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Genetic variability, frequency distribution and association analysis for high phenolic content in F2 population in rice (*Oryza sativa* L.)

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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.

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CITE THIS ARTICLE

Karunya N, Seshadri G, Sivakami R, Muthurajan R, Mannu J, Swaminathan M, Doraiswamy U . Genetic variability, frequency distribution and association analysis for high phenolic content in F2 population in rice (*Oryza sativa* L.). Plant Science Today (Early Access). https://doi.org/10.14719/pst.7031

Abstract

This study assessed genetic variability, heritability, and genetic advance as percentages of the mean, skewness, kurtosis, and trait relationships across nine quantitative and nutritional traits in an F2 population developed from the CO51 × *Mikuruvai* cross. Traits such as the number of productive tillers per plant and single plant yield demonstrated high genotypic and phenotypic variation, substantial heritability and genetic advance as a percentage of the mean. These findings highlight the role of additive gene action and underscore the potential for effective selection. Significant positive skewness for these traits further highlighted their potential for genetic improvement. Single plant yield demonstrated strong positive correlations with plant height, number of productive tillers per plant, panicle length, kernel length, kernel breadth, length-to-breadth ratio, and 1000-seed weight, identifying these as critical traits for yield enhancement. Furthermore, ten transgressive segregants were identified that surpassed the recipient parent CO51 in yield and the donor parent *Mikuruvai* in total phenolic content. These results provide a scientific basis for selecting superior genotypes and contribute to breeding programs to develop high-yielding rice varieties with enhanced nutritional quality.

Keywords

association analysis; genetic variability; kurtosis; rice; skewness

Introduction

Rice is a fundamental staple crop, supplying a significant proportion of daily caloric intake to over half the global population. Despite advances in agricultural productivity, approximately 735 million people globally still experience chronic malnutrition, predominantly in developing nations where rice is intricately tied to food security (1). Moreover, the double burden of malnutrition, characterized by the coexistence of undernutrition and the rising prevalence of diet-related non-communicable diseases (NCDs), including diabetes and cardiovascular disorders, has emerged as a critical public health concern worldwide (2). Epidemiological investigations have established inverse correlations between the incidence of chronic diseases such as certain cancers and coronary heart disease with the consumption of whole-grain rice, attributed to its abundant bioactive compounds, including phenolics, flavonoids, and γ-oryzanol (3–7). Notably, traditional rice land-

races exhibit elevated levels of these phytochemicals compared to commercially cultivated varieties, underscoring their potential for nutritional and therapeutic applications (8). Selecting suitable genotypes to improve yield and associated traits largely depends on the genetic diversity within the segregating population. Genetic diversity broadens the scope for identifying high-yielding varieties and provides opportunities to select genotypes with enhanced nutritional profiles, such as higher phenolic content. Analyzing genetic parameters and exploring trait interrelationships are crucial for understanding genetic variability in the breeding population.

The genotypic coefficient of variation (GCV) quantifies the extent of genetic variability, offering insights into the heritable fraction of total variability in a population (9). Heritability, a key parameter, indicates the proportion of phenotypic variance attributed to genetic factors, serving as a reliable measure for predicting the transmission of traits from parents to progeny. Traits with high heritability, combined with substantial genetic advance (GAM) as a percent of the mean, are particularly effective for selection as measures of distribution, kurtosis and skewness elucidate the underlying genetic effects and gene actions influencing specific traits (10, 11). Furthermore, association studies are crucial for assessing the correlation between component traits and yield, enabling breeders to identify traits with direct or indirect impacts on productivity. This study aimed to determine the critical characteristics affecting yield and grain quality by evaluating genetic variability, heritability, genetic improvement as a percentage of the mean, and trait correlations in a segregating population resulting from the cross CO51 × *Mikuruvai*. The results are anticipated to aid in developing high-yielding rice cultivars with enhanced phenolic content.

