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Evaluation of Parents for Selected Grain Quality Traits through a Specific Gene-Based Markers

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A B S T R A C T

Rice (*Oryza sativa* L.) is one of the most important cereal crops worldwide and plays a crucial role in global food security. Despite remarkable achievements in enhancing rice productivity, limited emphasis has been placed on improving grain quality traits that contribute significantly to consumer preference and export value. The present study was undertaken to investigate the genetic basis of yield and grain quality traits through quantitative trait loci (QTL) mapping in rice using segregating populations. A total of 304 $F_{2:3}$ lines derived from contrasting parental genotypes were evaluated for important quality and agronomic traits, while 96 polymorphic molecular markers were employed for genotypic analysis. Linkage map construction was performed using QTL IciMapping version 4.2, and QTL detection was carried out using composite interval mapping through Windows QTL Cartographer. Chi-square analysis was used to assess marker segregation, while linkage groups were established at a minimum LOD threshold of 3.0. Significant QTLs were identified using a LOD threshold of 2.5 and validated through permutation testing at a 5% significance level. The results revealed substantial variability among the parental lines and segregating generations for elongation ratio, elongation index, days to 50% flowering, days to maturity, and plant height. The F_1 hybrids generally exhibited superior performance for several traits, indicating the presence of heterosis, whereas the F_2 populations showed considerable segregation and transgressive variation. Several genomic regions associated with grain quality and agronomic traits were identified, explaining significant proportions of phenotypic variation. The findings provide valuable insights into the genetic architecture of economically important traits and offer useful molecular markers for marker-assisted selection and the development of high-yielding rice cultivars with superior grain quality characteristics.

Introduction

The rice is (*Oryza sativa* L.) belongs to the family Poaceae (Gramineae) and genus *Oryza*. The genus *Oryza* is distributed throughout the tropics and subtropics of the world. It has 25 species out of which 23 species are wild and only two species *viz., Oryza sativa* and *Oryza glaberrima* are cultivated. *Oryza sativa* is predominately cultivated in the Asiatic region whereas, *O. glaberrima* belongs to Africa.

The genus contains both diploid (2n = 24) as well as tetraploid (2n = 48) species. *O. sativa* is further classified into three sub-species based on geographical distribution and morphological traits: J*aponica, indica, and javanica*.

In India during the year 2020-21, rice was cultivated on an area of 43.60 million hectares with a record production of 121.46 million tones and with average productivity of 2.75 t/ha (Anonymous, 2021). West Bengal ranks first in rice production followed by Uttar Pradesh and Punjab (Anonymous, 2020). Despite having wheat as a major cereal crop and sugarcane as the principal commercial crop, Uttar Pradesh ranks second in total rice production in India.

Since the green revolution, there has been a significant increase in the production and productivity of rice. Advancement of technology, development of excellent hybrids, modern management techniques, and many more factors has led to enhanced rice production. The production of rice has increased from 30.45 MT in 1966-67 to 118.43 MT in 2019-20 (FAOSTAT, 2020). We are next only to China in rice production. It is unfortunate that despite achieving independency in rice production, the quality characteristics, that may increase the total export value of rice, have not been given due attention.

Conventional agriculture mainly focuses on yield enhancement, while very little attention has been given to the quality improvement of rice and with this drawback, crop improvement is still chasing quantity rather than quality improvement. Districts of Uttar Pradesh covering an area of about 5.87 million ha (13.34% area of the country) with the production of 15.57 million tonnes (Anonymous, 2020). Few districts of the state such as Binaur, Kushinagar, Peelibheet, Chandauli, Bagpat, Ambedkarnagar, and Varanasi are reported as high productive areas for rice cultivation because of appropriate climatic conditions and vast area of fertile Gangetic soil available in this area.

Material and Methods

The phenotypic data of the 304 $F_{2:3}$ lines for quality and yield traits and the genotypic data of all the 96 polymorphic markers were used for the construction of linkage map and mapping QTLs for different traits. The linkage map was prepared using QTL IciMapping Version 4.2 (Meng *et al.,* 2015).

