Assessment of Genetic Variability and Generation of Blast Resistant Fine Aromatic Rice Hybrids Within Resistant and Susceptible Land Races

Zakiul Hasan MAA[1], Mamunur Rashid M[1], Arifuzzaman M[2]*, Momotaj Begum[1] and Dilruba Shahrin Kabir[3]

[1]Department of Plant Pathology, Hajee Mohammad Danesh Science and Technology University, Bangladesh

[2]Department of Genetics and Plant Breeding, Hajee Mohammad Danesh Science and Technology University, Bangladesh

[3]Department of Entomology, University of Chattogram, Bangladesh

*Corresponding author: Aifuzzaman M, Department of Genetics and Plant Breeding, Hajee Mohammad Danesh Science and Technology University, Dinajpur 5200, Bangladesh

Abstract

A research work was conducted for generating blast resistant fine aromatic rice through crossing technique using landraces viz., BRRI dhan 34 as susceptible, Kataribhog and Radhunipagol as resistant sources during June to December, 2017 at experimental farm of Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh. The assessing data were based on different disease parameters at different days after transplanting (DAT) and yield. In field study, BRRI dhan 34 exhibited the highest number of spots (68.40), percent leaf blast severity (11.73), number of death leaf (6.33), neck blast (2.20), node blast (6.20), collar blast (4.33), death panicle (2.07) and yield per plant (68.64). The genotypic variance, phenotypic variance, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability, genetic advance and percent genetic advance were estimated where the PCV values were higher than corresponding GCV values for all of the selected characters. In comparison, the highest heritability value (99.49%) was estimated for collar blast per plant. Number of spots, percent leaf blast severity and neck blast asserted significant and positive correlation with, node blast, collar blast, number of death panicle, spikelet per panicle and yield. Among the disease parameters node blast per plant showed positive direct effect (0.227) on yield. Number of spots revealed highest indirect effect on yield via node blast (0.164). Number of spot (-0.014), death leaf (-0.162) and death panicle (-0.015) exhibited with negative direct effect on yield. In crossing, F1 generations (7) were developed from the cross BRRI dhan 34 × Kataribhog and BRRI dhan 34 × Radhunipagol. It is suggesting that for developing disease resistant fine aromatic rice, the number of spots and percent leaf blast severity responded highly genetical transmissible character resulting highly positive significant correlation in yield contributing linkage.

Keyword: Blast; BRRI dhan 34; resistance gene; cross

Introduction

Rice is the seed of the grass species *Oryza sativa* (Asian rice). As a cereal grain, rice is the most important human food crop in the developing world, directly feeding as staple food to more than half of the world's population than any other crop. Nearly sixty percent of the world's population [1], of which, more than 3 billion people [2] relied on rice every day in Asia. In Bangladesh, the traditional type of fine aromatic rice landraces are Kataribhog, Dadkhani, Radhunipagol, Badshabhog, Chinigura, Jirakatari, Balam, Phillipine katari, BRRI dhan 34, Zotakatari, Tajmohol katari, Behula bashor, Kalijira etc which have a high market price and socio-cultural importance in Bangladesh [3]. Blast of aromatic rice caused by *Magnaporthe oryzae* is a major disease under low land irrigated systems because of the long duration of crop. It is always a threat that leads to the reduction of rice yield along with misery and economic loss in many rice-growing areas of the world. The most destructive disease of aromatic rice are leaf blast, neck blast, node blast and collar blast where different types of blast caused 40% yield loss in Dinajpur region [4]. BRRI dhan 34 produced highest

yield (3.2 t/ha) (BRRI, 1996) and is predominant in Dinajpur district as other high yielding fine aromatic rice. Now a day, BRRI dhan 34 is becoming highly susceptible to blast disease and causes significant yield losses (about 60% loss) and reduce the market value in about 50% region [5].

So, envisaging the present situation, it is necessary to transfer blast resistance genes from traditional resistant variety to BRRI dhan 34 to ensure higher yields with quality rice grain through breeding technique. Therefore, the present research work was undertaken for developing new progeny from crossing of blast resistance (Kataribhog and Radhunipagol) and susceptible genotypes (BRRI dhan 34) and to find out the different blast disease severity in the selected three germplasms.