Materials and Methods

Seeds of CO51 and *Mikuruvai* were raised at the Tamil Nadu Agricultural University, Coimbatore. Parental seeds were sown, and crosses were made between CO51 and *Mikuruvai* to produce F1 seeds. These F1 plants were harvested individually and advanced to the F2 generation, where 200 segregants were grown with a spacing of 20 × 20 cm under standard management practices. Nine traits - plant height (cm), the number of productive tillers per plant, panicle length (cm), kernel length (mm), kernel breadth (mm), length-to-breadth ratio, single plant yield (g), and total phenolic content (mg GAE/100 g) were evaluated in each F2 plant.

Statistical analysis

The genotypic and phenotypic coefficients of variation (GCV and PCV) were calculated following the method recommended, with the variability classified into low (<10%), moderate (10–20%), and high (>20%) categories (12, 13). Since replicated trials could not be conducted for the segregants, environmental variance was derived from replicated trials of the parental lines. Broad-sense heritability was estimated using the standard procedure and categorized into high (>60%), moderate (30–60%), and low

(<30%) ranges (14). Genetic advance as a percent of the mean was computed as per the standard methods and classified into low (<10%), moderate (10–20%), and high (>20%) categories (15). Correlation analysis among yield-related traits and their interrelationships was conducted using the standard procedure (16). The studied traits' frequency distribution and association plots were visualized using R software version 4.4.1.

Results and Discussion

Yield and yield attributing traits

Variability among genotypes forms the cornerstone of effective selection, as it provides the genetic diversity necessary for identifying superior traits and improving desired characteristics. The extent of variability indicates a promising scope for selecting genotypes with enhanced performance for high phenolic content. Transgressive segregants are individuals in a segregating population that exhibit trait values beyond the range of their parental lines, resulting from recombining complementary alleles. These segregants hold immense potential in breeding programs as they combine favourable alleles from both parents, potentially surpassing existing genetic boundaries. Selecting transgressive segregants with high phenolic content is particularly advantageous, given the critical role of phenolics in plant defence, stress tolerance, and their associated antioxidant properties.

The analysis of the F2 population revealed substantial variability across the nine traits studied. Plant height (PH) ranged from 85.00 cm to 155.00 cm, with a mean of 123.56 cm. The number of productive tillers per plant (NPT) varied between 9.00 and 26.00, averaging 16.05. Panicle length (PL) ranged from 13.47 cm to 32.94 cm, with a mean of 22.90 cm. Kernel length (KL) spanned from 4.50 mm to 7.10 mm, with a mean value of 5.82 mm, while kernel breadth (KB) ranged from 1.60 mm to 2.90 mm, averaging 2.21 mm. The length-to-breadth (LB) ratio ranged between 1.67 and 4.18, with a mean of 2.70. The 1000-seed weight (TSW) ranged from 15.00 g to 25.75 g, with a mean of 20.02 g. Single plant yield (SPY) varied from 14.01 g to 35.79 g, with a mean of 20.93 g. Total phenolic content (TPC) exhibited a broad range, from 126.41 mg GAE/100 g to 310.53 mg GAE/100 g, with an average of 196.66 mg GAE/100 g. These range and mean values highlight the extent of genetic variation within the F2 population, providing a valuable foundation for selection and further genetic improvement.

Analysis of the segregating population revealed that the phenotypic coefficient of variation (PCV) remained marginally more significant when compared with the Genotypic Coefficient of Variation (GCV) for all characters considered, which represents the environmental impact on the expression of the characters (Table 1). The traits viz., number of productive tillers per plant, panicle length, and single plant yield exposed high GCV in addition to high PCV for a number of productive tillers per plant; for panicle length, single plant yield (17, 18, 19). Attributes namely, plant height, kernel length, kernel breadth, length-to-

