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PERFORMANCE OF YIELD AND YIELD CONTRIBUTING CHARACTERISTICS OF BC_2F_3 POPULATION WITH ADDITION OF BLAST RESISTANT GENE

Desempenho produtivo e características de produção de populações de BC_2F_3 com gene de resistência a brunose

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ABSTRACT

The study was carried out in the University Putra Malaysia (UPM) Rice Research Centre to evaluate the yield performance of newly developed selected blast resistant plants of BC_2F_3 generations derived from a cross between MR263, a high yielding rice variety but blast susceptible and Pongsu Seribu 1, donor with blast resistant (*Pi-7(t)* and *Pi-d (t)1, Pir2-3(t)* genes and *qLN2 QTL)*, Malaysian local variety. On the basis of assessed traits, the plants 12, 6, 7, 5, 21, 22, 5, 26, 11, 8, 10, 13 and 15 had the higher yield, blast resistant and good morphological traits. More than 70% heritability was found in days to maturity, plant height, tiller numbers per hill, and panicle per hill, 80% heritability was found in filled grain and yield per hill and more than 90% heritability was found in grain length, grain width and seed weight. Cluster analysis based on the traits grouped 30 plants along with MR263 into seven clusters. According to PCA, the first four principal components account for about 69.3% total variation for all measured traits and exhibited high correlation among the characteristics analyzed.

Index terms: Yield; rice; fungi resistance.

RESUMO

O estudo foi realizado no Centro de Arroz da Universidade Putra Malásia (UPM), para avaliar o desempenho produtivo de plantas resistentes a brunose do arroz recém desenvolvidas de BC_2F_3 gerações derivadas do cruzamento entre MR263, uma variedade de arroz de alta produtividade, embora suscetível a brunose e Ponsu Seribu 1, doador com resistência a brunose [*Pi- 7 (t) e Pi-d (t) 1, Pir2-3 (t)* genes e *qLN2 QTL*], e também uma variedade local da Malásia. Com base nas características avaliadas, as plantas 12, 6, 7, 5, 21, 22, 5, 26, 11, 8, 10, 13 e 15 apresentaram o maior rendimento, resistência a brunose e boas características morfológicas. Mais de 70% de herdabilidade foram encontradas em dias para maturação, altura da planta, número de perfilhos por cova, e panículas por cova, 80% de herdabilidade foram encontradas em grãos e rendimento por colina e mais de 90% de herdabilidade foi observada em grãos, largura de grãos e peso das sementes. A análise de agrupamento com base nos traços agruparam 30 plantas, juntamente com MR263 em sete clusters. De acordo com o PCA, os quatro primeiros componentes principais responsáveis por cerca de 69,3% da variação total para todas as características medidas apresentaram alta correlação entre as características analisadas.

Termos para indexação: Rendimento; arroz; resistência fúngica.

INTRODUCTION

Rice is one of the most important staple food crops of the world and over half of the global population depends on it for their feed (Sasaki, 2005). The world population will grow up to 8.5 billion till 2030 and 9 billion in 2050 for that to feed this growing population about 40% more rice will be required (Singh et al., 2013). But unfortunately, its production is constrained by considerable number of diseases caused by various pathogens which include bacteria, fungi, viruses and nematodes. The fungus *Pyricularia oryzae* Cavara [synonym *Pyricularia grisea* Sacc. the anamorph of *Magnaporthe grisea* (Herbert) Yaegashi and Udagawa] causes blast disease of rice is one of the most destructive and wide spread disease (Jia et al., 2000; Latif et al., 2011a; Singh et al., 2012; Tanweer et al., 2015). Blast disease produces significant yield loss in many rice growing countries and up to 75 and 50% losses have been reported from India and Philippines, respectively. The management of rice blast disease by

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the use of resistant cultivars which has proved to be most economical and environment friendly (Castano et al., 1990; Haq et al., 2002; Chandra et al., 2007; Latif et al., 2011b). But, with the appearance of new virulent races of the pathogen, this resistant can break down/over-come. So in this purpose we developed blast resistant lines through marker assisted backcrossing. The present study aimed to evaluate the agronomic characteristics and yield contributing characters of selected homozygous blast resistant plants of BC_2F_3 population derived from cross between MR263, a high yielding rice variety and donor, Pongsu Seribu 1, Malaysian local variety.

Plant breeders still use the approach although this is an old method. Morphological characters are important for the preliminary assessment of genotypes (Hien et al., 2007). In order to evaluate variation among genotypes, grain morphology was also taken into account as vital factor (Mathure et al., 2011). Morphological and physiological characterization is a traditional and general approach for the determination of genetic diversity as well as variation (Hien et al., 2007). However, they play crucial role in the selection and utilization of proper parents in breeding program. Moreover, yield and yield contributing characters are very helpful through which overall performance of genotypes could be determined. In this study, 30 selected homozygous blast resistant lines with MR263 were assessed and characterized for morphological traits with a view to gather knowledge for the further utilization. The present study deals with the following objectives: (i) to study genetic variation among blast resistant lines by quantitative traits and (ii) to estimate broad sense heritability, genetic advance.

MATERIAL AND METHODS

Plant materials

The experiment was conducted at UPM Rice Research Center, Serdang, Selangor, Malaysia. Newly developed 30 homozygous blast resistant plants out of 400 were selected from BC_2F_2 population derived from cross between MR263, a high yielding rice variety but blast susceptible and donor, Pongsu Seribu 1, Malaysian blast resistant variety. These 30 homozygous blast resistant plants were carried out to regenerate BC_2F_3 for the evaluation of yield and yield contributing characters.

Breeding strategy

Pongsu Seribu 1 having *Pi-7(t)* and *Pi-d (t)1, Pir2-3(t)* genes and *qLN2* QTL were used as a donor for blast resistance. This donor was crossed with the recurrent parent MR263, a popular rice variety with elite agronomic traits and high yielding but susceptible to blast disease. The lines derived from cross between MR263 and Pongsu Seribu 1, were named as MR263-BR-3, MR263-BR-4, MR263-BR-13 and MR263-BR-26. The carrying resistant 22 F_1 out of 100 plants were backcrossed with MR263 to produce the BC_1F_1 seeds. 42 heterozygous plants out of 200 were selected using the gene linked markers RM5961 and RM263 to identify the *Pi-7(t)* and *Pi-d (t)1, Pir2-3(t)* genes and *qLN2* QTL in BC_1F_1 generation as foreground selection. The four plants carrying resistance gene with highest resembling to recurrent parent were backcrossed to produce BC_2F_1 seeds independently in each of the backcrosses. 35 heterozygous plants out of 300 were selected using the same link markers (RM5961 and RM263). Foreground and phenotypic selections were carried out to select four best plants from each backcross to generate BC_2F_3 progenies following pedigree selection. The total scheme of work was shown in Figure 1.

