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Analysis of quantitative trait distribution and nutritional variability in Rice (*Oryza sativa* L.) F₂ population

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Abstract

Rice (*Oryza sativa* L.) is one of the most important staple food crops and serves as the primary energy source for more than half of the global population. Improvement of grain yield along with nutritional quality traits such as iron (Fe), zinc (Zn) and protein content has become an important objective in rice breeding programmes. The present investigation was undertaken to study the genetic architecture and frequency distribution of yield and nutritional traits in the F₂ population derived from the cross CO54 × Kalanamak. A total of 238 F₂ segregants were evaluated for seventeen quantitative traits related to yield and grain nutritional quality. Considerable variability was observed among the segregating plants for all the traits studied. High variance was recorded for total grains per panicle (2570.30), filled grains per panicle (1811.94) and plant height (230.99), indicating wider genetic variability. Skewness values ranged from -0.68 to 0.96, suggesting the involvement of additive and non-additive gene action. Positive skewness was observed for most traits including flag leaf width, protein content and number of productive tillers, indicating the possibility of obtaining superior segregants through selection. Most of the characters exhibited platykurtic distribution, revealing polygenic inheritance and broad variability within the population. The results indicated that the CO54 × Kalanamak F₂ population possesses substantial variability for yield and nutritional traits and can be effectively utilized for selection of superior recombinants and development of bio-fortified rice varieties.

Keywords: F₂ population, frequency distribution, skewness, kurtosis, nutritional, traits, biofortification

Introduction

Rice (*Oryza sativa* L.) is one of the most widely cultivated cereal crops and serves as the staple food for nearly half of the world's population. It plays a major role in food security, nutrition and livelihood of millions of people, particularly in Asian countries. Although rice is a rich source of carbohydrates, polished rice grains are generally deficient in essential micronutrients such as iron and zinc, resulting in widespread micronutrient malnutrition or "hidden hunger" among rice-consuming populations. Iron and zinc deficiencies affect nearly two billion people worldwide and lead to severe health complications including anemia, weakened immunity and impaired growth (Priyanka *et al.*, 2019) ^[1].

Bio-fortification has emerged as an effective and sustainable strategy to enhance the nutritional quality of staple crops through plant breeding approaches. Development of rice varieties with improved grain iron, zinc and protein content has therefore become an important objective in modern rice breeding programmes. Successful improvement of these complex traits depends largely on the availability of genetic variability and understanding of the inheritance pattern governing these traits. Segregating populations such as F₂ generations provide excellent opportunities for studying variability, recombination and gene action because they exhibit maximum segregation for quantitative traits (Deepika *et al.* 2025) ^[2].

Frequency distribution analysis helps in understanding the nature and extent of variability present in segregating populations. Statistical parameters such as skewness and kurtosis provide valuable information regarding the genetic architecture of quantitative traits. Skewness indicates the asymmetry of trait distribution and reflects dominance and epistatic gene interactions, while

kurtosis explains the nature of gene distribution and number of genes controlling a character. Traits showing platykurtic distribution are generally governed by many genes with broad variability, whereas leptokurtic distribution indicates fewer genes controlling the trait Riyanto *et al.*, (2024) ^[12].

Kalanamak is a traditional aromatic rice landrace known for its superior grain quality, aroma and high nutritional value, particularly iron content. CO54 is a high yielding rice variety with desirable agronomic traits. Hybridization between these contrasting parents offers opportunities for generating useful recombinants with improved yield and nutritional quality. Therefore, the present study was undertaken to analyze the variability, frequency distribution, skewness and kurtosis for yield and nutritional traits in the F₂ population of CO54 × *Kalanamak* cross.

