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Assessment of Variability and Genetic Diversity Study in an Advanced Segregating Population in Rice with Blast Resistance Genes Introgression

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ABSTRACT

Blast disease caused by a pathogenic fungus, *Magnaphorthe oryzae*, is the most destructive disease and has resulted in more than 50% of crop losses worldwide, including in Malaysia. The present study was conducted to investigate genetic variability among 36 advanced lines of MR264 × PS2 rice with blast resistance genes introduced at the Faculty of Applied Sciences, Universiti Teknologi MARA, Malaysia. Traits such as days of maturity, plant height, grain width, and seed setting rate exhibited negative skewness in this study, indicating a doubling of gene effects. Seed setting rate and 1000 grain weight showed positive kurtosis, indicating gene interactions. The phenotypic coefficient of variation (PCV) was slightly higher than the genotypic coefficient of variation (GCV) for all traits, indicating that environmental influences affect the expression of these traits. High heritability associated with high genetic advance as a percentage of the mean was observed for filled grains per panicle. In addition, the second-highest value for high heritability and the high genetic advance was observed for the number of tillers. Cluster and principal component analysis revealed that 36 advanced lines were grouped into four clusters based on ten agromorphological traits. Clusters A and C had higher mean values for most of the traits studied than clusters B and D. Desirable recombinants for higher yields with a broad genetic base can be generated by using cross lines from different clusters.

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1 Introduction

Rice (*Oryza sativa* L.) is the most important food crop in the world. Nearly 40% of the world's population consumes rice as a staple food (FAO 2019). Blast disease, also known as rice blight, is the most destructive rice disease caused by the fungus *Magnaphorthe oryzae*, resulting in more than 50% of crop losses worldwide (Hasan et al. 2015a; Chukwu et al. 2020). Conventionally, blast disease can be controlled with chemical pesticides and biocontrol agents (Rijal and Devkota 2020; Upadhyay and Bhatta 2020). However, due to environmental and food safety concerns, chemical control is not feasible, practical, and effective (Magar et al. 2015). Therefore, the development and use of resistant cultivars are considered as the most effective, economical, and environmentally friendly method to control the instability of the fungal pathogen *M. oryzae* (Xiao et al. 2019; Shahriar et al. 2020).

The success of a breeding programme always depends on the accessibility of a wide range of genetic resources and germplasm evaluation. A comprehensive study of genetic diversity within accessions would be useful to establish new varieties of breeding material (Govintharaj et al. 2016). Variability in genetic divergence for traits of interest is a major component of the breeding programme to expand the genetic pool of crops and requires an accurate assessment of heritability to prepare for a successful breeding programme (Roy and Shil 2020). A proper understanding of heritability helps plant breeders predicts the type of successful generation to make the appropriate selection and evaluate the extent of genetic improvement through selection. Traits with high genotypic coefficients of variation, as well as high heritability and genetic advance as a percentage of the mean, can be controlled by additive types of gene activity; therefore, traits can be improved by simple breeding methods.

Information on skewness and kurtosis provides information on the type of gene action (Savitha and Kumari 2015) and the number of genes controlling the trait. In addition, the studies on skewness and kurtosis are necessary to evaluate the extent to which the second generation affects the mean of the third generation and whether this parameter can be used for selection. The true genetic potential can only be determined in the performance of the progeny in the individual selection since the mean value of the progeny is a more reliable estimate than the individual values of the selected parents. If the kurtosis value for the traits has a positive sign, it indicates the presence of gene interactions, while a negative sign or a value close to zero for the kurtosis indicates the absence of gene interactions (Samak et al. 2011). A skewness with a positive sign indicates the presence of complementary gene action, whereas gene action is double (additive × additive) when the skewness has a negative sign (Vanitha et al. 2016).

Grain yield is a complex trait that depends on many components and responds poorly to direct selection. Knowledge of the relationship between grain yield and its components is useful for improving grain yield (Lakshmi et al. 2017). Information on the relationship between traits and the direct and indirect effects of each trait on yield is an added advantage in supporting the selection process. Correlation and pathway analyses determine the extent of the relationship between yield and its components and also reveal the relative importance of their direct and indirect effects, providing a clear understanding of their relationship with grain yield (Nikhil et al. 2014). Ultimately, this type of analysis could help growers develop their selection strategies to improve grain yield.

In the light of the above scenario, the present study was conducted to determine the genetic diversity and variability of yield and yield component traits of blast resistance genes introduced into segregating rice populations.

2 Material and Methods

2.1 Plant materials and Experimental setup

The experiment was conducted from March 2013 to September 2016 at the greenhouse of Agency Nuclear Malaysia Greenhouse in Selangor, Malaysia. The genetic material used in this experiment was MR264 as the recurrent parent (referred to as P1) and Pongsu Seribu 2 as the donor parent (referred to as P2). The parents were differentially resistant to blasts as shown in Table 1.

P1 and P2 were planted as parents in standard plastic pots (28 cm x 25 cm), each containing 5 plants. The F_1 generation was formed by crossing P1/P2, and the F_1 generation was obtained reciprocally. Four of the 32 F_1 plants developed as the female parent were backcrossed to MR264 to develop BC_1F_1 progeny. Six of 136 BC_1F_1 were backcrossed to produce BC_2F_1 plants. Selected BC_2F_1 plants were then allowed to self-pollinate to produce BC_2F_2 generation. Foreground and background selection was combined with strict phenotypic selection in each generation after pedigree selection (Figure 1). A total of 36 advanced lines were selected and used for this study (Figure 2). Standard cultivation methods recommended by IRRI (2015) were used to grow healthy plants.