Experimental design and management practices

From each plants, seeds were grown in plastic pots (28 cm x 25 cm) with randomized complete block design (RCBD) with 5 replications. MR263 variety was used as a control as a high yielding variety. We would like to mention here that only one seedling was transplanted in each pot. Management practices were done by Malaysian Agricultural Research and Development Institute (MARDI) recommended manual.

Molecular marker based screening of homozygous seedlings

Genomic DNA extraction

To extract genomic DNA, young and fresh leaves were collected of 4-week-old individual plants using the CTAB (Cetyltrimethylammonium bromide) method modified from Doyle and Doyle (1990) and Ashkani et al. (2013). About 1g of fresh leaf tissue was ground in liquid nitrogen and taken into a 2 ml micro centrifuge tube and then combined with 1000µl of CTAB buffer [100 mM Tris-HCl pH8.0, 20 mM EDTA (ethylenediaminetetraacetate) pH 8.0, 1.4 mM NaCl, 2% (w/v) CTAB, 2% (w/v) PVP (polyvinylepyrrolidone) and ß-mercaptaphenol] and 3 µl mercapethanol. The solution was then allowed to centrifuge at 500 rpm at

65 ºC for one hour (60 minutes) by gently shaking the tubes at 10-minutes interval to mix appropriately. Finally the whole samples were centrifuged at 13,000 rpm for 5 minutes to precipitate solid parts of the cell. Afterwards, the total amount of supernatant was transferred into a new 2 ml eppendorf tube. Then equal volume of chloroform:isoamyl alcohol (24:1, v/v) was added in each sample and the tube was gently inverted to homogenize the mixture and centrifuged at 13,000 rpm for 5 minutes to precipitate polysaccharides. The upper phase was then transferred into a new 2 ml eppendorf tube excluding green and white lower phase and again incorporated with 600 µl cold isopropanol and mixed gently by inverting the tube at least 50 times. All samples were then incubated at -20^{0}C for 30 minutes. After that it was centrifuged for 10 minutes at 13,000 rpm to get DNA pellet. Carefully pipetted off the supernatant without losing the pellet and let the pellet air-dried. The DNA pellet was then washed and dry out 1-2 times adding 600 µl of 4 ºC 75% ethanol until white DNA fiber appeared. Finally 50 µl of TE buffer (10 mM tris-HCl pH 8.0, 1 mM EDTA pH 8.0) and 1 µl of RNase (DNase free) were added and mixed by finger overtaxing to dissolve DNA completely and incubated at 37 ºC for 1 hour to break down RNA if any was present in the isolated DNA.

DNA identification and quantification

One µl of each DNA sample was put on NanoDrop spectrophotometry (ND-1000, NanoDrop Technologies Inc., Wilmington, DE, USA) and relative purity with concentration of the extracted DNA were estimated from the computer displayed data value. The final concentration of each DNA sample was diluted with 1xTE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0) to get required concentration and maintained in a refrigerator of -20 °C for PCR analysis.

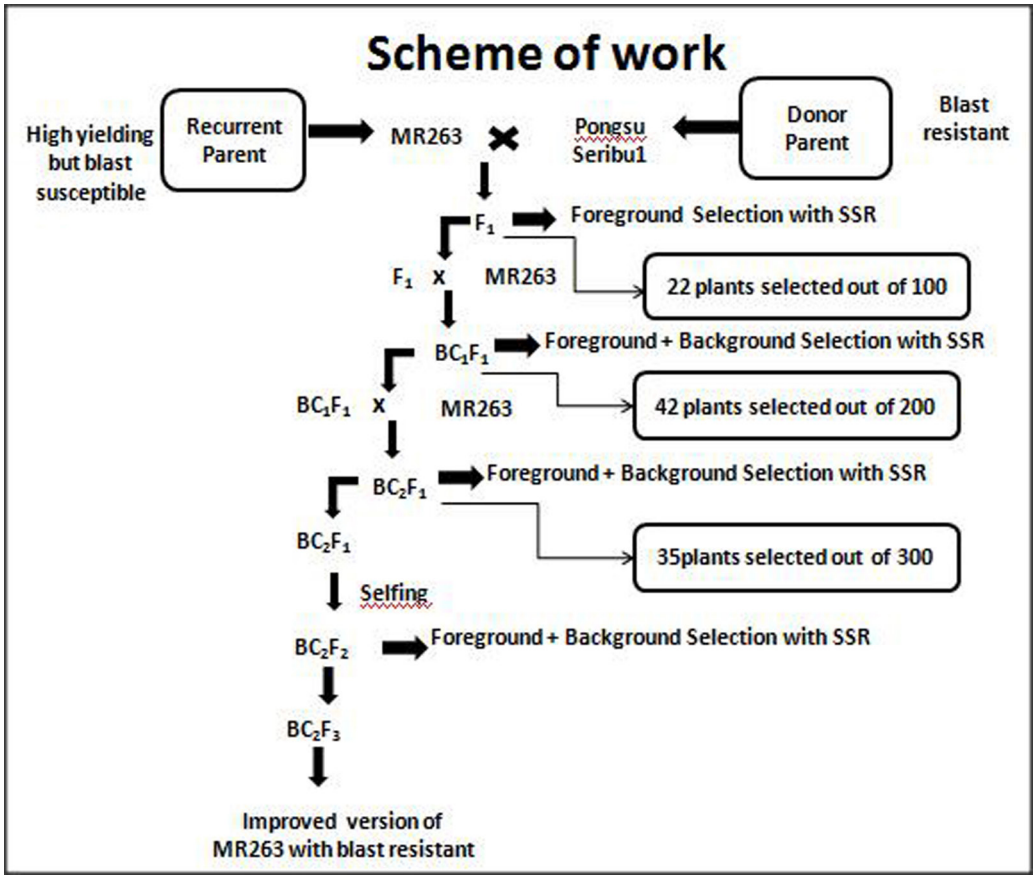

Figure 1: Schematic representation of development of blast resistant rice variety through marker assisted backcrossing (MABC).

