

Research Article

Revealing the higher degree statistics and transgressive segregation pattern of nutritional and agronomical traits in the segregating population derived from Samba Mahsuri and Sathi of rice (*Oryza sativa*)

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Abstract

Rice is the richest source of starch and carbohydrates but is deficient in major micronutrients such as iron and zinc. Slight enrichment with these micronutrients could help combat malnutrition. For a successful plant breeding program, genetic variability is crucial. Thus, the research aimed to analyze the description of statistics and transgressive segregation among the nutritional and agronomical traits in the F_2 rice population. In this context, 190 progenies from the F_2 population and parents were sown in *Kharif* 2020. Ten agronomical and two nutritional traits (grain iron and zinc content) were recorded from each genotype of the F_2 population. All the recorded data were subjected to descriptive analysis and transgressive segregants were recorded for grain iron and zinc content. Descriptive analysis revealed positive skewness for the number of effective tillers per plant (0.998), grain length-breadth ratio (0.256), thousand-grain weight (0.875), grain zinc content (0.232), and grain yield per plant (1.460). Negative skewness was recorded for days to fifty per cent flowering (-2.805), plant height (-0.396), panicle length (-0.150), grain breadth (-0.335), and grain iron content (-0.356). The number of filled grains per panicle, grain length breadth ratio, grain zinc, and iron content exhibited the platykurtic nature of the distribution curve. Concerning transgressive segregants of nutritional traits, ten were observed for grain zinc content and thirty for grain iron content in the F_2 rice population. These transgressive segregants for grain zinc and iron content might be used for developing advanced breeding lines, and skewness and kurtosis provide necessary genetic information for gene interaction.

Keywords: Kurtosis, Micronutrients, Rice, Skewness and Transgressive segregants

INTRODUCTION

Rice is the most important cereal crop that feeds most of the world's population and it is mainly in Asian countries, which account for nearly 90% of global rice consumption. India is the second largest rice-producing country after China with a cultivated area of 43.19 mil-

lion hectares, production of over 110.15 million tonnes and a yield is 2.55 tonnes/ha (Agricultural Statistics 2019). Rice is a deficient source of essential micronutrients such as iron (Fe) and zinc (Zn). Thus, the population only depends on rice-based food, such as in developing countries suffering micronutrient deficiency. Insufficient intake of these important micronutrients leads

to several abnormalities related to human health/ causes malnutrition (Bashir *et al.*, 2007). Fe and Zn deficiencies are the most prevalent micronutrient deficiencies in humans, affecting two billion people and causing more than 0.8 million deaths annually (Midya *et al.*, 2021). Zinc deficiency leads to the most important health risk factors in developing countries and worldwide. In children, zinc deficiency is commonly associated with diarrhea, pneumonia, stunting, and child mortality, whereas iron deficiency leads to a deficiency of blood hemoglobin in both children and adults; iron is very important for pregnant and lactating females (Swamy *et al.*, 2018). Poor people are unable to get supplements due to its high cost. Thus, even a small increase in the nutritional value of rice can highly contribute to human nutrition. From this point of view, developing such a variety that could be an alternative to supplements must be the prime aim of plant breeders. In addition, the presence of variability, gene interaction, and the nature of the base population is the prime base of any successful breeding program (Raza *et al.* 2019). Descriptive analysis is the best way to represent a huge amount of data in a summarized and proper way. The data's distribution and shape are described using a histogram and whisker-box plot. A histogram is a form of graphical representation that estimates the distribution of a continuous variable and provides a rough sense of the density of the data. However, skewness refers to the measure of asymmetry of the probability distribution of a random variable. Kurtosis, a high degree of statistics, represents the flatness or peakness of the frequency curve. Additionally, Skewness and kurtosis provide the nature of the gene interaction. On the other hand, descriptive statistics also explain box-and-whisker plots. Box-and-whisker plots explain the level of variation, the spread of the middle of the data, skew, and outlier (Kasanaboina *et al.* (2022). Histograms and Box-and-whisker plots are useful representations to understand whether the data was skewed or normally distributed and in proper shape (Shreffler & Huecker, 2021). Thus, the present research investigation focused on analyzing the description of statistics and identifying transgressive segregants for grain iron and zinc content in the F_2 rice population. That might be further helpful for developing biofortified rice varieties.