Table 1 Panicle characteristics of parents selected for crossing

Parents	Blast resistance level
MR264	Susceptible (score between 7-9)
Pongsu Seribu 2	Resistant (score between 1-3)

*Scoring was based on IRRI standard scoring for blast symptoms (IRRI 2015)

M	MR264	PS2	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12

(a) RM206

200bp

100bp

	A	B	H	H	A	A	H	A	H	H	H	H	A	H

M	MR264	PS2	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10

(b) RM224

200bp

100bp

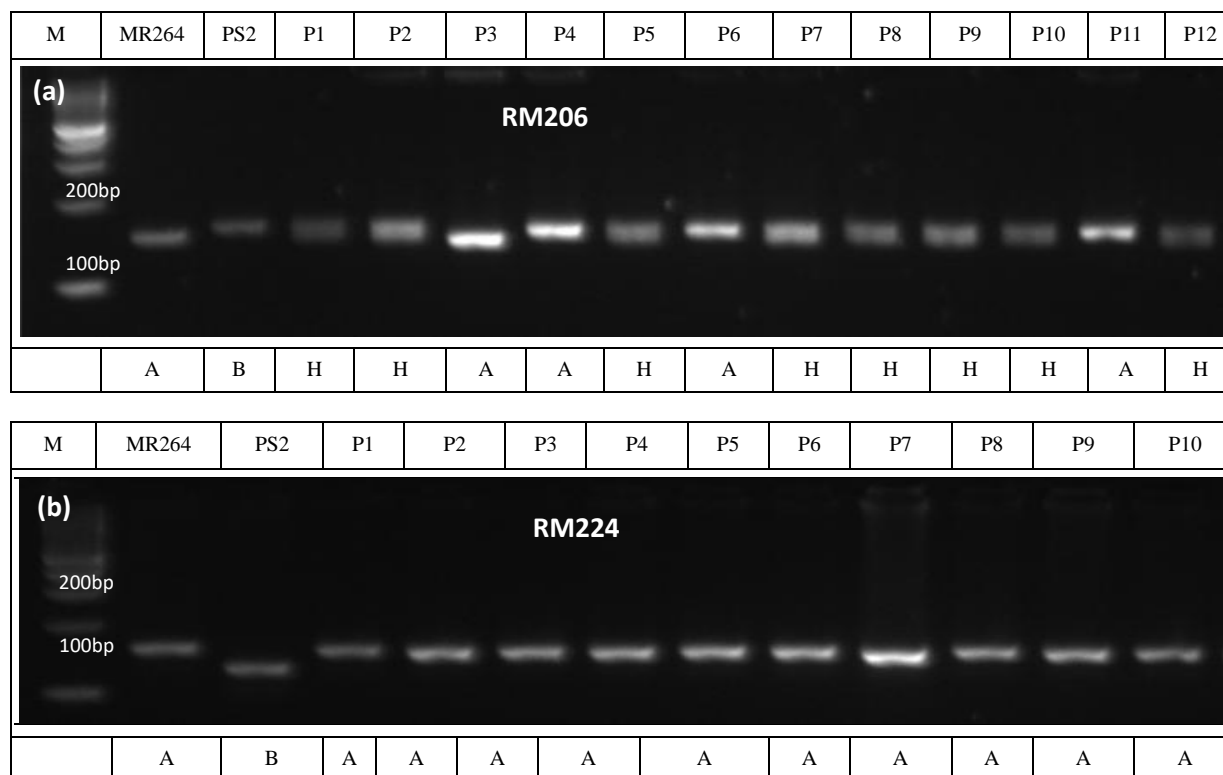|	A	B	A	A	A	A	A	A	A	A	A	A

Figure 1 Banding pattern in the BC_2F_2 population using (a) foreground markers; RM206 markers (b)background marker, RM224 (M=100 bp DNA ladder; P= plant no.)
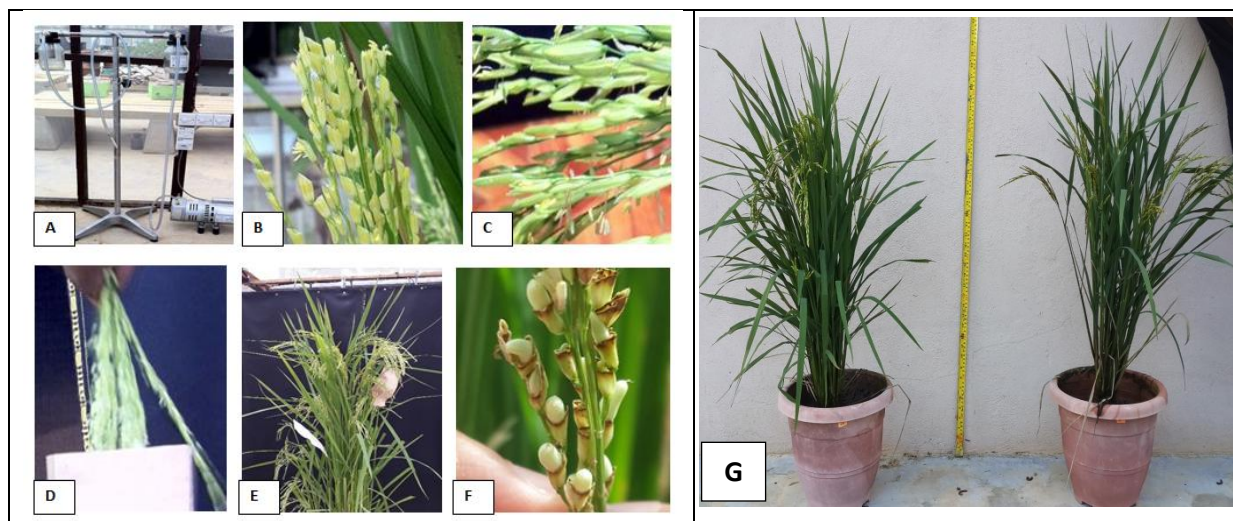
Figure 2 Hybridization process: A) Two selected parents Pongsu Seribu 2 and MR 264, B) Immature spikelets were immersed in hot water at 42.5ºC for five minutes, C) end of opened spikelets were snipped off with scissor, D) Pollen from a male parent; E) Fertilized seeds three weeks after pollination. G) Morphological features of plant height of the parental line and blast-resistant improved lines (BC_2F_3)

2.2 Field data collection

At the maturity stage, data were collected and recorded from thirty-six plants of the 234 BC_2F_3 populations according to the rice descriptors of IRRI (2015). The traits such as days to maturity (DOM, days), plant height (PH, cm), number of shoots per hill (NOT, n), panicle length (PL, cm), number of filled grains (FG, n), grain length (GL, cm), grain width (GW, cm), yield per hill (YH, g), 1000-seed weight (SW, g) and seed set rate (SS, %) were collected and recorded.

2.3 Statistical analysis

Data were used to estimate variability using simple measures such as mean and range, components of variation such as genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (GCV), genetic parameters such as heritability, and genetic advance as a percentage of mean, and genetic diversity. All parameters in this study were measured on the 36 BC_2F_3 plants using Microsoft Excel 2007 (Microsoft Crop., Redmond, WA, USA). To analyze the genetic relationships among the rice lines, the UPGMA (Unweighted pair group method with arithmetic means) algorithm and SAHN clustering (Oladosu et al. 2014) were used. The EIGEN and PROJ modules of NTSYS-pc and SAS version 9.2 were used to calculate the PCA of 36 rice lines. The association between the 10 quantitative traits was determined using the Pearson correlation coefficient. The equation for skewness and kurtosis was calculated according to the method presented by Herawati et al. (2021) and calculated as follows:

$$Skewness = \sum_{i=1}^{N} \frac{(Yi - \overline{Y})^3}{(N-1)S^3}$$