Polymerase chain reaction (PCR)

Genotype data was obtained by analyzing DNA with SSR markers using 15 µL PCR reactions containing 1 µl DNA 2 µl of forward and reverse primer, 7.4 µl Master mix and 4.6 µl of water. Using an Eppendorf single or dual 96-well thermal cycler. After initial denaturation for 5 min at 94 °C, each cycle comprised 1 min denaturation at 94 °C, 1 min annealing at 55 °C, and 2 min extension at 72 °C with a final extension for 5 min at 72 °C at the end of 35 cycles. The PCR products were loaded to well and were analyzed by electrophoresis on 3% metaphor agarose gel using horizontal Thermo Scientific Gel Electrophoresis tank. The gels were documented using Alpha Imager 1220 (Alpha Innotech, CA, USA). Microsatellite or Simple sequence repeat (SSR) markers were used for the rice lines selection (Temnykh et al., 2001; McCouch et al., 2002; Ashkani et al., 2011).

Data collection

Data were recorded on 10 quantitative characters of vegetative growth, grain qualities, yield and its attributes *viz.* days to maturity, plant height, number of tillers per hill, panicles per hill, panicle length, percentage of filled grain, grain length, grain width, yield per hill and 100-seed weight were measured.

Statistical analysis

The analysis of variance was done with the help of SAS 9.2 (2013) and the mean differences were adjudged by Tukey test at 5% level of significant.

Genetic parameters were estimated to determine genetic variation among genotypes and to assess genetic and environmental effects on various traits. Some formulas were given in order to calculate genetic parameters (Johnson et al., 1955).

Cluster and principal component analysis

Multivariate statistical analysis, such as cluster and PCA were used to assess the genetic diversity of quantitative traits using unweighted pair group method with arithmetic means (UPGMA) following the methods described by Mazid et al. (2013).

In this experiment, data were analyzed based on Euclidian distance method, Dice's and Jaccard's similarity coefficient using NTSYS-pc software (version 2.1). In order to determine genetic relationships among the rice genotypes the UPGMA algorithm and SAHN clustering were applied. The PCA of 41 rice accessions was calculated by EIGEN and PROJ modules of NTSYS-pc and Minitab software (version 15).

RESULTS AND DISCUSSION

Results have been presented in Tables 1 to 5 and Figures 2 to 7. Since interaction effect was not significant only main effect has been explained.

Molecular Marker based homozygous plants selection

Newly developed 30 homozygous blast resistant plants out of 400 were selected from BC_2F_2 population derived from cross between MR263, a high yielding rice variety but blast susceptible and donor, Pongsu Seribu 1, blast resistant, Malaysian local variety. These 30 homozygous blast resistant plants were also confirmed using gene linked markers (RM263 and RM5961) at BC_2F_3 (Figures 2 to 5).

Many blast-resistant varieties have been developed through conventional and molecular breeding. However, conventional breeding requires approximately ten years or more to develop a new variety from beginning to release. Many blast resistance genes (*Pi*) have been successfully introgressed through marker-assisted backcrossing (MABC). For example, the Pusa1602 (PRR78+*Piz5*) and Pusa1603 (PRR78+*Piz54*) lines have recently been developed through the incorporation of the blast resistance genes *Piz-5* and *Pi54* that were derived from the donor lines C101A51 and Tetep into the background of PRR78 (highly blast susceptible) through an MABC breeding strategy (Singh et al., 2012; Hasan et al., 2015). The leaf blast resistance line D521, the neck blast resistance line D524 and bacterial blight resistance have been developed through the introgression of the leaf resistance gene *Pi1*; the neck blast resistance gene *Pi2* derived from the donor BL122 into an elite, early-maturing maintainer line of hybrid rice disease susceptible to blast; and Ronfeng B hybrid rice through marker-assisted backcross breeding programs, respectively (Fu et al., 2012). In this study, our objective was to introgress blast resistant *Pi* genes (*Pi-7(t)* and *Pi-d (t)1, Pir2-3(t)* genes and *qLN2* QTL) from Pongsu Seribu 1, into the background of a popular and mega Malaysian rice variety, MR263 susceptible to blast through MABC and also to evaluate the agronomic performance of improved genotypes.

Analysis of agronomical recorded data

Days to maturity

Analysis results revealed significant variation in days to maturity with the highest in plant 11 (109 days) followed by plant 10 (105 days), 7 (104 days) and 29

(103 days) respectively, and the lowest days to maturity (97 days) was observed in plant number 3 (Table 1). The remaining progenies were observed less days to maturity than MR263 (103 days). Days to maturity in plant number 6 (~103 days) was observed very similar to MR263 (103 days) Table 1.

Table 1: Yield and yield contributing characteristics of 30 selected homozygous blast resistant plants.