MATERIALS AND METHODS

The study was conducted in the Research Farm of Bihar Agricultural University Sabour, Bhagalpur, Bihar in *Kharif* 2020. Standard agronomic practices for aerobic rice, including the application of recommended doses of manures and fertilizers, irrigation, and inter-culture operations, were properly followed (Tripathi *et al.*, 2015). The experimental material for the present study comprised parents and 190 F_2 progenies developed from a

cross Samba Mahsuri and Sathi collected from Bihar Agricultural University, Sabour, Bihar. In total, 192 genotypes, including parents, were sown in the wet bed nursery during *Kharif* 2020. All genotypes were transplanted in the main field after 21 days of sowing with a spacing of 20 cm between rows and 15 cm between the plants within the row.

Pre-harvest and post-harvest observations

Pre-harvest and post-harvest observations such as days to 50% flowering, plant height (cm), panicle length (cm), number of effective tillers per plant, number of filled grains per panicle, grain yield per plant (g), grain length (mm), grain breadth (mm), grain length/breadth ratio and test weight (1000 grain weight in gm) were recorded from each plant separately.

Estimation of grain iron and zinc content

In addition, two nutritional (Grain iron and zinc content in ppm) traits were recorded from the seeds of two panicles of each plant; average data of both panicles were used for analysis. The grain iron and zinc content was estimated using a procedure reported by Singh *et al.* (2017). Grains were cleaned to remove any surface contaminants. Uniform dried seeds (0.5 g) from two panicles were placed in labeled conical flasks separately. 10 ml of 70% concentrated nitric acid (Merck, analytical reagent grade) was added, and the mixture was subjected to pre-digestion overnight. The next day, 15 ml of a diacid mixture [nitric acid: perchloric acid (Merck, analytical reagent grade) in a ratio of 10:4] was added to the pre-digested sample. The digested sample was then cooled, and the volume was adjusted to 50 ml using double-distilled water. The diluted solution was filtered using number 42 quantitative circles (125 mm Ø filter paper) and transferred to clean, airtight tubes and bottles to estimate iron and zinc content. The grain iron and zinc content was measured using an Atomic Absorption Spectrophotometer (AAS, Perkin Elmer Precisely Analyst 400 AAS). Two replications were maintained, and their average was used to calculate the grain iron and zinc content using the following formula:

$$\text{Iron and Zinc content} = \text{Average ppm} \times \text{Dilution factor}$$

$$\text{Dilution factor} = \frac{\text{Final volume}}{\text{Seed sample in gm}}$$

Eq. 1

Iron and Zinc content of the grain sample were expressed in ppm. Morphological and biochemical data recorded from the F_2 population and parents were subjected to the higher degree statistical analysis using "XLSTAT2018.1 VERSION".

RESULTS

Skewness and kurtosis in the F_2 population

Skewness and kurtosis were included under four-

degree statistics in a complex matrix. The details of the skewness and kurtosis of the F_2 population derived by Samba Mahshuri and Sathi are shown in Table 1, Fig. 1 and Fig. 2.

Skewness and kurtosis

Skewness and kurtosis are advanced statistical measures that offer insights into the distribution and shape of a population using higher-degree statistics. In the present investigation, negative skewness was recorded for days to 50% flowering (-2.80), plant height (-0.39), panicle length (-0.15), grain breadth (-0.33), The grain iron content (ppm) (-0.35). Whereas positive skewness is required for the number of effective tillers per plant (0.99), number of filled grains per panicle (0.05), grain length (0.10), grain length/breadth (0.25), thousand-grain weight (0.87), grain zinc content (ppm) (0.23) and grain yield per plant (1.45).

Platykurtic nature of the distribution performed by most of the traits under study, such as plant height (0.06), panicle length (2.11), number of effective tillers per plant (1.39), number of filled grains per panicle (-0.09), grain breadth (-0.33), grain length/breadth (-0.05), grain zinc content (-0.70), grain iron content (-1.08). Whereas some other traits, such as days to 50% flowering (16.53), thousand-grain weight (8.15) and grain yield per plant (4.89), expressed leptokurtic nature and only one trait grain length (0.007) showed mesokurtic nature of population distribution.