$$Kurtosis = \sum_{i=1}^{N} \frac{(Yi - \overline{Y})}{(N-1)S^4}$$

3 Results and Discussion

3.1 Yield and it's component traits among 36 BC_2F_3 lines

Ten quantitative traits of rice yield were calculated for genetic variability (Table 1). Ten traits, including days to maturity, plant height, number of grains on hill[-1], panicle length, number of filled grains on panicle[-1], the yield on hill[-1], 1000-grain weight on hill[-1], and rate of seed formation, showed highly significant variation (P ≤ 0.05). The results obtained are in agreement with those of Sumanth et al. (2017) and Girma et al. (2018), which indicated significant differences among genotypes. Therefore, genetic improvement through selection is highly beneficial. High variability in breeding material helps to ensure that the subsequent generation has a better chance of producing superior recombinants.

Plant number 24 (P24) had the highest days to maturity (123), while P13 had the lowest days to maturity (112), which is statistically consistent with parent MR264. The highest plant height was observed in P19 (104 cm), while P4 had the lowest plant height (76 cm). This result is consistent with Mazid et al. (2013) and Hasan et al. (2015b), who found differences in plant height between the progeny of rice and the parent cultivar. The remaining plants showed differences in plant height, but these were not significant, indicating that they were very similar to the height of the parent plant MR264 (Table 1).

Tillering ability is one of the most important yield-determining traits of the rice plant. The number of shoots per hill varied from 28 to 10. The highest number of shoots (28) was found in plant No. 6 (P6), which was statistically similar to P3 and P4. The lowest number of tillers (10) was found in plant number 23, which was statistically similar to MR264. P7 and P8, P26 and P27, P30 and P32, P35 and P36 had a similar number of tillers. Other rice lines had different values but were statistically similar. It can be seen that the number of grains in the new progenies is close to MR264 (Table 1). The number of grains is crucial for high grain yield. The same result was also found in our recent study. Sharif et al (2013) also found significant differences in the number of grains on hill[-1] in pigmented rice.

In rice, yield is determined by indirect traits such as panicle length. In the present study, the highest panicle length (26 cm) was observed in plant number 4 (P4), followed by P2 (25.67 cm), while the shortest panicle length (21 cm) was observed in P3 and P26, which was statistically similar to P20 and P33. The study was in agreement with the reports of Sabri et al. (2020). Almost all progenies were statistically like MR264 (Table 1).