Progeny	Days to maturity	Plant Height (cm)	Tiller number/ hill	Panicles / hill	Panicle length (cm)	Filled grain (%)	Grain length	Grain width	Yield /hill (g)	100 seed wt (g)
MR263	103a-c	66.74a-d	28.8a-d	32.2ab	25.77a-d	70a-c	10.22a	2.1a	61.39ab	2.63a
P1	99.4bc	61.98d	28.6a-d	28.4a-c	22.48cd	72.2a-c	10.21a	2.1a	54.67a-c	2.62a
P2	100bc	64.14a-d	25.8a-d	25.8a-c	24.03a-d	62a-c	10.22a	2.1a	53.92a-c	2.65a
P3	96.8c	62.74cd	27.8a-d	27.6a-c	20.57cd	63.4a-c	10.21a	2.1a	51.35a-c	2.65a
P4	101.2a-c	63.24b-d	25.2a-d	25.2a-c	22.55cd	67.6a-c	10.21a	2.1a	51.4a-c	2.64a
P5	99bc	67.58a-d	25.2a-d	25.2a-c	21.86cd	74.2a-c	10.22a	2.1a	57.24a-c	2.63a
P6	102.8a-c	65.9a-d	27.2a-d	27.2a-c	24.58a-d	58.2c	10.21a	2.09a	59.27a-c	2.64a
P7	104.4a-c	72.26a-d	33.6a-c	32ab	22.69b-d	67.8a-c	10.22a	2.09a	56.04a-c	2.64a
P8	103.2a-c	68.06a-d	23cd	23bc	25.29a-d	70.8a-c	10.21a	2.09a	57.1a-c	2.62a
P9	98.4bc	65.54a-d	34.2a-c	33ab	25.42a-d	65a-c	10.21a	2.09a	53.79a-c	2.61a
P10	105.8ab	69.74a-d	28.4a-d	28a-c	25.41a-d	73a-c	10.22a	2.09a	51.04a-c	2.65a
P11	109.4a	62.5d	31.4a-d	30.6a-c	24.41a-d	72.6a-c	10.22a	2.09a	55.82a-c	2.57a
P12	100.6ac	63.88a-d	36.4a	35.2a	23.22a-d	71.4a-c	10.22a	2.1a	62a	2.58a
P13	98.4bc	66.5a-d	29.6a-d	29.2a-c	19.12d	70.6a-c	10.21a	2.1a	55.68a-c	2.62a
P14	97.2bc	65.36a-d	33.4a-c	33ab	22.6b-d	66a-c	10.21a	2.1a	51.24a-c	2.65a
P15	101.00a-c	66.4a-d	32.2a-d	32ab	23.7a-d	68.4a-c	10.22a	2.1a	54.84a-c	2.63a
P16	101.4a-c	64.82a-d	20d	20c	22.97a-d	68.8a-c	10.22a	2.09a	48.25c	2.62a
P17	103.8a-c	67.34a-d	26.4a-d	26.2a-c	21.99cd	74a-c	10.21a	2.09a	49.6bc	2.18a
P18	104.4a-c	63.88a-d	29.2a-d	28.8a-c	23.9a-d	69a-c	10.22a	2.09a	53.41a-c	2.61a
P19	100.4bc	68.3a-d	35.6ab	33.8ab	21.84cd	71.8a-c	10.21a	2.1a	56.65a-c	2.58a
P20	101.00a-c	65.36a-d	27.6a-d	27.6a-c	25.5a-d	59.6bc	10.21a	2.1a	53.76a-c	2.59a
P21	100.6a-c	78.28a	29.2a-d	28.2a-c	22.51cd	77.8a	10.22a	2.1a	57.78a	2.64a
P22	99bc	64.14a-d	28.2a-d	27.8a-c	23.88a-d	66.8a-c	10.22a	2.1a	53.1a-c	2.62a
P23	101.2a-c	67.34a-d	31a-d	30.2a-c	29.9ab	75ab	10.22a	2.1a	51a-c	2.61a
P24	100.6a-c	66.82a-d	26.4a-d	25.4a-c	20.68cd	68.2a-c	10.21a	2.09a	52.84a-c	2.61a
P25	98.4bc	67.12a-d	31a-d	30.4a-c	25.57a-d	67.6a-c	10.22a	2.09a	51.16a-c	2.62a
P26	97.2bc	64.3a-d	28a-d	28a-c	25.14a-d	74.2a-c	10.21a	2.1a	54.93a-c	2.63a
P27	101.00a-c	66.28a-d	27.8a-d	27.8a-c	24.75a-d	77.8a	10.22a	2.09a	49.79bc	2.65a
P28	101.4a-c	77.62ab	23.8cd	23.8bc	30.44a	72.2a-c	10.22a	2.09a	53.43a-c	2.61a
P29	103.8a-c	59.1d	29.2a-d	28.8a-c	22.86b-d	63.4a-c	10.22a	2.1a	50.16a-c	2.65a
P30	101.4a-c	77.37a-c	24.8a-d	27a-c	26.77a-c	73.4a-c	10.21a	2.09a	52.22a-c	2.62a
Mean	101.16	65.89	28.8	28.43	23.95	69.44	10.21	2.1	54.01	2.62
CV%	3.56	9.093	17.88	15.87	12.62	9.47	0.065	0.395	9.07	2.33

Means with different lower case letters are significantly different at $P<0.05$.

Table 2: Estimation of genetic variables of 10 morphological characteristics of 30 selected homozygous blast resistant plants.

Traits	Mean	MSG	MSE	σ^2_G	σ^2_P	PCV (%)	GCV (%)	h_B^2 (%)	GA (%)
Days to maturity	101.16	57.22	9.006	24.107	33.113	5.6884	4.85359	72.80	8.53105
Plant height	66.8027	97.18272	11.9	42.6414	54.5414	11.0553	9.77511	78.18	17.805
Tiller number/Hill	28.8	81.10666	12.543	34.2818	46.8248	23.76	20.3301	73.21	35.8344
Panicles/Hill	28.43	76.46	13.3754	31.5423	44.9177	23.5739	19.7547	70.22	34.1016
Panicle length (cm)	23.95	38.847	7.14	15.8535	22.9935	20.0215	16.6248	68.94	28.437
Filled grain	69.44	127.66	13.314	57.173	70.487	12.0905	10.8889	81.11	20.202
Grain length(mm)	10.21	0.000436	0.000018	0.00021	0.00023	0.14757	0.14159	92.07	0.27988
Grain width (mm)	2.1	0.000358	0.000008	0.00018	0.00018	0.64418	0.62994	95.62	1.269
Yield/ hill (gm)	54.01	91.489	9.004	41.2425	50.2465	13.1244	11.8905	82.08	22.1914
100 seed weight	2.62	0.00557	0.00014	0.00272	0.00286	2.0394	1.98877	95.09	3.99515

Notes: MSG – Genotype mean squares; MSE – Error mean squares; σ^2_G – genetic variance; σ^2_P – Phenotypic variance; PCV-Phenotypic coefficient of variation; GCV-Genetic coefficient of variation; h_B^2 – Broad-sense heritability; GA- Genetic advanced.

Table 3: Groups of 30 blast resistant plants with MR263 based on 10 yield and yield according to cluster analysis.

Groups	Plants
Cluster I	MR263, P18, P7, P11
Cluster II	P1, P26, P13, P3, P4, P5, P2, P22, P29, P6, P20, P8, P24
Cluster III	P9, P14, P12, P15, P19
Cluster IV	P10, P27, P16, P25, P23
Cluster V	P21
Cluster VI	P28, P30
Cluster VII	P17

Table 4: Mean values of 10 morphological characters for seven groups revealed by cluster analysis on 30 blast resistant plants with MR263 variety.