Box-and-whisker plot for yield and yield attributing traits

Box-and-whisker plot represents the spread of the data set in the population under study (Figure. 2). For days to 50% flowering/plant showed less spread of data, the first quarter (Q1) had slightly higher spread of data than other quarters, the second quarter is more concentrated than others, mean and median lied on the same place and availability of outlier below the minimum was recorded. The data on plant height (cm) exhibited a wider spread, with the first quartile (Q1) showing slightly more variability than the other quartiles. The median was positioned above the mean, and there were no outliers. The third quartile had the most concentration

of data. In contrast, the panicle length (cm) data displayed a more moderate spread. The median was slightly higher than the mean, and outliers were present above the maximum and below the minimum values. The third quarter was found to be more concentrated than others. The trait, number of effective tillers per plant, showed comparatively average spread of data, median located below the mean value, and availability of outlier above the maximum. The second quarter was found to be more concentrated than the others. Maximum dispersed data was observed in the fourth quarter. The Box-and-whisker plot for the number of filled grains per panicle depicted the maximum spread of data, and the box and whiskers in the plot showed a nearly equal distribution of the data. The third quarter was more concentrated than others; the median was located slightly above the mean value, and the availability of outliers above the maximum was also recorded. Grain length (mm) showed a moderate data spread, the median was located very slightly below the mean value, and the availability of outliers above the maximum and below the minimum was recorded. Whiskers were found to be more dispersed than boxes. Grain breadth (mm) showed comparatively average spread of data. The Median lied right on the mean value, and the availability of outlier above the maximum and below the minimum was recorded.

The first quarter was more dispersed, while the second quarter was denser than the others. The grain length-to-breadth ratio exhibited a relatively wide data spread, with the median slightly below the mean. Outliers were present above the maximum value. The second quartile was more concentrated than the others, whereas the fourth quartile exhibited the greatest spread. For thousand grain weight, the spread of data was comparatively less. The median was found slightly below the mean value, with the availability of outliers above the maximum and below the minimum. The second quartile was found to be more concentrated than others, whereas the fourth quartile showed the maximum spread of data. Similarly, grain yield per plant showed a comparatively moderate spread of data. The median was found below the mean value, with the availability of outliers above the maximum. The second quartile

Table 1. Skewness and kurtosis of morphological and qualitative traits in F_2 population of a cross Samba Mahsuri and Sathi of rice crop

Statistic	DFF	PHT	PL	NETPP	NFGPP	GL	GB	GLB Ratio	TGW	GZC	GIC	GYPP
Skewness (Pearson)	-2.805	-0.396	-0.150	0.998	0.051	0.108	-0.335	0.256	0.875	0.232	-0.356	1.460
Kurtosis (Pearson)	16.537	0.067	2.111	1.398	-0.097	0.007	1.085	-0.057	8.157	-0.710	-1.083	4.899

DFF = Days to 50% flowering per plant, PHT = Plant height (cm), PL (cm) = Panicle length (cm), NETPP = Number of effective tillers per plant, NFGPP = Number of filled grains per panicle, GL = Grain length (mm), GB = Grain breadth (mm), GLB = Grain length/breadth ratio, TGW =Thousand grain weight (g), GZC (ppm) = Grain zinc content (ppm), GIC(ppm) = Grain iron content (ppm), GYPP (g) = Grain yield per plant (g)