Table 1. Genetic variability parameters of different biometrical traits in F2 generation of CO51 × *Mikuruvai*

| Traits	PH	NPT	PL	KL	KB	LB ratio	TSW	SPY	TPC
Minimum	85.000	9.000	13.470	4.500	1.600	1.667	15.004	14.010	126.410
Maximum	155.000	26.000	32.940	7.100	2.900	4.176	25.750	35.790	310.530
Mean	123.555	16.050	22.898	5.817	2.211	2.696	20.017	20.931	196.656
PCV	12.101	29.644	24.967	11.918	16.587	18.848	13.184	22.998	13.055
GCV	8.775	23.009	24.392	11.794	15.136	17.604	8.485	21.415	11.090
h^2	52.585	60.243	95.446	97.919	83.264	87.232	41.416	86.707	72.166
GAM	13.109	36.789	49.090	24.041	28.451	33.870	11.248	41.077	19.408
Skewness	-0.608**	0.517**	0.072	-0.185	0.125	0.465**	-0.279	0.758**	0.398*
Kurtosis	0.367	-0.588	-1.265**	-1.023**	-1.122**	-0.234	-1.009**	0.083	1.454**

***Significant at 5 % level and ** Significant at 1 % level; PH** - Plant height, **NTP**- Number of productive tillers per plant, **PL**–Panicle length, **KL**–Kernel length, **KB**–Kernel Breadth, **LB** ratio–Length breadth ratio, **TSW** –Thousand seed weight, **SPY**–Single plant yield. **PCV**–Phenotypic coefficient of variation, **GCV**–Genotypic coefficient of variation, **h²**– Heritability, **GAM**–Genetic advance as per cent of mean.

breadth ratio, 1000-seed weight and total phenolic content exhibited moderate range for both GCV along with PCV, which were in agreement with various research for plant height; kernel length, kernel breadth and 1000- and seed weight (21–23).

All the characters under study had high heritability (Table 1). The higher range of heritability along with higher GAM in traits *viz.,* number of productive tillers per plant, panicle length, kernel length, kernel breadth, length to breadth ratio along with single plant yield recognized indicates the existence of cumulative gene effect signifying that advancement of those traits might be effectual by adopting clear-cut selection strategies. Research suggests similar findings for several productive tillers per plant, panicle length, kernel length, kernel breadth, length-to-

breadth ratio, and single plant yield (20, 22–24). A Moderate range of GAM was observed for plant height, 1000-seed weight and total phenolic content.