Groups	Days of maturity	Plant Height (cm)	Tiller number/ hill	Panicles / hill	Panicle length (cm)	Filled grain (%)	Grain length	Grain width	Yield /hill (g)	100 seed wt (g)
Cluster I	105.30	66.35	34.36	33.40	24.19	69.85	10.22	2.09	58.81	2.64
Cluster II	100.33	64.45	26.85	26.67	23.29	66.72	10.21	2.10	54.15	2.63
Cluster III	99.52	65.90	31.70	30.90	23.36	68.52	10.21	2.10	53.99	2.61
Cluster IV	101.56	67.06	29.48	28.92	25.72	72.44	10.22	2.09	50.25	2.63
Cluster V	100.60	78.28	20.00	20.00	22.51	77.80	10.22	2.10	57.78	2.61
Cluster VI	101.40	77.50	24.30	25.40	28.61	72.80	10.22	2.09	52.83	2.62
Cluster VII	103.80	67.34	26.40	26.20	21.99	74.00	10.21	2.09	49.60	2.18

Table 5: Eigenvectors and eigenvalues of the first four principal components.

Variables	PCA1	PCA2	PCA3	PCA4
Eigenvalue	2.5568	1.8401	1.4084	1.1182
Variation%	25.6	18.4	14.1	11.2
Cumulative%	25.6	44	58.1	69.2
Days to maturity	0.226	-0.47	-0.2	-0.445
Plant Height(cm)	0.455	-0.032	0.156	0.204
Tiller number	-0.478	-0.423	-0.056	0.136
Panicles /hill	-0.476	-0.418	-0.044	0.155
Panicle length (cm)	0.26	-0.333	0.234	0.203
Filled grain (%)	0.311	-0.134	-0.018	0.498
Grain length	0.12	-0.444	0.359	0.128
Grain width	-0.311	0.3	0.351	0.22
Yield /hill(gm)	0.026	-0.08	0.358	-0.598
100 seed wt(gm)	-0.102	0.039	0.703	-0.077

Plant height

The highest plant height (78.28 cm) was observed in plant number 21 followed by plant number 30 and 28 respectively and the lowest (59.1cm) was seen in plant number 29. They had highly significant difference. This result was in consistent to those of Mazid et al. (2013) who observed variable plant height among forty-one rice genotypes from various origins. Although the height of the rest of the plants was different but did not differ significantly and very close to the height of MR263. Meaning is that plant height of newly developed plants is near about same with MR263 (Table 1).

Number of tillers hill^{-1}

Significant variations were also observed for number of tillers hill^{-1} with the highest tiller number in plant 12 (36) followed by plant number 19 (35), 9 (34) and 7 (33) respectively and the lowest tiller number was recorded in plant number 16 (20). The rest of the progenies had different values but were statistically similar. It indicates that tiller number of new progenies is similar to MR263 (Table 1). Tiller is a unique character to rice production. With decreasing tillers hill^{-1}, yield will be decreased considerably. The same results were also observed in our present study. Mondal, Islam and Siddique (2005) also found significant differences in number of tillers hill^{-1} among the lines.

Panicle length

Increasing panicle length might have increased grain yield of rice indirectly by increasing panicle length. The highest panicle length was measured in plant 28 (30.44 cm) followed by plant 23 (29.9 cm), 30 (26.77 cm) and 25 (25.57 cm) respectively and lowest panicle length was recorded in plant 13 (19.12 cm) which were statistically differed. Similar results were also recorded by Idris and Matin (1990). The most of the progenies were statistically similar to MR263 (Table 1).

Panicles hill^{-1}

Highly significant variations were recorded in number of panicle per hill with the highest in plant number 12 (35) followed by plant number 19 (34), 14 (33) and 7 (32) respectively and the lowest tiller number was seen in plant number 16 (20). The rest of the progenies had different values but were statistically non-significant. It indicates that tiller number of new progenies is similar to MR263 (Table 1).

Percentage of filled grains

Number of filled grains panicle^{-1} is the most important yield attributing traits differed significantly among the progenies. Plant number 21 and 27 had the highest number of filled grains (77.8%) followed by plant number 23 (75%) and 5 (74.2%) respectively and the lowest filled grain was measured in plant number 6 (58.2%) which differed statistically. This result is also similar with the findings of Dutta, Mia and Khanum (2002).The remaining measured values of progenies were different but statistically non-significant (Table 1).

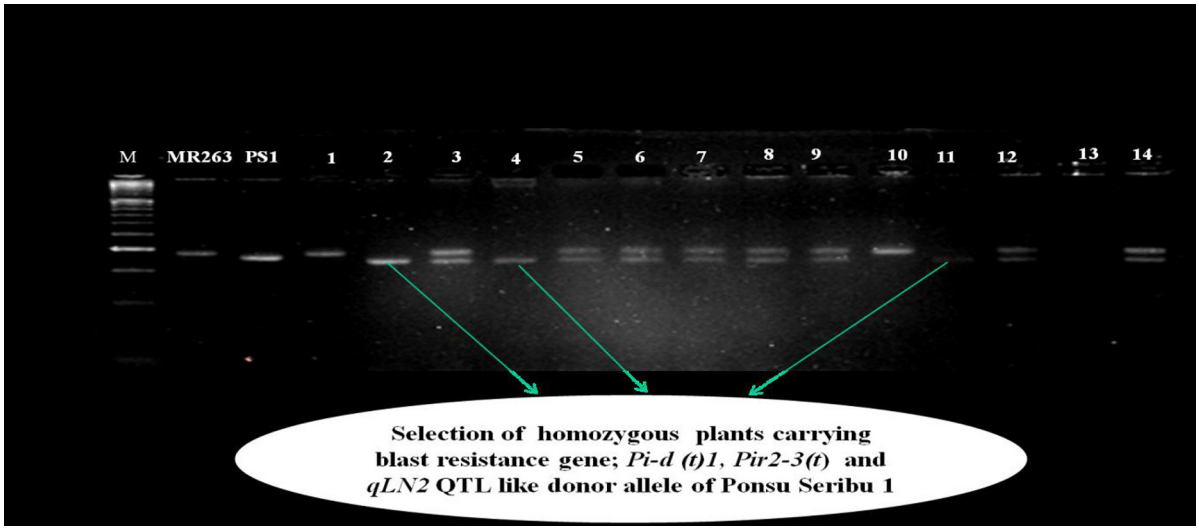

Figure 2: Selection of homozygous plants carrying blast resistant gene same as donor allele of Pongsu Seribu 1 (PS1) at BC_2F_2 population using gene linked marker RM263.