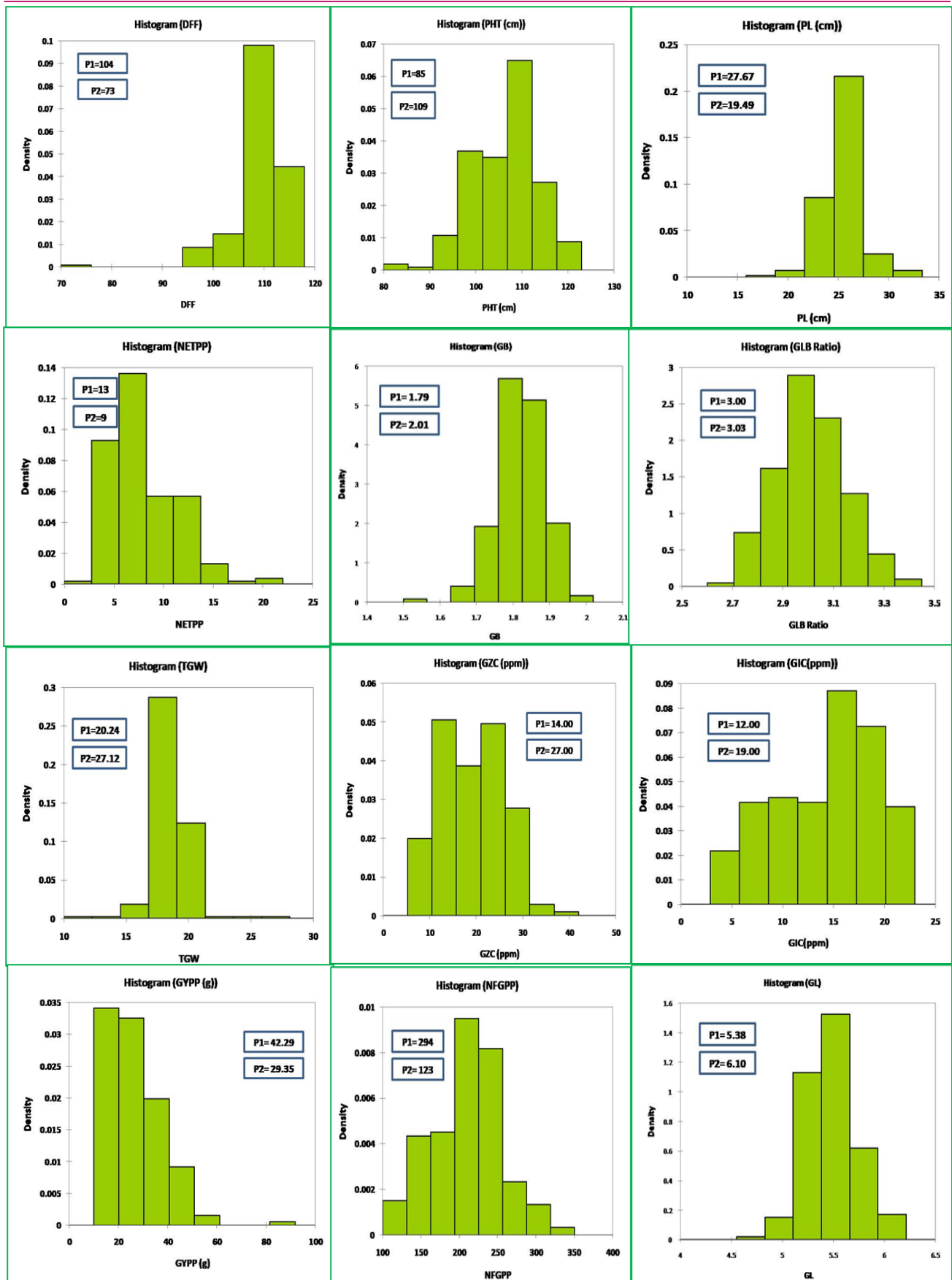


Fig. 1. Histogram of agronomical and nutritional trait analysis in F_2 population of a cross Samba Mahsuri and Sathi of rice crop