Materials and Methods

Plant materials and experimental location

Three fine aromatic rice cultivars including BRRI dhan 34, Kataribhog and Radhunipagol were collected from Saidpur, Nilphamari to develop high yielding blast resistant fine aromatic rice line through crossing technique. The research work was carried out under the department of Plant Pathology at Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur from July/2017 to December/2017.

Experimental design and layout

The research plot was laid out in Randomized Complete Block Design (RCBD) with three replications. The research plots were designed by three replications with nine plots considering the measurement 2.0 m × 1.52 m for each plot along with row to row distance 61 cm, plant to plant distance 40 cm and the wide of in-between drains was 30 cm and.

Transplantation of seedlings

Seeds were sown in the seeding tray at 3 days intervals from each other land races in. After 7 days of transplantation, the raised bed with three blocks was prepared. Each block contained three plots.

Mechanism of crossing, emasculation and pollination

In the study, Radhunipagol and Kataribhog were used as donor parent of blast disease resistance genes where BRRI dhan 34 was used as elite parent. To develop F1 generation, five plants of each cultivar were transplanted to fifteen earthen pots and identified by using paper tagging, because the rate of successful crossing is higher in-vitro condition than the field. Emasculation was done at the evening of the previous day of crossing by clipping off top 1/3rd and bottom 1/3rd portions in the panicle of BRRI dhan 34 leaving the middle spikelet. Again, top 1/3rd portion was clipped-off from each spikelet in a slanting position. Then six anthers were removed and proper care was taken during emasculation for not to damage the gynoecium. To prevent contamination from the foreign pollen, the emasculated panicle was fully covered with a butter paper bag. For successful crossing, flower synchronization is very important. If each of the cultivars does not flower at the same time, successful crossing will not be accomplished. That's why seedlings of different

ages were transplanted. In case of pollination, at the next day morning (approximately at 9.00 AM), the bloomed panicle from the desired male parent was taken. The top portion of the butter paper bag which was cut to expose the panicle to disperse the pollen. After ensuring the abundant disbursement of pollen, the opened portion of the butter paper bag was closed. After ensuring pollination, the bag was removed.

Collection of data

The crossings among selected three cultivars were done in at flowering stage of synchronization. Data on different disease parameters such as number of death leaf per plant, number of spots per plant, percent leaf blast severity, neck blast per plant, node blast per plant, collar blast per plant, death panicle per plant and yield per plant.

Statistical Analysis

At first the data were plotted on MS excel sheet and analysed using the software package of R version 3.4.2. The data were subjected to statistical analysis for Randomized Block Design (RBD) as described by Panse and Sukhatme [6]. Genotypic and phenotypic components of variance were computed according to the formula given by Lush [7] and Chaudhary and Prasad [8]. Broad sense heritability was estimated as the ratio of genotypic variance to the phenotypic variance and was expressed in percentage [9]. Genetic advance (GA) was computed according to the formula given by Johnson et al. [10]. Simple correlation co-efficient (r) among different important characters of selected three fine aromatic rice cultivars were estimated with following formula Miller et al. [11], and Singh and Chaudhury, [12]. The path coefficient analysis was analyzed by using Wright's [13,14] formula as was described by Deway and Lu [15].

Results

Among three different fine aromatic rice cultivars viz. BRRI dhan 34, Kataribhog and Radhunipagol; the mean performances of seven characters were separated by DMRT test at 5% level of probability (Table 1). The highest number of spots (68.40) was observed in BRRI dhan 34 whereas the lowest (16.27) was in Radhunipagol. The highest percentage of leaf blast severity 11.73 was found in BRRI dhan 34 whereas the lowest (3.73) was in Radhunipagol. In case of number of death leaf per plant, BRRI dhan 34 had higher number of death leaf (6.33) over Kataribhog (5.87) and Radhunipagol (5.67). Neck blast is the major constraint for yield of rice out of other blast symptoms. The data on neck blast per plant was found higher in case of BRRI dhan 34 (2.20) and lower in case of Radhunipagol (0.33). But Kataribhog and Radhunipagol were statistically at par. The highest number of infected node (2.20) as well as collar (4.33) per plant due to blast was recorded at BRRI dhan 34 whereas Kataribhog and Radhunipagol showed the minimum infestation. The highest number of death panicle per plant (2.07) was estimated in BRRI dhan 34 and the lowest (0.40) was recorded in Radhunipagol. Regarding every traits, the decisive inference was that BRRI dhan 34 was more susceptible to blast disease over the other two cultivars.