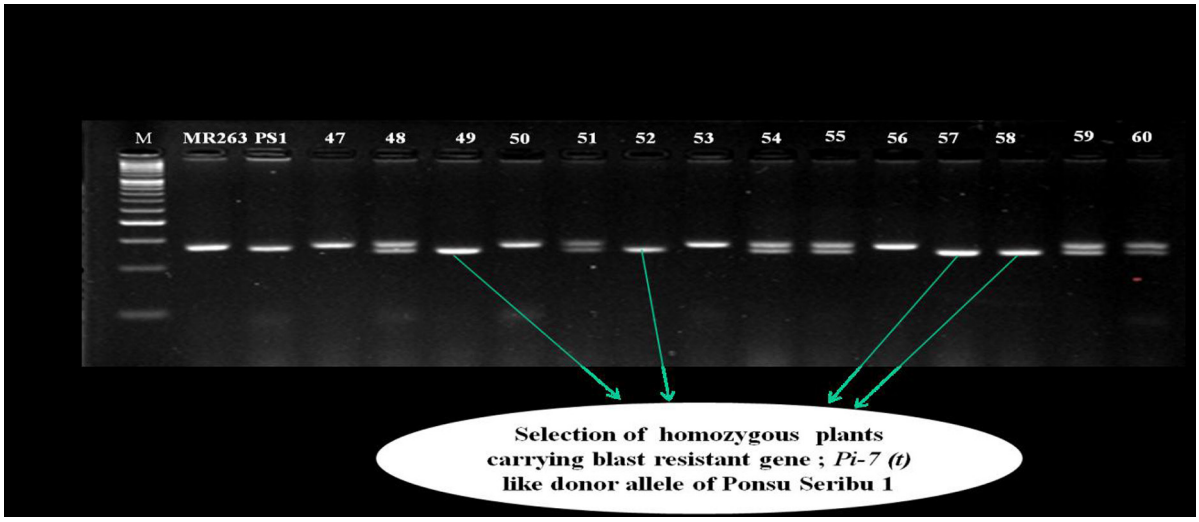

Figure 3: Selection of homozygous plants carrying blast resistant gene same as donor allele of Pongsu Seribu 1 at BC_2F_2 population using gene linked marker RM5961.

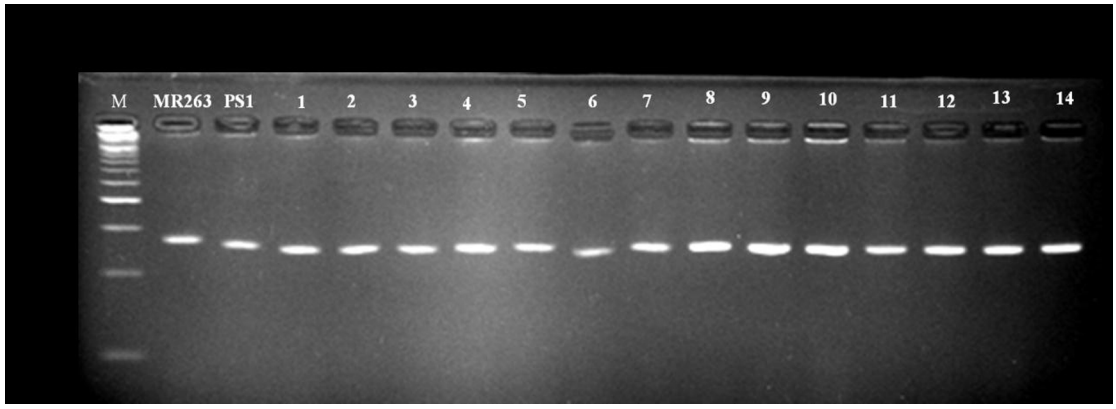

Figure 4: Marker assisted foreground selection at BC_2F_3 families for genes using gene linked marker RM5961. MR263- recurrent parent; PS1- Pongsu Seribu 1 (blast resistant parent) and M-50bp ladder.

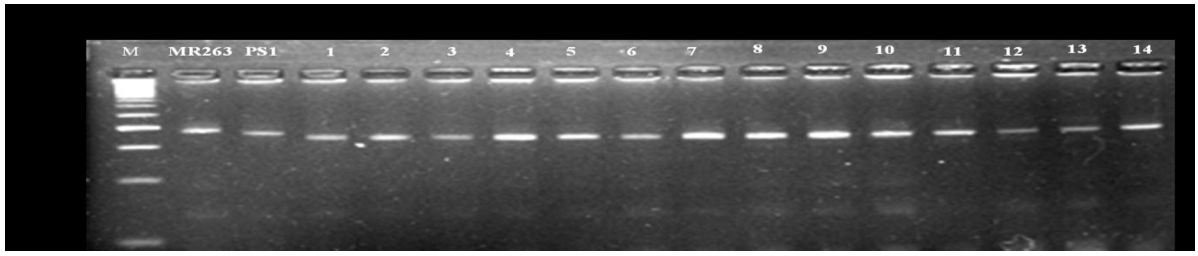

Figure 5: Marker assisted foreground selection at BC_2F_3 families for genes using gene linked marker RM263. MR263- recurrent parent; PS1- Pongsu Seribu (blast resistant parent) and M- 50bp ladder.

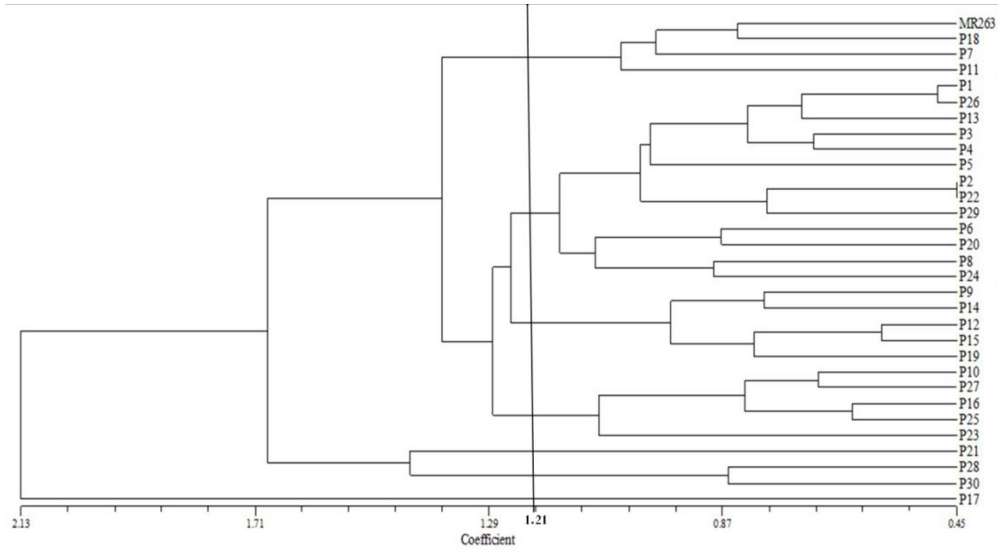

Figure 6: The dendrogram of 30 blast resistant plants with MR263 based on 10 measured yield and yield contributing characteristics.