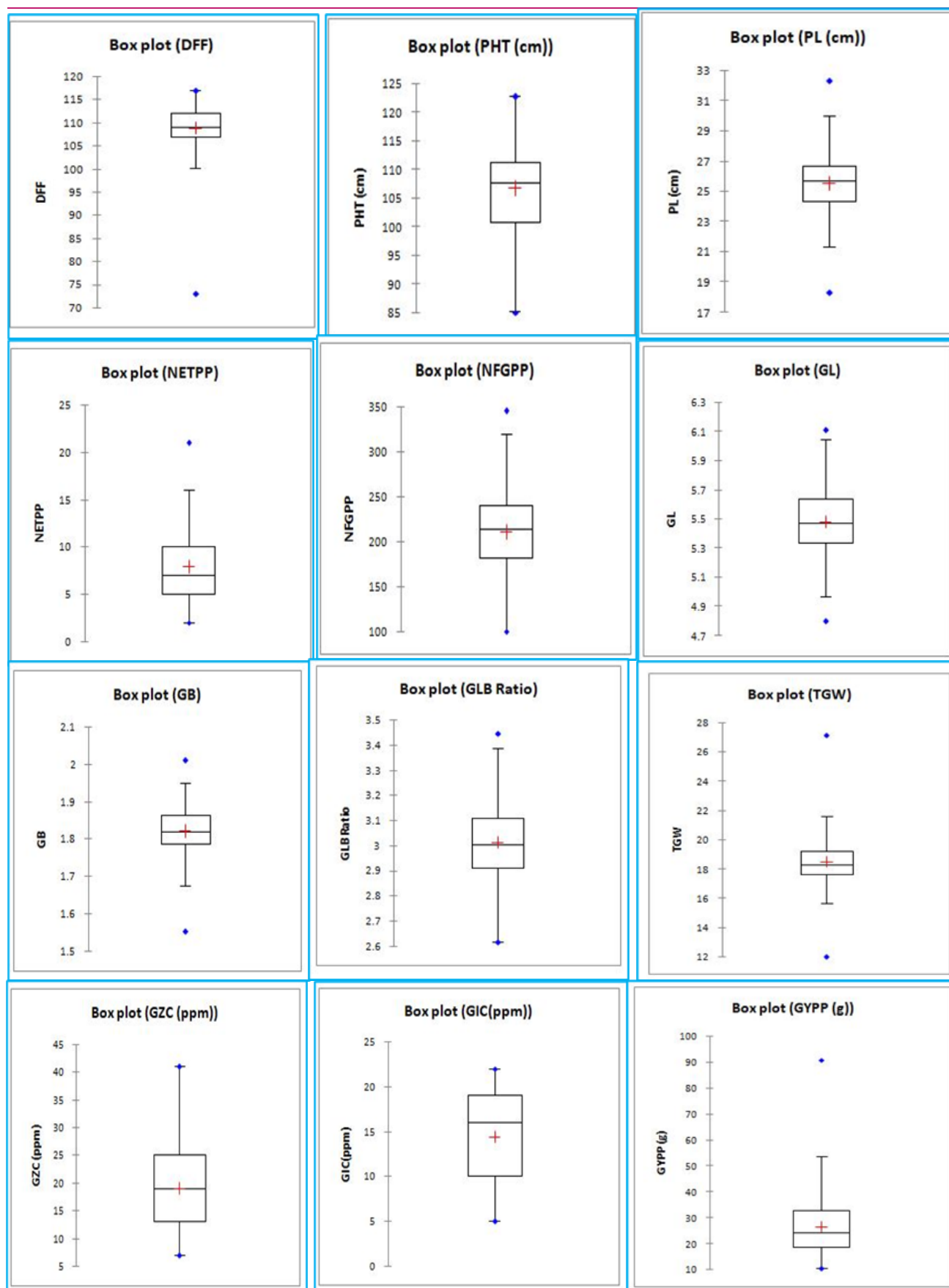


Fig. 2. Box and whisker plot of agronomical and nutritional trait analysis in F_2 population of a cross Samba Mahsuri and Sathi of rice crop

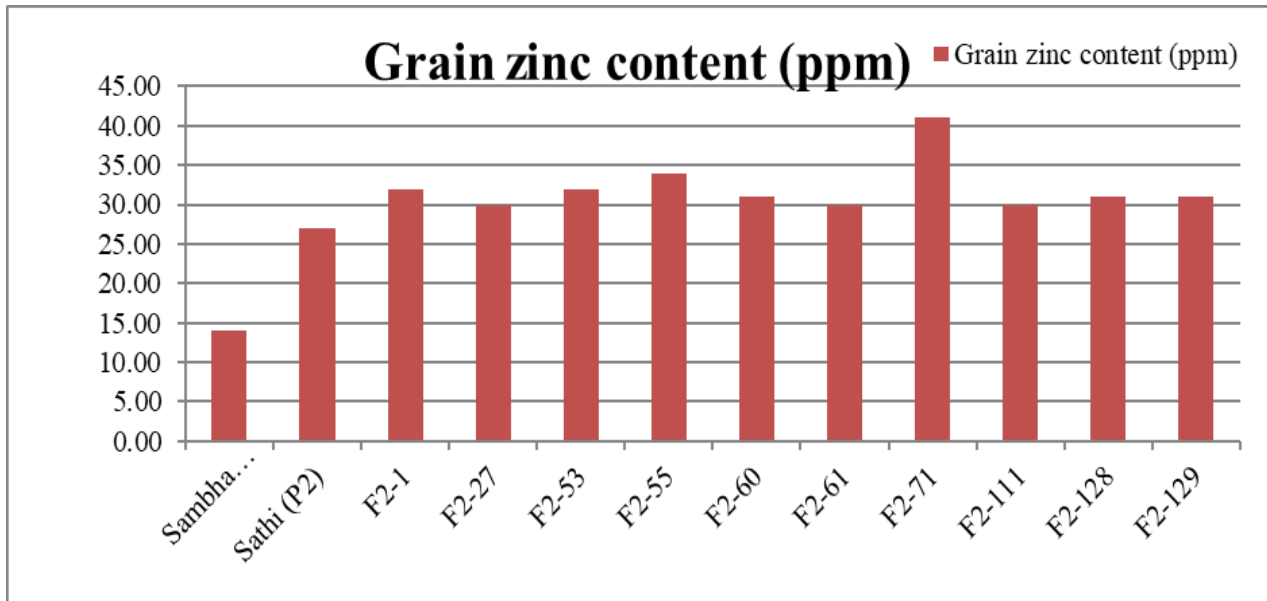


Fig. 3. Transgressive segregants for grain zinc content in the F_2 population of Samba Mahsuri (P1) and Sathi (P2) of rice crop

was found to be more concentrated than others, while the fourth quartile showed a maximum spread of data.