Table 1: Mean performance of different disease parameters of selected three fine aromatic rice land races.

Parameters	BRRI dhan 34	Kataribhog	Radhunipagol	Range
Number of spots per plant	68.40 a	24.07 b	16.27 b	68.40-16.27
Percent leaf blast severity	11.73 a	5.73 b	3.73 b	11.73-3.73
Number of death leaf per plant	6.33 a	5.87 b	5.67 b	6.33-5.67
Node blast per plant	6.20 a	2.20 b	1.73 b	6.20-1.73
Neck blast per plant	2.20 a	0.47 b	0.33 b	2.20-0.33
Collar blast per plant	4.33 a	1.07 b	1.13 b	4.33-1.07
Death panicle per plant	2.07 a	0.53 b	0.47 b	2.07-0.47

Genetic variability

The genotypic variance (Vg), phenotypic variance (Vp), genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability, genetic advance (GA) and genetic advance as percent (GA%) of selected parameters were analyzed to evaluate the variability existed among the land races (Table 2). The highest Vg and Vp value (8001.23 and 8056.67, respectively) were found in number of spots per plant whereas the lowest (0.61 and 1.52, respectively) in number of death leaf per plant. Very high PCV and GCV value (396.45 and 395.43, respectively) were found for collar blast per plant and second highest PCV and GCV value (389.41 and 388.07, respectively) were found for number of spot per plant. The lowest PCV and GCV value (38.29 and 24.24, respectively) were found for collar number of death leaf per plant.

Table 2: Estimation of genetic variability parameters on different characters in selected three fine aromatic rice lines.

Characters	Vg	Vp	GCV (%)	PCV (%)	h2b (%)	GA	GA %
Number of spots per plant	8001.23	8056.67	388.07	389.41	99.31	18362.76	796.64
Death leaf per plant	0.61	1.522	24.24	38.29	40.08	101.86	31.61
Node blast per plant	39.54	52.72	272.92	315.14	75	1121.8	486.89
Neck blast per plant	5.25	5.43	229.13	233.02	96.67	464.04	464
Collar blast per plant	23.35	23.47	395.43	396.45	99.49	992.89	812.51
Percent leaf blast severity	313.34	319.94	356.38	360.11	97.94	3608.79	726.55
Death panicle per plant	3.79	4.09	190.49	197.88	92.67	386.07	377.76
Yield per plant	167.67	175.27	20.92	21.39	95.66	2608.86	42.15

Here, Vg= Genotypic Variance, Vp= Phenotypic Variance, GCV= Genotypic coefficient of variation, PCV= Phenotypic coefficient of variation, h2b=Heritability in broad sense, GA= Genetic advance.

The transmission ability and expressivity of the characters were envisaged through heritability estimates and corresponding genetic advance; thereby, heritability and genetic advance were estimated. The highest heritability value (99.83%) was estimated for plant height whereas the lowest heritability value (40.08%) was estimated for number of death leaf per plant. After all, the highest genetic progress was 812.51%for collar blast per plant and 796.64% for number of spots per plant.