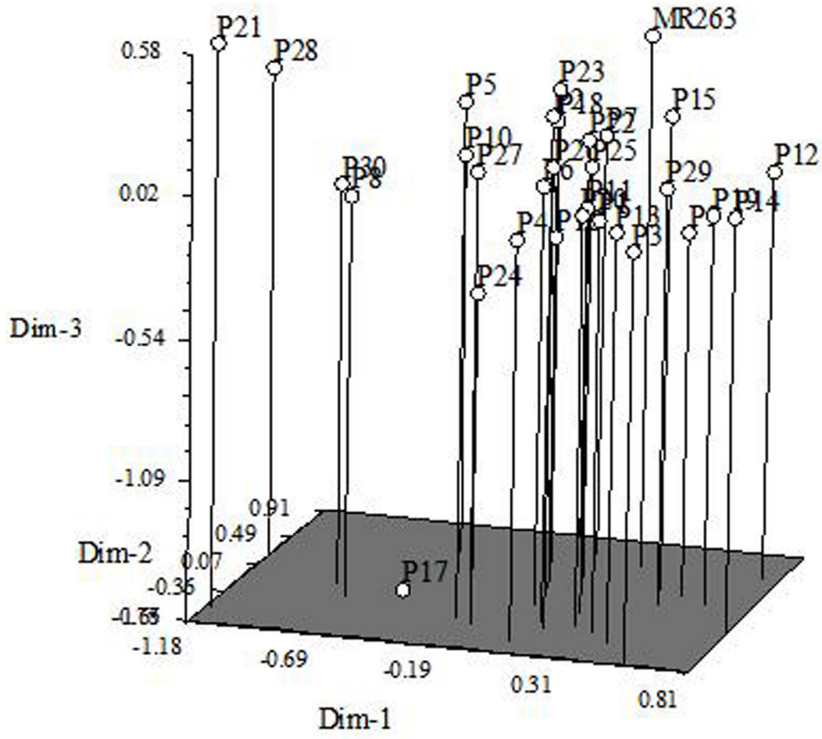

Figure 7: The 30 blast resistant plants with MR263 based on 10 morphological characteristics.

Grain length

Statistically non-significant differences were observed for grain length in MR263. It indicates that it is controlled by genetically (Ashraf; Khalid; Ali, 1999). Meaning is that the genetic components of newly developed lines have become same with parental lines, MR263 (Table 1).

Grain width

Grain widths were also non-significantly differed among all the progenies of MR263. It means that there was no environmental effect on seed widths (Ashraf; Khalid; Ali, 1999) (Table 1).

Grain yield per hill

Yield/hill had shown variability among the studied progenies. The highest yield/hill was recorded in plant 12 (62 g) followed by plant number 6 (59.27 g), 21 (57.78 g) and 5 (57.24 g) respectively and lowest yield/hill was observed in plant number 16 (48.25 g) which was statistically significant. This result agrees with the results of Dutta, Mia and Khanum (2002) and Mondal Islam and Siddique (2005). The rest of the progenies had different values but were statistically similar. It indicates that yield/hill of new progenies were near about similar with MR263 (Table 1).

100 seed weight (g)

Thousand-grain weight, an important yield determining component, is a genetic character least influenced by environment (Ashraf; Khalid; Ali, 1999). Results revealed that the 100 seed weight of all progenies had no significant difference. It means that seed weight did not affect by environmental factors (Table 1).

Phenotypic coefficient of variation (PCV), Genetic coefficient of variation (GCV) and the estimation of genotypic heritability

The estimation of genotypic, phenotypic and heritability for each and every trait was calculated and presented in Table 2. The level of variation was different among various quantitative traits (Table 2). The highest phenotypic coefficient of variation (PCV) was observed in number of tillers per hill (23.76%) followed by panicles per hill (23.57%), panicle length (20.02%) and yield per hill (13.12%) respectively while the lowest value was

recorded in grain length (0.15%). The highest genetic coefficient of variation (GCV) was also noted for number of tillers per hill (20.33%) followed by panicles per hill (19.75%), panicle length (16.62%) and yield per hill (11.89%) respectively while the lowest value was recorded for grain length (0.14%). Some researcher observed high GCV and PCV for number of filled grains per panicle and yield (Akhtar et al., 2011; Zahid et al., 2006) Moreover, high GCV and PCV were also observed for number of tillers per hill (Pandey; Anurag, 2010; Habib et al., 2005). The results of high GCV as well as PCV were supported by Shahidullah et al., (2010) who found the same result in aromatic rice genotypes. High GCV and PCV for the traits like number of unfilled grains per panicle and total number of spikelet's per panicle were observed by Ghosh and Sharma (2012).

Among the traits, days to maturity, plant height, tiller numbers per hill, and panicle per hill showed more than 70% heritability (Table 2). Whereas more than 80% heritability was found in filled grain and yield per hill. The rest of the traits, grain length, grain width and seed weight showed more than 90% heritability. So it indicates that the more heritability, the more genetic transfer of the desired traits to the generation. Present study showed that these traits are easily inherited characters to the next generation as they possess high heritability and are less affected by environmental factors. High heritability was found in the present study for the traits such as yield per hill, total number of tillers per hill, panicles per hill, panicle length, number of filled grains per panicle, days to maturity, 100 grain weight. The results were mostly in accordance with the findings of Iftekharuddaula et al., (2001) who reported high heritability for days to maturity, number of filled grains per panicle and 1000 grain weight. Almost same result for days to maturity and number of filled grains per panicle was also reported (Akhtar et al., 2011). Approximately similar results were also recorded by Habib et al., (2005). High heritability for yield per hill was also in agreement with Pandey and Anurag (2010). High heritability for the following traits such as total number of spikelets per panicle, yield per hill, days to 50% flowering, flag leaf length, 1000 grain weight, were also supported by Ghosh and Sharma (2012). Moderate heritability for plant height was noticed in this study which got conformity with findings of Ghosh and Sharma (2012).