Box-and-whisker plot for nutritional traits

The box-and-whisker plot for grain zinc content (ppm) indicated relatively higher data variability, with the median aligning with the mean. Outliers were observed at both the minimum and maximum values. In the box plot, the second quartile (Q2-Q1) was equal to the third quartile (Q3-Q2), while the whisker from Q3 to the maximum was longer than the whisker from Q1 to the minimum. The fourth quartile showed the greatest data spread. Similarly, the grain iron content (ppm) displayed a relatively wider spread, with the median above the mean and outliers at both the minimum and maximum values. In the box plot, the second quartile (Q2-Q1) was greater than the third quartile (Q3-Q2), while the whisker from Q3 to the maximum was shorter than the whisker from Q1 to the minimum. The interquartile range (Q3-Q1) indicated that the middle 50% of the data was more dispersed than the rest.

Transgressive segregants in the F_2 population

Transgressive segregants are the appearance of extreme phenotypes in the segregating generation. In the present study, ten transgressive segregants, namely, F₂-1, F₂-27, F₂-53, F₂-55, F₂-60, F₂-61, F₂-71, F₂-111, F₂-128 and F₂-129 recorded for grain zinc content are mentioned in Figure 3. However, thirty transgressive segregants namely, F₂-26, F₂-27, F₂-48, F₂-50, F₂-52, F₂-56, F₂-59, F₂-65, F₂-67, F₂-73, F₂-76, F₂-78, F₂-79, F₂-80, F₂-87, F₂-88, F₂-89, F₂-92, F₂-93, F₂-95, F₂-99, F₂-105, F₂-106, F₂-129, F₂-130, F₂-147, F₂-165, F₂-179, F₂-183 and F₂-185 recorded for grain iron content are mentioned in Figure 4.

DISCUSSION

Skewness and kurtosis

Instead of a lower degree of statistics, third and fourth degrees could have better potential to explain the genetics of the traits, *i.e.* skewness and kurtosis in segregating the F_2 population. The study of the distribution of the data using skewness generates important information about the nature of gene action (Fisher *et al.* 1938), and kurtosis provides information about the number of genes involved in regulating the expression of traits (Robson, 1956). The distribution peak at the left and longer right tail showed positively skewed; the peak towards the right and longer left tail represented negatively skewed data. The peak at the center and no longer tail toward the left and/or right side is represented as normally distributed symmetrical data. Positive skewness is coupled with complementary gene interactions and lack of a dominant allele, while negative skewness is linked with duplicate gene interactions and influence of dominant allele. Skewness equal to zero or near zero showed a normal distribution that leads to the absence of gene interaction (Savitha and Kumari 2015; Sheshaiyah *et al.* 2018; Reddy *et al.* 2019). Regarding kurtosis, normal distribution showed a kurtosis value of exactly zero known as mesokurtic. A distribution, known as platykurtic, showed less than 3 kurtosis value (more precisely kurtosis < 0), while a kurtosis value of more than 3 (more precisely kurtosis > 0) is represented as leptokurtic. The traits showed leptokurtic distribution; fewer genes control these, whereas platykurtic population distribution showed that the trait was controlled by a large number of genes (polygenic in nature). The peakedness shape of the population follows the order leptokurtic>mesokurtic>platykurtic (Choo and Rein-