A significant difference was observed in grains per panicle among all progenies, with values ranging from 210 to 96. The highest number of filled grains (210.67) was obtained from plant number 2 (P2), while the lowest number of filled grains (96) was from P18. The highest yield/ha (44.67 g) was recorded from plant number 6, followed by P4 (43 g), while the lowest value (33.67 g) was observed from P33 (Table 1). The highest amount of total grain weight per hill belonged to P2, while P16 and P33 had the lowest amount of total grain weight per hill. The 1000-grain weight varied significantly among genotypes, ranging from 29.67 to 16.67 g. P1, P20, and P23, and P27, P23, and P30 had similar total grain weight per hill, 22 and 20 g, respectively. The highest percent seeding rate was recorded by P3 (96.33%), which is statically similar to plant numbers 5, 11, and 12. Plant number 36 (P36) had the lowest seeding rate (54.33%). This result is also in agreement with the results of Roy and Shil (2020).

Seed length also varied among progenies, ranging from 11.33 to 7.67 mm. Plant number 34 had the longest (11.33 mm) seeds, while P10 had the shortest (7.67 mm) seeds, similar to P12 and P17. The highest width length (2.07 mm) was found in MR264, while the lowest value (1.83 mm) was observed in P2. The other measured values of progeny length were different but not statistically significant. This indicates that the newly developed lines do not show genetic variation with the parental MR264 lines and are not controlled by environmental factors. This was also noted by Hasan et al. (2015b) and Sabri et al. (2020).

Table 2 Mean performance of 36 BC$_2$F$_3$ rice lines for 10 agronomic traits.

Progeny	Days of maturity	Plant height(cm)	Tiller number/hill	Panicle length (cm)	Filled grain (%)	Grain length	Grain width	Yield /hill (g)	1000 seed wt (g)	Seed setting rate (%)
MR284	112.00[ab]	83.00[ac]	24.33[ab]	21.33[c]	164.67[ab]	8.00[a]	2.07[a]	40.00[ac]	23.33[ab]	91.00[ac]
P1	113.33[c]	84.00[b]	23.67[c]	23.33[b]	160.00[c]	8.33[a]	2.03[a]	40.00[b]	22.00[c]	90.33[b]
P2	113.00[c]	80.33[b]	24.67[c]	25.67[b]	210.67[c]	10.67[a]	1.83[a]	41.80[b]	29.67[c]	93.89[b]
P3	114.33[c]	77.33[b]	27.00[c]	25.00[b]	193.33[c]	10.67[a]	1.97[a]	42.00[b]	26.00[c]	96.33[b]
P4	118.00[c]	76.00[b]	27.00[c]	26.00[b]	207.00[c]	10.00[a]	2.00[a]	43.00[b]	27.33[c]	92.67[b]
P5	115.67[c]	82.33[b]	21.33[c]	21.00[b]	206.33[c]	8.33[a]	1.97[a]	40.00[b]	24.33[c]	96.33[b]
P6	115.67[c]	76.67[b]	28.00[c]	23.33[b]	177.33[c]	8.67[a]	1.93[a]	44.67[b]	26.33[c]	94.33[b]
P7	117.67[c]	77.33[b]	24.00[c]	25.00[b]	179.33[c]	8.33[a]	1.90[a]	41.00[b]	26.67[c]	91.00[b]
P8	116.67[c]	78.67[b]	24.00[c]	22.33[b]	173.67[c]	10.00[a]	1.97[a]	41.07[b]	25.33[c]	91.00[b]
P9	117.00[c]	105.67[b]	20.67[j]	26.67[b]	105.00[c]	8.00[a]	1.90[a]	40.67[b]	20.67[c]	52.00[b]
P10	116.67[c]	95.67[b]	13.33[c]	23.67[b]	117.67[c]	7.67[a]	2.00[a]	41.00[b]	22.00[c]	76.33[b]
P11	117.00[c]	95.00[b]	21.33[c]	22.67[b]	99.33[c]	8.33[a]	1.87[a]	39.67[b]	19.33[c]	96.33[b]
P12	113.67[c]	98.00[ac]	19.00[c]	21.00[b]	108.0[c]	7.67[a]	2.00[a]	40.33[b]	18.67[c]	96.33[b]
P13	112.00[c]	92.00[b]	18.67[c]	20.00[b]	100.00[c]	9.00[a]	2.03[a]	37.00[b]	19.33[c]	82.33[b]
P14	116.00[c]	93.67[b]	17.33[c]	22.23[b]	107.67[c]	8.67[a]	1.97[a]	39.27[b]	21.00[c]	81.40[b]
P15	118.00[c]	100.33[b]	16.00[c]	25.00[b]	114.33[c]	8.33[a]	1.97[a]	41.00[b]	20.00[c]	79.67[b]
P16	117.67[c]	99.67[b]	21.00[c]	24.67[b]	98.00[c]	9.33[a]	1.97[a]	35.27[b]	16.67[c]	88.00[b]
P17	113.33[c]	100.67[b]	16.67[c]	21.00[b]	105.00[c]	7.67[a]	1.87[a]	38.80[b]	19.67[c]	90.00[b]
P18	116.33[c]	103.67[b]	14.00[c]	20.33[b]	96.00[c]	8.33[a]	2.00[a]	36.47[b]	19.30[c]	76.00[b]
P19	114.00[c]	104.00[b]	18.33[c]	22.00[b]	97.67[c]	9.00[a]	2.00[a]	36.33[b]	19.00[c]	87.67[b]
P20	118.00[c]	102.00[b]	15.00[c]	20.33[b]	115.00[c]	9.67[a]	1.97[a]	36.67[b]	22.00[c]	81.67[b]
P21	116.00[c]	98.00a[c]	13.33[c]	24.67[b]	179.67[c]	8.33[a]	2.00[a]	41.33[b]	21.00[c]	82.00[b]
P22	115.33[c]	98.00[ac]	14.33[c]	23.67[b]	185.00[c]	9.00[a]	1.97[a]	41.00[b]	22.33[c]	80.33[b]
P23	121.67[c]	60.00[b]	10.33[c]	25.33[b]	196.67[c]	9.00[a]	2.03[a]	39.00[b]	22.00[c]	90.33[b]
P24	123.00[c]	95.33[b]	10.67[c]	24.00[b]	179.33[c]	10.00[a]	1.97[a]	41.00[b]	21.33[c]	81.00[b]
P25	115.00[c]	92.33[b]	11.00[c]	23.67[b]	173.33[c]	8.67[a]	2.10[a]	44.00[b]	20.00[c]	75.67[b]
P26	121.67[c]	96.33[b]	12.33[c]	20.00[b]	146.00[c]	9.67[a]	2.03[a]	40.00[b]	21.00[c]	81.00[b]
P27	118.00[c]	96.00[b]	12.33[c]	21.67[b]	155.33[c]	11.33[a]	1.97[a]	42.33[b]	20.00[c]	80.00[b]
P28	115.67[c]	95.33[b]	17.67[c]	22.00[b]	162.67[c]	10.00[a]	2.03[a]	36.00[b]	19.67[c]	83.67[b]
P29	116.33[c]	95.33[b]	14.33[c]	22.00[b]	157.00[c]	9.67[a]	2.00[a]	39.00[ac]	20.33[ab]	90.33[b]
P30	121.67[c]	95.33[b]	15.00[c]	22.33[b]	118.33[c]	10.00[a]	2.00[a]	40.33[b]	20.00[c]	77.67[b]
P31	115.00[c]	102.00[b]	15.67[c]	21.33[b]	109.33[c]	10.33[a]	1.97[a]	37.00[b]	18.33[c]	87.67[b]
P32	114.33[c]	97.67[b]	15.00[c]	22.00[b]	134.33[c]	11.00[a]	1.97[a]	36.67[b]	17.33[c]	79.33[b]
P33	118.33[c]	98.67[b]	13.00[c]	20.67[b]	114.67[c]	11.00[a]	1.97[a]	33.67[b]	16.67[c]	74.33[b]
P34	116.67[c]	98.67[b]	18.00[c]	23.00[b]	124.67[c]	11.33[a]	1.97[a]	36.67[b]	18.67[c]	72.33[b]
P35	119.00[c]	99.33[b]	16.33[c]	21.67[b]	121.33[c]	10.67[a]	1.93[a]	35.00[b]	17.33[c]	65.67[b]
P36	115.00[c]	95.00[b]	16.33[c]	22.00[b]	108.00[c]	10.67[a]	1.93[a]	38.33[b]	18.00[c]	54.33[b]
Mean	117.04	93.27	18	22.86	143.65	9.32	1.96	39.11	21.11	83.04
CV%	4.10	4.72	11.78	7.69	5.80	17.20	8.20	4.62	9.34	2.49