Materials and Methods

The current investigation was carried out in the experimental field of Department of Plant Breeding and Genetics, Tamil Nadu Rice Research Institute, Aduthurai, Tamil Nadu. Based on documented results, (Table 1 & 2) the parents were chosen based on yield and grain iron and zinc content and crossed and developed mapping populations during 2023. The F₂ generations resulting from the cross between ADT₅₆ × *Kalanamak*, were grown. Twenty six days old seedlings were transplanted in to the main field with a spacing of 30 cm x 30 cm. A total of 222 possible F₂ segregants of ADT₅₆ × *Kalanamak* cross were identified and labeled for further assessment. The recommended agronomic practices and crop protection measures were followed during the crop growth period. Each F₂ plant was harvested separately. Observations were recorded from selected plants of each of the cross combinations and each of the randomly selected individual plant was tagged before anthesis. Data on all the seventeen characters namely DM-Days to Maturity, PH- Plant Height, NPT-number of productive tillers per plant, FL-Flag leaf length, FW-Flag leaf width, KL- kernel length; KB- kernel breadth; LBR-length-to-breadth ratio, PL-Panicle Length, PW-Panicle Weight, NB-Number of branches per panicle, FG-Filled Grains, UFG-Unfilled Grains, TGP-Total number of Grains per Panicle, SPY – Single Plant Yield, FEB- Fe content in brown rice and ZNB-Zn content in brown rice and PCB- Protein content in brown rice were analyzed at Tamil Nadu Rice Research Institute, quality lab, with ED-XRF. The recorded data was subjected to statistical analysis mean, variance, Skewness and Kurtosis using R studio software.

Estimation of grain Fe and Zn content

Each individual plants are harvested separately and analyzed the grain nutritional content. Required amount of seeds were de-husked and milled using non-metallic de-husker (Krishi international 810 de-husker) at the grain quality laboratory, Tamil Nadu Rice Research Institute, Aduthurai, Tamil Nadu. Samples were polished for 30-45 sec and cleaned using tissue paper. Only full, undamaged grains free from debris were analysed. The grain iron and zinc content in brown rice samples were estimated using a non-destructive, energy-dispersive x-ray fluorescence spectrometry (ED-XRF) instrument (Hitachi X-supreme) following Harvest plus guidelines (Stangoulis *et al.*, 2007 and Deepika *et al.* 2025) ^[15, 2]. Each sample of brown rice (5g) was weighed and transferred to sample cups. The sample cups were gently shaken for uniform distribution of samples and then set aside for analysis. The concentration of grain iron and zinc was expressed in milligram per kilogram (mg kg⁻¹) or parts per million (ppm) of grains.

Estimating grain protein content using Near-Infrared (NIR) spectroscopy involves scanning samples to absorbance at specific wavelengths (900-2500nm). Grain samples are cleaned to remove impurities, dust and damaged kernels. Samples grind into fine flour using a laboratory mill. Samples are placed on a sample compartment and NIR analyzer passes near-infrared light on the samples and device measure the reflected or absorbed light, which is affected by molecular bonds (Sharma *et al.*, 2025).

Results and Discussion

The F₂ population of CO54 × *Kalanamak* exhibited substantial variability for all the studied traits (Figure 1), indicating wide segregation and recombination among the progenies. Days to maturity ranged from 113.30 to 153.80 days with a mean value of 130.49 days. Plant height varied from 94.50 cm to 172.60 cm with an average of 122.19 cm. Number of productive tillers per plant ranged from 9.50 to 38.20 with a mean value of 20.88. Among grain quality traits, kernel length ranged from 5.50 to 7.60 mm with a mean of 6.32 mm, while kernel breadth ranged from 1.50 to 2.60 mm with a mean value of 2.17 mm. Length to breadth ratio varied from 2.23 to 3.90 with an average value of 2.96.

Yield contributing traits also exhibited wider variation. Filled grains per panicle ranged from 72.60 to 284.60 with a mean of 186.14, whereas total grains per panicle varied from 108.80 to 380.30 with a mean of 242.85. Single plant yield ranged from 9.20 g to 45.70 g with an average of 28.23 g. The nutritional traits showed promising variability among the segregants. Grain iron content ranged from 9.12 to 15.84 mg kg⁻¹ with a mean of 12.41 mg kg⁻¹. Zinc concentration ranged from 18.69 to 32.56 mg kg⁻¹ with a mean value of 25.57 mg kg⁻¹, while protein content varied from 7.21 to 12.83% with an average of 9.43%. The wider range observed for yield and nutritional traits suggested the existence of substantial genetic variability in the population. Such variability provides opportunities for effective selection of superior recombinants possessing high yield and enhanced nutritional quality. Similar findings were earlier reported by Riyanto *et al.* (2024) ^[12], Das *et al.* (2021) ^[1], Perween *et al.* (2022) ^[9], Patel *et al.* (2020) ^[8], Lakshmi *et al.* (2017) ^[5].