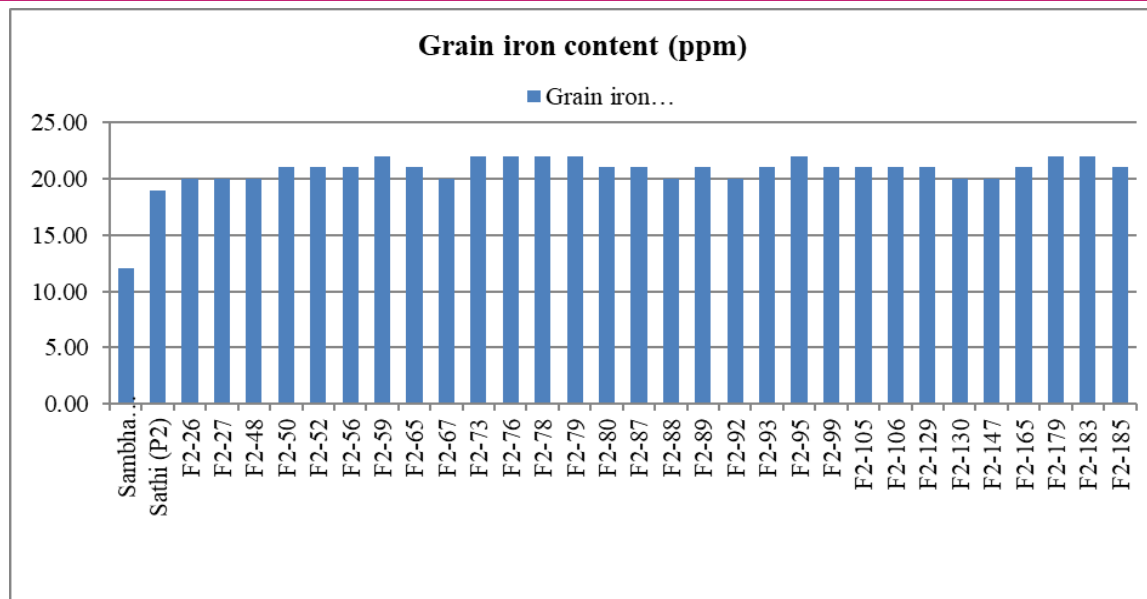


Fig. 4. Transgressive segregants for grain iron content in the F_2 population of Samba Mahsuri (P_1) and Sathi (P_2) of rice crop

bergs, 1982; Kiran K. 2012; Savitha and Kumari 2015; Reddy *et al.* 2019; Li *et al.* 2021). Selection intensity largely depends upon gene interaction, which is higher under complementary than duplicate gene interaction. So prior knowledge of gene interaction is required to know the nature and behavior of the population (Choo and Reinbergs, 1982; Savitha and Kumari 2015).

The respective box plot of the present study showed that plant height, panicle length, number of effective tillers per plant, number of filled grains per panicle, grain length, grain length-breadth ratio, thousand-grain weight, and grain yield per plant showed median value slightly displaced from the mean value and had great variation. Similarly, Kumar *et al.* (2014) investigated the F_2 population derived from the cross between high-yielding (PAU201) and iron-rich (Palman 579) *indica* rice varieties exhibited large variation for various morphological traits, including grain yield per plant and iron and zinc contents.

In the present investigation, positive skewness was recorded for traits such as the number of effective tillers per plant, grain length-breadth ratio, thousand-grain weight, grain zinc content, and grain yield per plant in segregating population indicating that population was found to be distorted from normal distribution as well as the presence of complementary gene action for these traits. Thus, extreme selection is necessary for vast genetic gain in these traits. While negative skewness was recorded for days to fifty per cent flowering, plant height, panicle length, grain breadth, and grain iron content indicated the presence of duplicate gene action and distorted from the normal distribution. Hence, mild selection is required to achieve productive outcomes in rapid genetic gain for these traits. The number of field grains per panicle showed near to zero value, indicat-

ing no gene interaction and normal distribution for this trait. Savitha and Kumari (2015) reported similar findings regarding the presence of genetic interactions for yield and yield-attributing traits like the number of productive tillers per plant, panicle length, hundred-grain weight and single plant yield in the six crosses of F_2 and F_3 segregating generations derived from the following genotype Veeradangan, Kavuni, Kathanellu, Navara and six improved semi-dwarf high yielding varieties viz., IR 72, ADT 39, ADT 45, ASD 16, TPS 4 of the rice. Similarly, Baisakh *et al.* (2020) reported normal phenotypic distribution with skewness for all the yield-related traits panicle number per plant, grains per panicle, and grain yield per plant studied using an $F_{2:3}$ population derived from Cocodrie $\times$ Nagina 22 (N22) of rice cultivar. Kumar *et al.* (2014) also recorded skewed frequency distribution curve for grain iron and zinc content towards "PAU201" having high concentration of iron and zinc. Thus, knowledge about the gene action of micronutrients is helpful in biofortification program.