Table 3 Variability parameters of different biometrical traits in a BC$_2$F$_3$ population in rice.

Traits	Mean	MSG	MSE	σ2P	σ2G	PCV (%)	GCV (%)	h2B (%)	GA (%)	Skewness	Kurtosis
DOM	117.04±2.17	81.24	23.07	42.46	19.39	5.57	3.76	45.66	5.24	-0.191	-0.38
PH	93.27±9.37	388.52	19.37	142.42	123.05	12.79	11.89	86.40	22.77	-0.93	1.16
NOT	18.00±4.73	71.17	4.50	26.72	22.23	28.72	26.19	83.17	49.20	0.25	-0.742
PL	22.86±1.83	10.43	3.09	5.53	2.45	10.29	6.84	44.18	9.37	0.30	-0.71
FG	143.65±36.6	4124.92	69.39	1421.23	1351.84	26.24	25.60	95.12	51.42	0.36	-1.15
GL	9.32±0.50	3.65	2.57	2.93	0.36	18.37	6.42	12.23	4.63	0.37	-0.14
GW	1.96±0.04	0.03	0.03	0.03	0.00	8.24	0.88	1.15	0.20	-0.17	-0.58
Y	39.11±2.18	32.57	3.26	13.03	9.77	9.23	7.99	75.00	14.26	0.02	-0.35
SW	21.11±1.81	28.83	3.89	12.20	8.31	16.55	13.66	68.14	23.23	0.71	0.43
SS	83.04+10.85	331.12	4.29	113.23	108.94	12.81	12.57	96.21	25.40	-1.11	1.51

Data presented as Mean ± SE. Notes: Genetic variance (S2 g), error variance (S2 e), phenotypic variance (S2 P), genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), broad sense heritability(h2B), and genetic advance (GA).days to maturity (DOM, days), plant height (PH, cm), number of tillers per hill (NOT, n), panicle length (PL, cm), number of filled grains (FG, n), grain length (GL, cm), grain width (GW, cm), yield per hill (YH, g), 1000-seed weight (SW, g) and seed setting rate (SS, %).