Cluster analysis

The standardized recorded data were employed to calculate the Euclidean distances among the 30 selected homozygous progenies along with MR263 variety and an UPGMA dendrogram was constructed using these values (Figure 6). In this dendrogram, 30 progenies along with MR263 based on 10 measured characteristics were grouped into seven clustered at 1.21 dissimilarity coefficients. In this case, cutoff point was set at 1.21 only for the convenience of discussion.

Cluster I, II, and III had namely 4, 13 and 5 plants and clusters IV, V, VI and VII had 5, 1, 2 and one plants respectively (Table 3). The highest and nearly highest yield and yield component traits such as number of panicles per hill, panicle length, yield per hill, 100 grain weight was under cluster I. Cluster V consisted of progeny with the highest average of plant height and filled grain %. Cluster III had the highest days to maturity. Cluster I expressed better yield performance than remaining clusters (Table 4).

The 30 selected homozygous blast resistant plants with MR263 grouped into seven clusters based on morphological traits at distant coefficient of around 1.21 which implies level of morphological diversity in the rice genotypes. Result of this assay unveiled the better resolution power of quantitative traits for grouping of the rice genotypes. On the basis of 18 morphological traits 58 rice varieties were clustered in to four groups in a study conducted by Ahmadikhah, Nasrollanejads and Alishah (2008). The genetic distance was approximately 0.75. In their study, a group consisted of merely one member and group B, C and D had 14, 20 and 23 members respectively. Moreover, 23 rice populations were clustered into 10 different groups based on 20 morphological traits. The last group was the largest comprising of seven members on the other hand the smallest groups such as one; two and seven had only one member (Veasey et al., 2008).

Principal component analysis

It was found from the principal component analysis that the similar genotypes were grouped together (Figure 7). Cluster analysis was mostly confirmed by PCA and three dimensional plot (3D) (Figure 7) evidences of it. According to PCA the first four principal components account for about 69.3% of total variation for all measured traits and exhibited high correlation among the characteristics analyzed (Table 5). The same way it was substantiated for physiological traits where first three PCs expressed 66.60% of total variation, with PC1 explaining 26.10% of the variation, PC2 21.90% and PC3 18.60% of the total variation. The first 10 principal components were accounted for 67% of total variation found by Caldo et al., (1996) in his study. This implied a strong correlation among traits which were studied. About 82.7% of total

variation among 32 upland rice varieties was also noticed (Lasalita-zapico et al., 2010) where almost 66.9% variation showed by PC1 and 15.87% by PC2. It was noticed from the eigen vectors analysis that 25.6, 18.4, 14.1and 11.2% variation of measured traits could be explained in respect by the first four principal components.

As stated before, PC1 depicted 25.6% of total variation (Table 5). Among the 10 morphological traits six traits were positively and four traits were negatively correlated to PC1. The positively correlated traits were days to maturity (0.226), plant height (0.455), panicle length (0.26), filled grain (0.311) grain length (0.12) and yield/hill (0.026). The negatively associated traits were tiller number/hill (-0.478), panicles per hill -0.476), grain width (-0.311) and 100 seed weight (-0.102). The first PC increases with the decrease of negatively correlated traits but with the increase of positively correlated traits. This indicated that rising in one trait among the positively correlated traits will influence the other to increase. The PC1 could be viewed as a measure of the quality of the positively correlated traits. Moreover, first PC most strongly correlated to tiller number (-0.478).

Similarly, PC2 showed 18.4% of total variation. Among 10 traits, 8 traits were negatively and rests of the traits were positively correlated in this component. The days to maturity (-0.47), plant height (-0.032), tiller number/hill (-0.423), panicles/hill (-0.418), panicle length (-0.333), filled grain (-0.134), grain length (-0.444) and yield /hill (-0.08) were the negatively correlated traits. On the other hand, grain width (0.3) and the 100 seed weight (0.039) were positively correlated traits. Thus, the PC2 decreases with the increases of positively correlated traits but the increases of negatively correlated traits. It could be stated from the correlation value that PC2 was mainly a measure of days to maturity (-0.47) which was most strongly associated to this principal component (Table 5).

The third PC explained 14.1% of total variation. It was observed that in the third PC, 6 traits were positively correlated which were plant height (0.156), panicle length (0.234), grain length (0.359), grain width (0.351), yield/hill (0.358) and 100 seed weight (0.703). The rest of the four traits were negatively correlated which were days to maturity (-0.2), tiller number/hill (-0.056) panicle number/hill (-0.044) and filled grain (-0.018). This PC rises with the rising of positively correlated traits but with the decline of negatively correlated traits. The PC3 could be said a measure of panicle length on the basis of correlation value (0.234) as it was most strongly correlated to this PC (Table 5).

The fourth PC described 11.2% of total variation which was the lowest among the 4 PCs. This PC consisted of 3 negatively as well as 7positively correlated traits. The negatively correlated traits were days to maturity (-0.445), yield/hill (-0.598) and 100 seed weight (-0.077). On the contrary, the 7 positively correlated traits were plant height (0.204), tiller number/hill (0.136), panicles/hill (0.155), panicle length (0.203), filed grain (0.498), grain length (0.128) and grain width (0.22). On the basis of correlation value number of tillers per hill most strongly and negatively correlated with this PC (Table 5).

CONCLUSIONS

After all analysis and discussions we conclude that most of the traits including number of filled grains , panicle length,100 grains weight, number of tillers per hill and number of panicles per hill revealed relation to the yield per hill. Several of the studied traits were highly heritable in present study as well as other studies conducted by different scientists from different countries of the world. The current study unveiled that yield per hill, total number of spikelet's per panicle and number of filled grains have high heritability and genetic advance which are regarded as essential for trait selection by the scientists. Therefore, this is a good prospect for rice breeder to select economically vital traits with little environmentally influenced errors. The selected 30 homozygous plants of BC_2F_3 showed homozygous in comparison with the recurrent parent MR263. The maximum progenies of BC_2F_2 families observed the similarity like MR263. The results revealed that the values of filled grains, grain length, grain width, yield per hill, grain weight, panicle length were higher or equal to MR263. Very few progenies showed less performance than MR263. It indicates that the measured traits of developed selected blast resistant plants tend to MR263.

Some better plants were identified from the cluster analyses based on measured traits. Some plants could be selected for future breeding program from the previous discussion based on evaluated traits. On the basis of assessed traits the following plants could be used as parents which might produce higher yield, blast resistant and good morphological traits having plants, such as plant number 12, 6, 7, 5, 21, 22, 5, 26, 11, 8, 10, 13 and 15.

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