Variability analysis

The variance estimates revealed considerable variability among the F₂ segregants for different quantitative traits. The highest variance was observed for total grains per panicle (2570.30) followed by filled grains per panicle (1811.94) and plant height (230.99). Moderate variance was observed for days to maturity (157.23), flag leaf length (75.47) and single plant yield (47.64). Higher variance for yield-related traits indicated greater dispersion of values around the mean and reflects the presence of substantial genetic variability within the population. Such variability is highly useful for identifying desirable segregants in early generations.

Skewness analysis

Skewness analysis (Figure 1) provides information regarding the symmetry of trait distribution and gene action involved in trait inheritance. In the present study, skewness values ranged from -0.68 to 0.96. Positive skewness was observed for most of the traits including days to maturity, number of productive tillers, flag leaf length, flag leaf width, kernel length, length-to-breadth ratio, panicle length, number of branches, zinc content and protein content. The highest positive skewness was recorded for

flag leaf width (0.96), indicating the presence of more individuals on the lower side of the distribution along with a few superior transgressive segregants.

Positive skewness generally indicates complementary gene interaction and the possibility of improving the trait through intense selection in advanced generations. Traits such as zinc content and protein content with positive skewness are particularly important for biofortification breeding because superior segregants can be effectively isolated from the population.

Negative skewness was observed for kernel breadth (-0.68), filled grains per panicle (-0.22) and single plant yield (-0.24). Negative skewness suggests dominance-based gene interactions and indicates that selection may be effective from the lower end of the distribution. The skewness pattern observed in the present study indicated the involvement of both additive and non-additive gene action in governing the traits. Similar observations were reported earlier by Sahu *et al.* (2023) [13], Patel *et al.* (2020) [8], Nikhitha *et al.*, (2020) [7], Khandappagol *et al.* (2019) [4], Priyanka *et al.* (2019) [11].

Kurtosis analysis

Kurtosis explains the peakedness or flatness of trait distribution and indirectly indicates the number of genes controlling a trait. In the present investigation, most of the traits exhibited negative kurtosis values, indicating platykurtic distribution.

Traits such as days to maturity (-1.21), number of branches per panicle (-0.93), iron content (-0.91), zinc content (-0.81) and protein content (-0.77) showed platykurtic distribution. Platykurtic distribution suggests polygenic inheritance and broad variability within the population. Such traits are usually governed by many genes with small individual effects. Kernel length (0.06) and single plant yield (0.45) exhibited near mesokurtic distribution, indicating approximately normal distribution of segregants. The broad distribution pattern observed for most traits suggested the usefulness of the F₂ population for effective selection and improvement of yield and nutritional quality traits. The results are in agreement with the findings of and Sowmiya *et al.* (2023) [14], Manoj Kumar *et al.* (2022) [6], Priyanka *et al.* (2019) [11], Prabhu *et al.* (2015) [10].

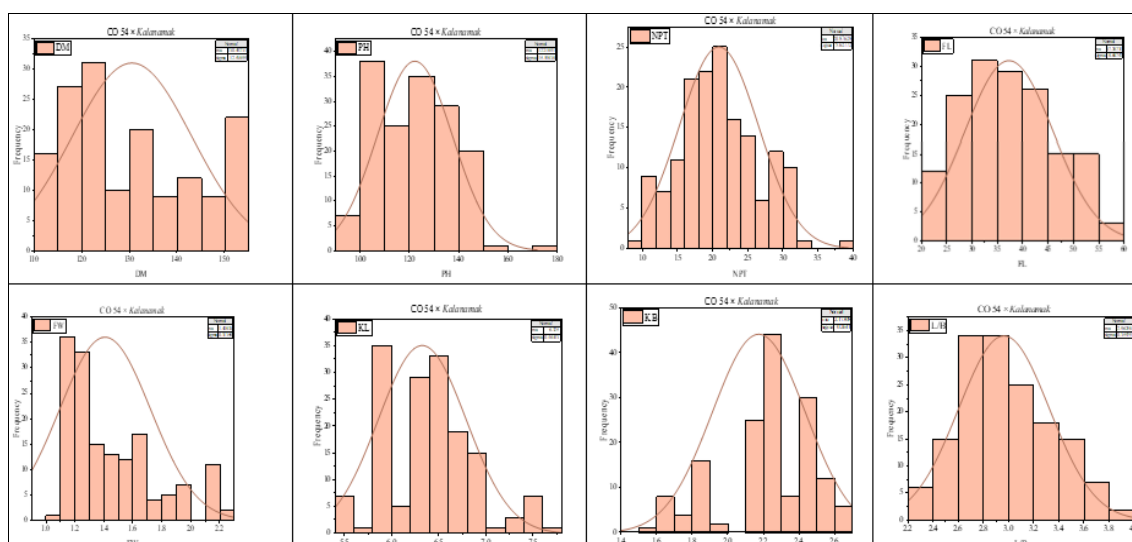
Table 1: Parents used for development of CO54 x *Kalanamak* F₂ population