3.2 Studies on the variability in BC$_2$F$_3$

The values of the mean, range, genotypic and phenotypic variance, phenotypic and genotypic coefficient of variance, heritability, genetic advance, and genetic advance as a percentage of the mean for ten different traits are shown in Table 3.

Day of maturity, plant height, grain width, and seed setting rate had negative skewness in this study, indicating double gene effects, while all other traits had positive skewness, indicating complementary gene effects. Similar results were previously reported by Raghavendra and Hittalmani (2015). Plant height, 1000-seed weight, and seed setting rate showed positive kurtosis, indicating gene interactions, while other yield and yield-related traits showed no gene interactions. Savitha and Kumari (2015) reported that plant height and 1000- seed weight showed positive kurtosis in rice. Moreover, Nurhidayah et al. (2021) reported the negative kurtosis values in grain width and gran length.

3.3 Phenotypic and genotypic variance

The highest phenotypic and genotypic variance was observed in the number of grains per panicle (1351.84% and 1421.23%, respectively), plant height (123.05% and 142.42%, respectively), and seed set rate (108.94% and 113.23%, respectively) while moderate phenotypic and moderate genotypic variance was observed in days to maturity (42.46% and 19.39%, respectively), plant height (142.42% and 123.05%, respectively), and number of shoots (26.72% and 22.23%, respectively). Low phenotypic and genotypic variance was observed in grain length (2.93% and 0.36%, respectively) and grain weight (0.03% and 0.00%, respectively). This study shows that phenotypic variation was

higher than genotypic variation for all studied traits, indicating a significant influence of environment on the expression of these traits. Previous rice studies have found higher phenotypic variation for various agromorphological traits in rice populations, including Bhandari et al. (2015); Adhikari et al. (2018); Herawati et al. (2021); and Mahesh et al. (2022).

3.4 Genotypic and phenotypic coefficient of variation

The GCV was lower than the PCV for all traits studied, suggesting that the observed variation was not only controlled by genotype but also influenced by environmental factors on trait expression. High GCV and high PCV were observed for the number of prickers (28.72% and 26.19%, respectively) and the number of grains per panicle (26.24% and 25.60%, respectively). High GCV and PCV levels were also observed in the number of grains filled as reported by Mallimar et al. (2015), Rashid et al. (2017), and Gyawali et al. (2018). In contrast, some researchers observed high GCV and PCV for the number of shoots per hill (Pandey and Anurag 2010; Sabri et al. 2020).

The moderate phenotypic and moderate genotypic variance was observed for 1000-seed weight (16.55% and 13.66%, respectively) and seed set rate (12.81% and 12.57%, respectively), indicating the possibility of improvement in these traits through further selection in segregating generations. The low phenotypic and genotypic variance was observed in days to maturity (5.57% and 3.76%, respectively), grain weight (8.24% and 0.88%, respectively), and yield per hill (9.23% and 7.99%, respectively). The results are in agreement with those of Habib et al. (2005) and Osman et al. (2012) for the same traits. In addition, Anis et al. (2016) and Nishanth et al. (2017) also reported a small difference in GCV and

PCV values for submergence tolerance among 20 rice cultivars and 525 germplasm lines, respectively, for some evaluated traits. In BC$_2$F$_3$ lines, the recorded variability parameters were very low because the strict selection was performed in BC$_2$F$_2$ generation and it was effective (Govintharaj et al. 2016). The current study showed that the variability in the studied traits is very broad.

3.5 Heritability and genetic advance

In this study, most of the traits had high heritability except for grain length and grain width, which had the lowest heritability percentage of 12.23% and 1.15%, respectively. High heritability and genetic advance percentage of mean were observed for the number of filled grains per panicle (95.12% and 51.42%, respectively) and seed setting rate (96.21% and 25.40%, respectively), indicating that the presence of additive gene action for trait expression can be improved by simple selection. Previous results of Akhtar et al. (2011) and Sohrabi et al. (2012) also confirmed the results of the present study.

Moderate heritability and moderate genetic advance as a percentage of mean were observed for plant height (86.40% and 22.77%, respectively), 1000-seed weight (68.14% and 23.23%, respectively), and the number of shoots (83.17% and 49.20%, respectively), indicating that simple selection would be effective in advancing the later generation. These results are consistent with Ghosh and Sharma (2012) for plant height.

On the other hand, moderate heritability and low genetic advance as a percentage of the mean were recorded for yield per hill (75.00% and 14.26%, respectively), indicating that environment influences phenotypic expression. Therefore, direct selection of these traits is less effective. Low heritability and genetic advance as a percentage of the mean were observed for grain width (1.15% and 0.20%, respectively), indicating that non-additive gene action has a large influence. The lowest genetic advance for grain width was also found in the study of Umarani et al. (2017) and Rashid et al. (2017).

3.6 Genetic diversity analysis

A total of 36 BC$_2$F$_3$ lines based on ten agromorphological phenotypic traits were classified into four main clusters, with 8 lines in the first cluster (designated C-A), 6 in C-B, 5 in C-C, 17 in C-D (Table 4 and Figure 3). The lines classified in cluster A had higher mean values for the majority of the studied characteristics. The B cluster had lower mean values than the C and D clusters for all characteristics except grain width. This study provides information to select better recombinants for different traits. Thus, this information could be used to generate greater variability in future rice breeding programs.