Chi-square values were calculated for each marker to detect segregation deviations (at P=0.05) from the expected Mendelian ratio. Linkage groups (LG) were developed using an independence test of the LOD score with a minimum threshold set to „3.0‟ which shows that the probability of occurrence of linkage between any two markers is 1000 times more than no linkage (Stam P, 1993). Kosambi mapping function was used to convert recombination frequency (in percent) between adjacent markers into map interval distance in centi Moran (cM). The default settings of the software were used: LOD = 3.0, k-Optimality-by Rec-2OptMAP, window size = 5. QTLs were detected using Windows QTL Cartographer Version 2.5_011 (Wang *et al.,* 2012) by employing composite interval mapping (CIM). CIM was carried out using the default settings (standard model 6, walk speed of 1cM, default value 5, window size of 10 with forward regression analysis). The linkage between the markers and the QTL was detected by a statistical test called the Logarithm of Odds (LOD) score method using a computer program.

The LOD score was calculated using the following formula:

$$LOD\ score = log10 = \frac{Probability\ of\ certain\ degree\ of\ linkage}{Probability\ of\ independent\ assortment}$$

Generally, there is a direct one-to-one transformation between LOD scores and Likelihood Ratio Statistic (LRS) scores. The conversion was calculated by the formula:

$$Likelihood\ Ratio\ Statistic\ (LRS) = 4.6 \times LOD$$

The LOD profiles were used to identify the most likely position for a QTL in relation to the linkage map, it is the position where the highest LOD value is obtained. A typical output from interval mapping was a graph with markers comprising linkage groups on X-axis and the test statistic on the Y-axis. The peak or maximum must also exceed a specified significance level to declare the QTL as „real‟ (i.e., statistically significant).

A default Likelihood Ratio (LRS) of 11.5 and an appropriate threshold value of LOD 2.5 as documented by publications of QTL analysis (Sabouri & Biabani, 2009; Swamy *et al.,* 2018) was used to declare QTLs as significant.

A permutation test (Churchill & Doerge, 1994) was used at 0.05 confidence level and 1000 permutations of trait data to identify QTLs with LRS of 11.5 that satisfied this stricter empirical threshold. The percentage of total phenotypic variation (R^2) explained by each QTL and the additive effects were estimated.

Result and Discussion

Elongation ratio (ER)

The observation of mean values elongation ratio ranges from 1.69 to 1.84 and are depicted in table 4.1.1. It is clear from the table that the mean values for the two parents differed significantly. The F_1 hybrid (1.83) recorded higher mean values compared to parent 1, but slightly lower than parent 2. While the mean value of the F_2 generation (1.80) was lower than the observation of the F_1 generation. The mean value of BCP_1 (1.76) was lower than the F_1 but higher than the recurrent parent P_1, whereas the mean value of BCP_2 (1.75) was lower than the F_1 and the recurrent parent P_2.

Elongation index (EI)

The mean values of six different generations for the elongation index are presented in table 4.1.1 and they ranged from 0.92 to 1.18. The mean values of both parents varied significantly from each other. The mean values of F_1 (1.18) outperformed both the parents, while the mean values of F_2 generations (1.07) were smaller than the mean values of the F_1 generation. The mean value of BCP_1 (0.95) was lower than the F_1 hybrid but higher than the recurrent parent P_1, whereas the mean value of BCP_2 (1.14) was lower than the mean values of F_1 as well as the recurrent parent P_2.

Days to 50 per cent flowering (DF)

The trait days to 50% flowering mean values are depicted in table 4.1.2 and it ranged from 101.73 days to 121.90 days, and the parental mean values differed significantly from each other. The F_1 mean (111.00 days) was intermediate between both the parents, while the mean value for the F_2 generation (108.72 days) was lower than the mean value of the F_1 hybrid. The mean values of the BCP_1 (105.04 days) and BCP_2 (115.83 days) generations were in between the mean value of F_1 and their respective recurrent parents.

Day to maturity (DM)

The observed mean days to maturity ranged from 132.50 to 152.90 days, with the parental values differing significantly from each other (Table 4.1.2). The F_1 mean (141.13 days) was intermediate between the two parents, while the mean value of the F_2 generation (138.99 days) was lower than that of the F_1 hybrid. The mean value of BC_1P_1 (135.50 days) was lower than the F_1 mean but higher than that of the recurrent parent P_1, whereas the mean value of BC_1P_2 (146.25 days) was higher than the F_1 mean but lower than that of the recurrent parent P_2.