The biological implications of skewness and kurtosis provide valuable insights into the genetic control of traits and the potential for selection. Skewness indicated the direction and extent of asymmetry in trait distribution, while kurtosis reflected the shape of the distribution, particularly the presence of outliers. The frequency distribution pattern of nine quantitative traits based on skewness and kurtosis in segregants disclosed a significantly positively skewed number of productive tillers per plant, length-to-breadth ratio, single plant yield and total phenolic content (Fig. 1). The positive skewness indicated the presence of complementary epistatic gene action for these
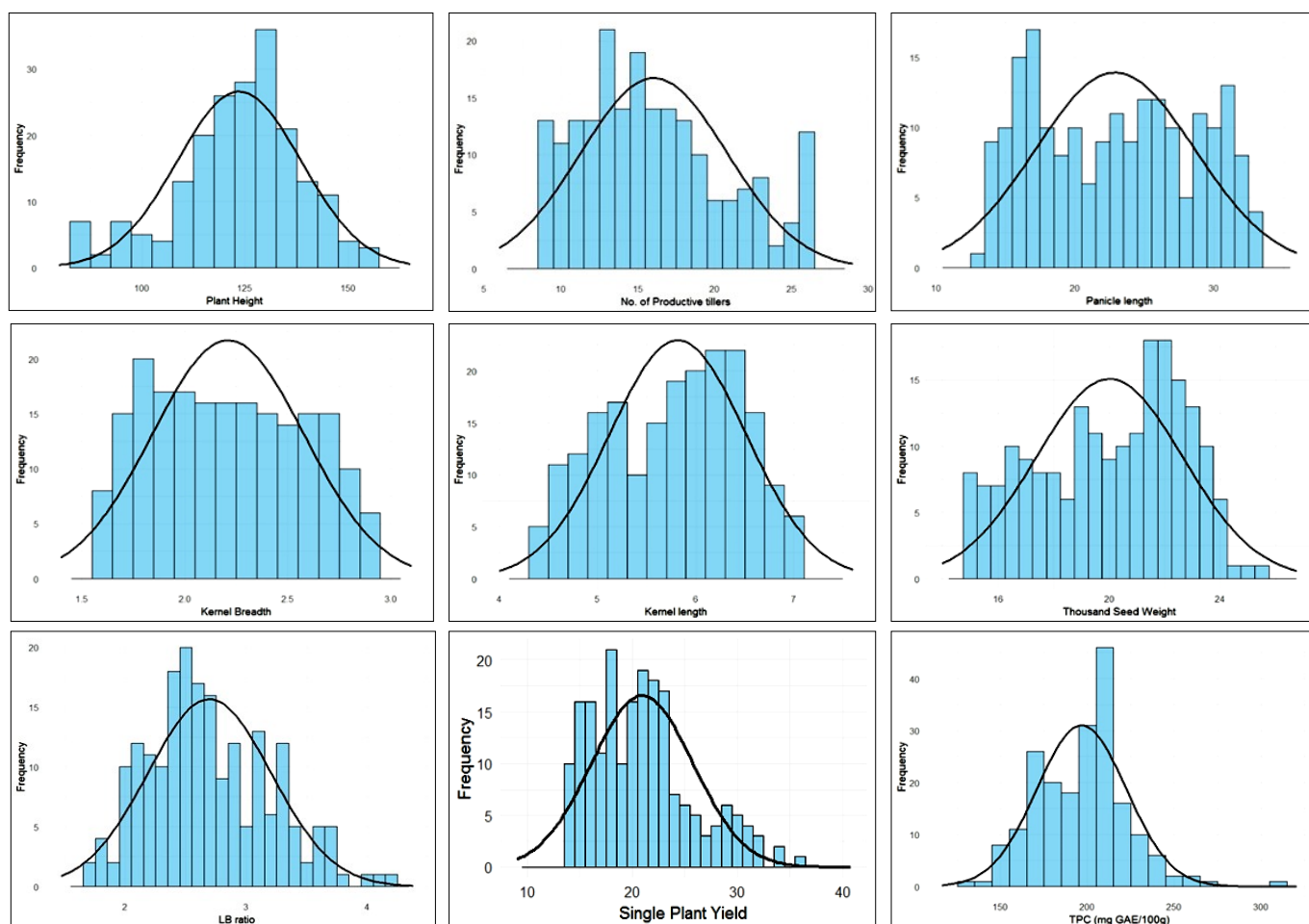
Fig. 1. Frequency distribution for biometrical traits in F2 generation of CO51 × Mikuruvai cross.

traits. The gain will be slower with the mild selection and faster with intensive selection for that particular trait in the segregating generation. Complementary epistatic gene action occurs when alleles at two or more loci interact, so both are required to express a distinct phenotype. This interaction suggests that breeders must combine specific alleles from different parents to achieve the desired trait expression, making hybridization and strategic crosses essential. A significant negative skew was detected in plant height. The negative skewness indicated the presence of duplicate epistatic gene action; the gain is faster with mild selection and less rapid with intense selection for these traits. Significant platykurtic distributions were witnessed for panicle length, kernel length, kernel breadth and1000–seed weight, indicating more than one gene governing these traits.

In contrast, duplicate epistatic gene action occurs when two or more loci have similar effects, and the presence of either can produce the desired phenotype. This redundancy allows for greater flexibility in selection and increases the likelihood of retaining favourable traits across generations. In both cases, understanding these gene interactions helped breeders design crosses and selection schemes that capitalized on these genetic mechanisms to enhance trait improvement while preserving genetic diversity. Selection of plants focused on one trait may directly or indirectly influence the performance of another trait, mainly when concerned with complex attribute yield, in which direct selection was designated to be less effective. In this study, association analysis disclosed that single plant yield had a significant positive correlation with plant height, number of productive tillers per plant, panicle length, kernel length, kernel breadth, length-to-breadth ratio and 1000-seed weight (Table 2 and Fig. 2).