The platykurtic nature of distribution exhibited by the number of filled grains per panicle, grain length breadth ratio, grain zinc, and iron content indicated that these characters were controlled by many genes (polygenic). The shape of the population distribution was found to be nearly flat, not to be more peaked for these traits. Platykurtic with left-skewed distribution was also recorded by Savitha and Kumari (2015) for days to 50 per cent flowering, plant height, number of productive tillers per plant, panicle length, hundred-grain weight, and single plant yield in rice. The findings of the present study were supported by Sheshaiah *et al.* (2018), who reported positive skewness and platykurtic distribution for days to flowering, number of tillers per plant, pro-

ductive tillers per plant, days to maturity, panicle length, grain yield per plant and L:B ratio using 226 F₂ individuals developed from the cross of rice variety 'Jyothi × Kiruwana'.

However, in the present study, plant height and grain length were found close to zero, indicating the mesokurtic nature of the population; the shape of the distribution was found to be neither flat nor peaked they lied between these two. Remaining traits such as days to 50% flowering, panicle length (cm), number of effective tillers per plant, number of filled grains per panicle, grain yield per plant (g), grain breadth (mm), grain length/breadth ratio and test weight, grain iron content were found to be greater than zero indicating the leptokurtic nature of the population and peaked shape of distribution was recorded for such traits; a fewer gene controlled these traits. Sheshaiah *et al.* (2018) reported a similar finding in the rice F₂ population of Jyothi × Kiruwana' for grain yield and its component characters rice. Similarly, Rani *et al.* (2016) reported negatively skewed platykurtic distribution recorded for the traits total number of tillers, total number of productive tillers, flag leaf length, flag leaf width, panicle length, total number of spikelets per panicle were governed by a large number of genes and majority of them displaying dominant and dominant based duplicate epistasis. Consequently, rapid genetic gain through selection is expected in crop improvement program for these traits.

Transgressive segregation

The main cause of transgressive segregants is the presence of epistasis, overdominance, masking of rare lethal recessive alleles by a desirable dominant allele, accumulation of desirable alleles from both of the parents and complementary action of additive alleles. Mostly transgressive segregation provided by genetic cause is heritably stable (Nirubana *et al.* 2019). In the present study, ten transgressive segregants were observed for grain zinc content in rice, whereas thirty transgressive segregants were observed for grain iron content. In the parental line, one of the parents Sathi recorded 27 ppm zinc and 19 ppm iron content in grains of rice; however, transgressive segregant showed 30-41 ppm zinc and 20-22 ppm iron content in rice grains. Transgressive segregants for grain iron and zinc content showed low grain-yielding capacity. Mallimar *et al.* (2017) also reported transgressive segregants for grain iron and zinc content in the F₂ family of two populations, such as Swarna × Ranbir basmati and Swarna × BR2655. They found four transgressive segregants for iron content and three for zinc content in the rice Cross Swarna × Ranbir basmati. However, in the case of Swarna × BR2655, thirteen segregants for iron content and two for zinc content were reported. Similarly, in the present study, more transgressive segregants were reported for grain iron content than grain zinc content. These micronutrient concentrations negatively

correlate with yield; therefore, selecting genotypes in advanced generations that offer high iron and zinc content with minimal yield reduction is crucial. Transgressive segregants for iron and zinc in rice are also reported by Kumar *et al.* (2014); Wattoo *et al.* (2019); Korada *et al.* (2020); Shaikh *et al.* (2020) and Li *et al.* (2021).

The present study revealed the genetic nature of nutritional and yield-related traits and identified more transgressive segregants for iron and zinc content. The further careful selection of these genotypes will lead to the development of rice biofortified lines.

Conclusion

The presence of variability is a prerequisite for crop improvement, and this prospect of investigating the F₂ rice population had ample variability for agronomical traits and nutritional traits. Hence, this population may be used further in crop improvement programmes. Most characters, including grain iron and zinc content, were polygenic and showed the platykurtic nature of kurtosis. Most of the characters, including grain zinc content, showed complementary gene action described by positive skewness of distribution that responded better to selection. Iron and zinc deficiency can be addressed in developing countries by developing biofortified rice varieties, where rice is consumed as a staple food. Although, as per the present study, iron and zinc were found to be positively correlated with each other, there is a need to develop such a variety that should be rich in zinc and iron.

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Conflict of interest

The authors declare that they have no conflict of interest.

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