Table 4 Mean value of 10 quantitative traits for 4 groups constructed by cluster analysis

| Groups	Days of maturity	Plant height (cm)	Tiller number/hill	Panicle length(cm)	Filled grain (%)	Grain length	Grain width	Yield /hill (g)	1000 seed wt (g)	Seed setting rate (%)
Cluster A	117.46±3.41	84.33±13.29	18.37±6.62	23.12±1.90	174.79±26.38	8.79±0.68	1.98±0.05	40.46±2.19	23.47±2.13	88.46±5.84
Cluster B	117.11±38.89	95.00±28.46	15.53±4.47	22.73±7.35	146.45±48.55	9.59±3.22	1.99±0.66	40.26±13.20	20.80±6.45	73.59±26.52
Cluster C	115.22±26.10	87.62±21.49	20.77±7.60	23.80±5.86	171.64±53.87	9.87±2.41	1.97±0.44	40.30±9.56	20.93±6.93	83.14±23.54
Cluster D	116.92±2.53	98.88±2.89	15.71±1.74	21.88±0.66	122.17±17.14	10.38±0.75	1.98±0.02	36.83±2.02	19.42±1.26	79.38±8.35
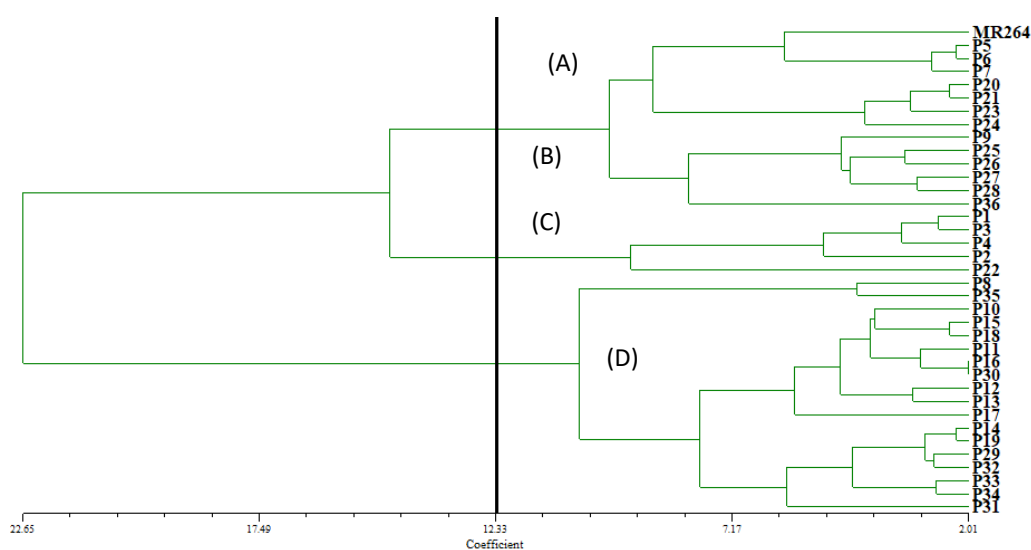
Figure 3 Dendrogram generated by cluster analysis of agro-morphological among 36 BC$_2$F$_3$ rice lines

Table 5 Principal components for rice lines based on ten characters

| Variable	PC1	PC2	PC3
Eigenvalue	3.91805724	1.99821384	1.19673420
Variation (%)	39.18	19.98	11.97
Cumulative (%)	39.18	59.16	71.13
Characters	Latent vectors (loading)		
1000 seed wt (g)	0.81010	0.42638	0.00590
Yield /hill (g)	0.79555	-0.34195	0.03248
Filled grain (%)	0.78954	0.23863	0.33615
Seed setting rate (%)	0.58326	0.12315	-0.55082
Tiller number/hill	0.55668	0.29005	-0.14776
Plant height (cm)	-0.87142	0.10121	-0.08219
Days of maturity	-0.47634	0.71528	0.14880
Panicle length (cm)	0.47040	0.49931	0.32276
Grain width	0.37766	-0.84407	0.03253
Grain length	-0.07266	-0.24414	0.78949
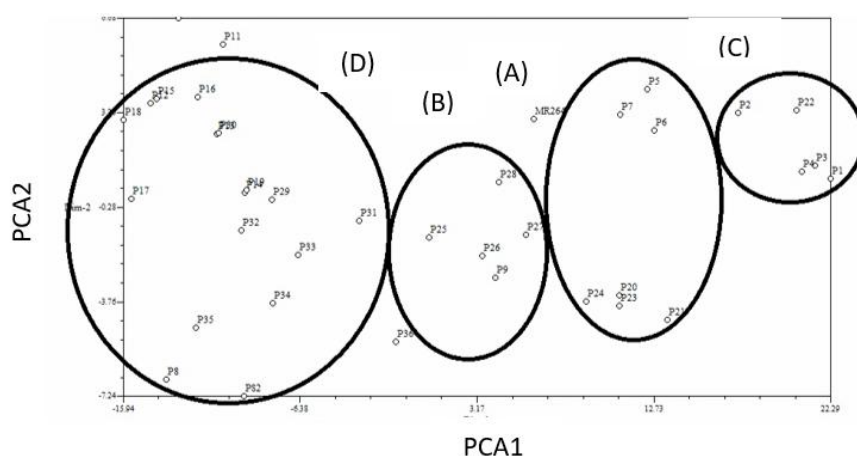
Figure 4 Graphical representation of the PCA analysis among 36 BC$_2$F$_3$ rice lines for identification of the genetic diversity and the traits responsible for the main source of variability.