Table 2. Phenotypic correlation among the biometrical traits in F2 generation of CO51 × *Mikuruvai*

| Traits	PH	NPT	PL	KL	KB	LB ratio	TSW	SPY	TPC
PH	1.000	0.245**	0.832**	-0.123	0.143*	-0.215**	0.066	0.393**	0.088
NPT	0.245**	1.000	0.221**	-0.042	0.133	-0.127	0.159*	0.534**	0.044
PL	0.832**	0.221**	1.000	-0.097	0.218**	-0.272**	0.039	0.431**	0.045
KL	-0.123	-0.042	-0.097	1.000	0.204**	0.458**	0.057	0.243**	0.057
KB	0.143*	0.133	0.218**	0.204**	1.000	-0.763**	0.133	0.164*	0.042
LB ratio	-0.215**	-0.127	-0.272**	0.458**	-0.763**	1.000	-0.073	0.022	0.015
TSW	0.066	0.159*	0.039	0.057	0.133	-0.073	1.000	0.349**	0.053
SPY	0.393**	0.534**	0.431**	0.243**	0.164*	0.022	0.349**	1.000	0.057
TPC	0.088	0.044	0.045	0.057	0.042	0.015	0.053	0.057	1.000
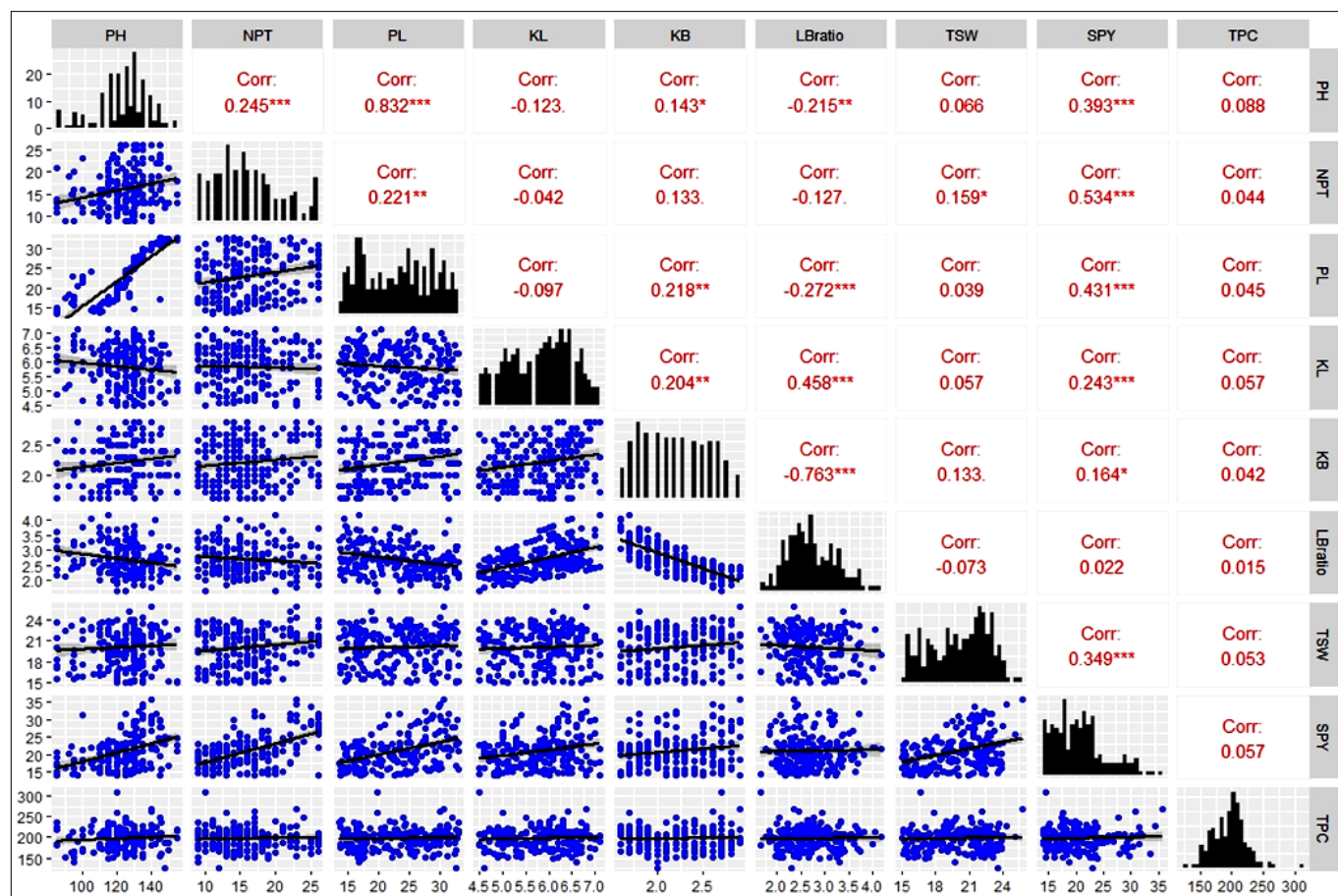
Fig. 2. Phenotypic correlation among the biometrical traits in F2 generation of CO51 × *Mikuruvai* cross.