Plant height (PH)

The observation of mean values for plant height are presented in table 4.1.2 and they varied from 100.13 cm to 161.40 cm. For the trait PH, both parents differed significantly from one another.

The F_1 mean value (160.27 cm) was nearer to the second parent, while the mean value for the F_2 generation (131.92 cm) was significantly lower than the observation mean value of the F_1 hybrid.

The observation value of BCP_1 (123.85 cm) was lower than the mean value of F_1 but nearer to the mean values of its recurrent parent P_1, while the mean value of BCP_2 (158.13 cm) was lower than the F_1 hybrid as well as its recurrent parent P_2.

Panicle length (PL)

The mean values for the trait panicle length varied from 22.33 cm to 30.73 cm, and a significant difference existed between both the parents for the trait (Table 4.1.2). The F_1 mean (29.73 cm) was nearer to the second parent, while the mean value for the F_2 generation (25.80 cm) was lower than the observation mean value of the F_1 hybrid. The observation mean value of BCP_1 (25.00 cm) was in between the mean value of F_1 and its recurrent parent P_1, while the mean value of BCP_2 (27.72 cm) was lower than the mean values of F_1 as well as its recurrent parent P_2.

Number of effective tillers per plant (ET)

The mean values for the number of effective tillers per plant are expressed in table 4.1.2, and they ranged from 11.74 to 17.03 tillers per plant. The parental mean value for the trait ET. The mean values of F_1 (17.03) outperformed both the parents, while the F_2 mean values (11.97) were lower than both the parents as well as their F_1. The mean values of the BCP_1 (12.70) and BCP_2 (11.74) generations were lower than the mean value of F_1 as well as the mean values of their respective recurrent parents.

Number of spikelet's/panicle (NSP)

The observation means values for the number of spikelet's ranged from 170.83 to 199.27 and are presented in table 4.1.2. The mean values of differed significantly both the parents in their per se performance. The F_1 mean (188.77) was in between the mean values of both the parents, while the F_2 mean (170.83) was lower than the mean values of both the parents as well as their F_1 hybrid. The mean values of BCP_1 (185.49) were lower than both the F_1 hybrid as well as the recurrent parent P_1, whereas the mean value of BCP_2 (179.63) was lower than F_1 but higher than the recurrent parent P_2.

1000 grain weight (TW)

The mean values for the six different generations of 1000 grain weight are depicted in table 4.1.2 and they ranged from 10.69 g to 15.20 g. For TW, the two parental mean values differed significantly from each other. The F_1 mean value (15.12 g) showed superior performance over parent 2 and was similar to the mean value of parent 1.

The mean value of the F_2 generation (12.45 g) was lower than the observation mean value of the F_1 generation. The mean value of BCP_1 (12.73 g) was lower than that of F_1 as well as the recurrent parent P_1, whereas the mean value of BCP_2 (12.02 g) was lower than F_1 but higher than the recurrent parent P_2.

Grain yield per plant (GYPP)

The mean values for the economic trait grain yield per plant varied from 14.98 g to 33.89 g, and both the parents differed significantly from each other (Table 4.1.2). The mean values of the F_1 generation (33.89 g) outperformed both the parents, while the mean values of the F_2 generation (14.98 g) were significantly lower than the mean values of F_1 as well as both the parents. The mean values of BCP_1 (23.24 g) and BCP_2 (15.27 g) generations were lower than the mean value of F_1 as well as the mean values of their respective recurrent parents.

In conclusion, the earlier reported gene-specific markers; nksbad2, BADEX7_5, Aro7, and SSI used in the present study were able to differentiate the parents for aroma, cooking, and eating quality traits hence these validate the presence of *for* gene (badh2) and GBSSI gene in the parents.

The scaling test results showed significance for either one of the four scales or in combination, along with a significant χ^2 value of joint scaling, suggesting the presence of non-allelic interaction for all the grain quality and yield traits.

Author Contributions

Bharat Lal Maurya: Investigation, formal analysis, writing—original draft. Alok Kumar Singh: Validation, methodology, writing—reviewing. Ramesh Kumar Maurya:—Formal analysis, writing—review and editing. N. V. Durga Prasad Rao: Investigation, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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