3.7 Principal component analysis (PCA)

Principal component analysis (PCA) is a powerful tool and the most basic statistical technique for multivariate data reduction. The results of PCA showed that all three principal components were significant (eigenvalue > 1) and accounted for 71.23% of the total variation (Table 5 and Figure 4). PC1 accounted for 39.18%, PC 2 for 19.98%, and PC3 for 11.97% of the total variation. PCA revealed the same grouping pattern as in the cluster analysis, indicating that there was significant variation in this study. Partitioning of PC 1, PC 2, and PC 3 showed that 36 lines were scattered in all quarters, indicating a high degree of genotypic variation among lines. Lines 5, 6, 7, 20, 21, 23, 24 and traits such as 1000 seed weight, number of filled grains, and panicle length showed greater variation than any other traits. An almost parallel finding was also made by Manohara et al. (2020) and

Okasa et al. (2022), who found about 68.4% and 83.46% of the total variation, respectively, in all the traits studied and a high correlation among them.

3.8 Interrelations among the morphological traits

Knowledge of the correlation between different traits is crucial for developing an implicit breeding strategy for any crop. Yield traits are quantitatively inherited and influenced by genetic effects and genotype-environment interaction. Therefore, direct selection for yield development can be complicated and tedious, and sometimes the key trait is expressed late. For some reason, the indirect selection is much easier and preferable. Accordingly, it is reasonable to determine and use highly correlated traits (Ahmadikhah et al. 2008; Silva et al. 2016).

Traits	DOM	PH	NOT	PL	FG	GL	GW	Y	SW	SS
DOM	1									
PH	0.307*	1								
NOT	-0.261*	-0.416*	1							
PL	0.081	-0.228*	0.254*	1						
FG	-0.049	-0.652*	0.241*	0.319*	1					
GL	0.0175	-0.023	-0.048	-0.072	0.055	1				
GW	-0.314*	-0.249*	-0.042	-0.177	0.121	-0.006	1			
Y	-0.445*	-0.542*	0.264*	0.196*	0.481*	0.025	0.290*	1		
SW	-0.132*	-0.566*	0.545*	0.300*	0.682*	-0.039	0.026	0.439*	1	
SS	-0.170	-0.482*	0.385*	-0.042	0.408*	-0.143	0.0557	0.236*	0.428*	1
Indicator	-1	-0.8	-0.6	-0.4	-0.2	0.2	0.4	0.6	0.8	1

Figure 5 The phenotypic correlations among the traits.

Note: ∗significant at 0.05 probability level. Strength of a particular association between two traits is indicated by the color assigned to a point in the heat map grid. Positive correlation is indicated by red, while negative correlation is indicated by blue

Pearson's correlation coefficient was estimated for 10 morphological traits in 36 BC_2F_3 lineages (Figure 5). Most of the traits showed a positive correlation. A positive and strong correlation was found between filled grain per panicle and 1000-grain weight (r=0.6821), followed by the number of shoots and 1000-grain weight (r=0.5456). The results are in agreement with the study of Nor et al. (2013), which demonstrated a positive and strong association between filled grain per panicle and 1000-grain weight in F_1 rice lines from MR264 x PS2. These traits also showed moderate to high heritability and genetic advantage as a percentage of the mean. Therefore, the results suggest that 1000-grain weight and number of grains per panicle are important yield-enhancing traits, and selection based on these traits would be most effective.

Yield hill^{-1} was highly significant (P ≤ 0.05) and positively correlated with the number of filled grains panicle^{-1}(0.4819), 1000-grain weight hill 1 (0.4393), seed set rate (0.2360), and the number of panicles hill^{-1} (0.1964). The seeding rate was significantly positively related to the number of tillers, yield hill^{-1}, seeding rate, 1000-grain weight, and the number of filled grains. This study was also supported by Kumar & Singh (2018) and Zarbafi et al. (2019). This shows that traits other than filled grains per panicle and 1000-grain weight can be used for direct selection. The significant phenotypic correlation between traits was due to genetic cause, possibly pleiotropic effect rather than linkage between genes affecting different traits (Pandey et al. 2012).

A significant negative correlation was also observed between plant height and yield (-r= 0.5420), between plant height and 1000-grain weight (r=-0.5420), between filled grain and plant height (r=-0.6525), and plant height and days to maturity (r=-0.4453), which was also reported by Kumar & Singh (2018).

Conclusion

Marker-assisted backcrossing plays an important role in generating genetic variation and creating lines with superior traits. The significant correlation between the number of grains per panicle, grain weight per panicle, and the number of planters indicated that simultaneous selection for these traits would be very effective. The multivariate approach used in this study could help growers better select lines that should be recommended for release based on their high yields. The combination potential of the high-yielding line from cluster A could be further investigated. Thus, the observed genotypes can be crossed to produce desirable traits with higher heterotic potential.

Acknowledgments

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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