Parents	Genotype	Parentage	Features	Source
P1	CO54	CB04110 /CB 05501	White Medium slender rice. Moderately resistant to Blast, Sheath rot, Brown Spot and BPH	Coimbatore, TNAU
P2	<i>Kalanamak</i>	Landrace	Short duration, finest quality, resistant to blast. Cooked rice is fluffy, soft, non-sticky, sweet and easily digestible with relatively longer shelf-life. It had 15.6 mgkg ⁻¹ of Fe. It is famous for taste, palatability and aroma.	TRRI, Aduthurai

Table 2: Descriptive statistics of CO54 X *Kalanamak* cross

Parameters	DM	PH	NPT	FL	FW	KL	KB	L/B	PL	PW	NB	FG	TGP	SPY	FEB	ZNB	PCB
Mean	130.49	122.19	20.88	37.27	1.41	6.32	2.17	2.96	29.58	2.98	11.34	186.14	242.85	28.23	12.41	25.57	9.43
Maxi	153.80	172.60	38.20	56.20	2.20	7.60	2.60	3.90	35.60	4.40	14.80	284.60	380.30	45.70	15.84	32.56	12.83
Mini	113.30	94.50	9.50	22.20	1.00	5.50	1.50	2.23	25.10	1.53	8.20	72.60	108.80	9.20	9.12	18.69	7.21
Standard Deviation	12.54	15.20	5.63	8.69	0.31	0.46	0.26	0.36	2.29	0.65	1.75	42.57	50.70	6.90	1.63	3.21	1.43
Standard Error	1.00	1.22	0.45	0.70	0.03	0.04	0.02	0.03	0.18	0.05	0.14	3.41	4.06	0.55	0.13	0.26	0.11
Variance	157.23	230.99	31.67	75.47	0.10	0.22	0.07	0.13	5.24	0.42	3.08	1811.94	2570.3	47.64	2.67	10.32	2.05
Skewness	0.42	0.21	0.28	0.26	0.96	0.42	-0.68	0.31	0.10	0.03	0.27	-0.22	0.01	-0.24	0.10	0.17	0.47
Kurtosis	-1.21	-0.49	-0.30	-0.84	-0.08	0.06	-0.16	-0.40	-0.66	-0.76	-0.93	-0.22	-0.14	0.45	-0.91	-0.81	-0.77

DFF - Days to Fifty percent Flowering, PH - Plant Height, NPT - number of productive tillers per plant, FL - Flag leaf length, FW - Flag leaf width, PL - Panicle Length, PW - Panicle Weight, NB - Number of branches per panicle, FG - Filled Grains, UFG - Unfilled Grains, TNP - Total number of Grains per Panicle, HSW - Hundred Seed Weight, SPY - Single Plant Yield, KL - Kernel Length, KB- Kernel Breadth, LB - Length to Breadth ratio, HP- Hulling percentage; MP- milling percentage; HRR- head rice recovery; KL- kernel length; KB- kernel breadth; LBR-length-to-breadth ratio; FEB- Fe content in brown rice and FEP- Fe content in polished rice