Similar outcomes were found for plant height; panicle length; for several productive tillers per plant; for kernel length; for 1000- seed weight (23, 26–28).

The results of this study show that traits such as the number of productive tillers and single plant yield exhibited high GCV, PCV, heritability, and genetic advance as a percentage of the mean, along with a substantial positive skewness, indicating the presence of cumulative gene action and the potential for effective selection. A significant positive correlation was observed between single plant yield and traits such as plant height, number of productive tillers per plant, panicle length, kernel length, kernel breadth, length-to-breadth ratio, and 1000-seed weight, all of which are favourable for improving yield.

Total phenolic content

Despite the moderate genetic variability observed for total phenolic content, the trait indicated the presence of substantial genetic control, evidenced by high broad-sense heritability. The moderate genetic advance as a percent of the mean further supported the potential for significant genetic improvement through directional selection. Similar studies revealed high genetic variability, heritability, and advancement (29, 30). The positive skewness for total phenolic content indicated the presence of complementary epistatic gene action, and the gain would be slower with the mild selection and faster with intensive selection in the segregating generation. Significant leptokurtic distribution was observed in total phenolic content, indicating that few genes govern this trait. The positively skewed and leptokurtic distribution patterns also suggested the presence of transgressive segregants with higher total phenolic content, which can be utilized in breeding programs. These factors collectively indicate that total phenolic content, despite its moderate variability, is a suitable target trait for selection. Ten transgressive segregants exhibited higher yield than the receipt parent CO51 and higher total phenolic content than the donor parent *Mikuruvai* (Fig. 2).

Conclusion

The absence of phytonutrients in the elite breeding material of staple rice has posed challenges in tackling global nutritional security concerns. With their vast collection of genetic material, Gene banks offer a valuable resource of bioactive compounds, mainly phenolic acids, which can be compared to superfoods. These health-beneficial traits must be integrated into high-yielding rice varieties without compromising texture, taste, and aroma preferences to ensure broader consumer acceptance.

The present study revealed significant variability among the genotypes for all traits studied. The high heritability coupled with high genetic advance as a percent of mean observed for yield-contributing traits and their considerable positive correlations with single plant yield emphasize the effectiveness of cumulative gene action and the feasibility of concurrently improving these traits. Furthermore, identifying transgressive segregants exhibiting

superior yield and elevated phenolic content underscores the scope for developing rice genotypes that integrate enhanced nutritional quality with superior agronomic performance. These findings provide a robust scientific basis for advancing breeding programs focused on the simultaneous improvement of yield and total phenolic content in rice.

Authors' contribution

KN and GS conceptualized the study, developed the methodology, conducted formal analysis, performed investigation and data curation and prepared the original draft; RS, RM, JM, MS, UD supervised, validated, acquired funding and reviewed and edited the manuscript, with all authors approving the final manuscript.

Compliance with ethical standards

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

Ethical issues: None

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