice-based diets. Furthermore, the fact that eight of these genotypes also possess intermediate amylose content adds to their value, as this amylose range is associated with desirable cooking characteristics, such as

fluffiness and tenderness, which are favored by consumers.

The high-yielding and nutritionally enriched genotypes identified in this study serve as valuable candidates for incorporation into breeding programs aimed at enhancing the nutritional profile of rice varieties, particularly targeting the improvement of the Indrayani variety. By leveraging these genotypes, breeders can work towards developing rice varieties that not only meet yield expectations but also address nutritional deficiencies, thereby contributing to improved food security and health outcomes for populations reliant on rice as a dietary staple as well as for better monetary returns.

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