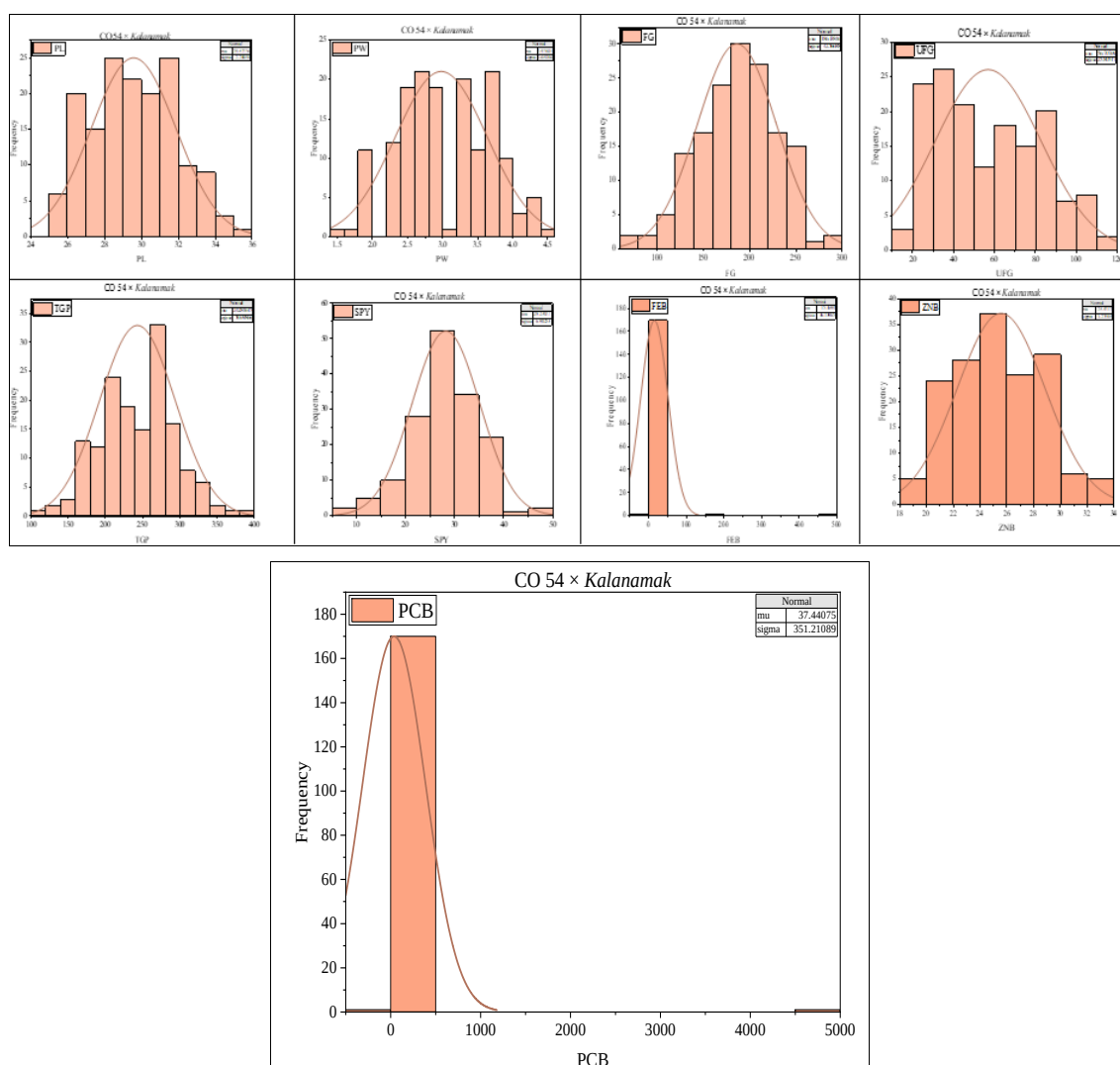


Fig 1: Distribution pattern of yield and nutritional characters in CO54 X *Kalanamak* of F_2 segregating generation of rice

Conclusion

The F_2 population derived from the cross CO54 × *Kalanamak* exhibited wide variability for yield and nutritional traits, indicating substantial scope for selection and genetic improvement. High variance observed for total grains per panicle, filled grains and plant height suggested the presence of broad genetic variability among segregants. Skewness and kurtosis analyses revealed the involvement of both additive and non-additive gene actions with most traits exhibiting polygenic inheritance. Nutritional traits such as grain iron, zinc and protein content showed promising variability, highlighting the potential of this population for developing bio-fortified rice varieties. The study demonstrated that early generation evaluation through frequency distribution analysis is highly useful for identifying superior segregants and improving breeding efficiency in rice.

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Conflict of Interests

The authors declare that they have no conflict of interest

Author's Contributions

All authors listed have made a substantial, direct and intellectual contribution to the work and approved it for publication.

Ethical approval: None

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