Correlation among the disease parameters

To determine the component on which selection could be based for improvement of blast resistant fine aromatic rice genotypes under consideration, the correlated characters and the values of correlation co-efficient are presented in Table 3. Number of death leaf per plant showed significant and positive correlation with number of death panicle per plant (r=0.191*). Number of spots per plant asserted significant and positive correlation with percent leaf blast severity (r=0.640***), neck blast per plant (r=0.623***), node blast (r=0.719***), collar blast (r=0.754***), number of death panicle (r=0.629***) and yield per plant (r=0.574***).Percent leaf blast severity substantiated significant and positive correlation with neck blast (r=0.485***), node blast (r=0.556***), collar blast (r=0.480***), number of death panicle per plant (r=0.409***) and yield per plant (r=0.415**). Neck blast per plant exhibited significant and positive correlation with node blast (r=0.656***), collar blast (r=0.695***), number of death panicle per plant (r=0.377***) and yield per plant (r=0.601***). In case of node blast per plant, it showed significant and positive correlation with collar blast (r=0.784***), number of death panicle per plant (r=0.556***) and yield per plant (r=0.624***). In case of collar blast per plant, it asserted significant and positive correlation with number of death panicle per plant (r=0.517***) and yield per plant (r=0.629***). Number of death panicle per plant showed significant and positive correlation with yield per plant (r=0.427**).

Table 3: Correlation value of different disease parameters of selected three fine aromatic rice land races.

Characters	NSP	LBS (%)	NKB	NB	CLB	DPP	YPP
DLP	0.262	0.253	0.073	0.245	0.293	0.191*	0.006
NSP		0.640***	0.623***	0.719***	0.754***	0.629***	0.574***
LBS (%)			0.485***	0.556***	0.480***	0.409**	0.415**
NKB				0.656***	0.695***	0.377*	0.601***
NB					0.784***	0.556***	0.624***
CLB						0.517***	0.629***
DPP							0.427**

Path coefficient studies

The results of the path co-efficient analysis are presented in Table 4. Number of spots per plant exhibited negative direct effect (-0.014) with yield per plant. It had positive indirect effect via node blast per plant, neck blast per plant, collar blast per plant and percent leaf blast severity whereas had negative indirect effect with number of death panicle and number of death leaf per plant. Percent leaf blast severity exhibited positive indirect effect via node blast per plant, neck blast per plant and collar blast per plant.

Table 4: Path coefficient of different disease parameters on yield in three fine aromatic rice genotypes.

Characters	NSP	LBS (%)	DLP	NKB	NB	CLB	DPP	Total correlation with YPP
NSP	-0.014	0.007	-0.042	0.027	0.164	0.062	-0.009	0.574
LBS (%)	-0.009	0.011	-0.04	0.022	0.127	0.039	-0.006	0.415
DLP	-0.004	0.003	-0.162	0.003	0.055	0.024	-0.002	0.006
NKB	-0.009	0.006	-0.011	0.044	0.15	0.057	0.017	0.601
NB	-0.01	0.006	-0.039	0.029	0.227	0.065	0.127	0.624
CLB	-0.011	0.005	-0.047	0.03	0.177	0.083	0.043	0.629
DPP	-0.009	-0.006	-0.002	0.017	0.127	0.043	-0.015	0.427

Residual Effect (R) = 0.44

Legends: NSP = Number of spots per plant, LBS = Percent leaf blast severity, DLP = Number of death leaf per plant, NKB = Neck blast per plant, NB = Node blast per plant, CLB = Collar blast per plant, DPP = Death panicle per plant, YPP = Yield per plant.

Number of death leaf per plant had highly negative direct effect (-0.162) with yield per plant. Neck blast per plant had positive indirect effect via number of death panicle, node blast per plant, collar blast per plant and percent leaf blast severity. On the other hand, node blast per plant exhibited positive indirect effect via number of death panicle, neck blast per plant, collar blast per plant and percent leaf blast severity whereas negative indirect effect with number of death leaf per plant and number of spots per plant. Collar blast per plant showed positive indirect effect via number of death panicle, neck blast per plant, node blast per plant and percent leaf blast severity. It showed negative indirect effect with number of death leaf per plant, number of spots per plant. Number of death panicle per plant exhibited negative direct effect (-0.015) on yield per plant. It had positive indirect effect via neck blast per plant, node blast per plant and collar blast per plant but had negligible negative indirect effect with number of death leaf per plant, number of spots per plant and percent leaf blast severity. The effect of path coefficient values on yield particularly the disease parameter through the host pathogen interaction mechanism or resistance mechanism looks good, but more research is needed. Legends: NSP = Number of spots per plant, LBS = Percent leaf blast severity, DLP = Number of death leaf per plant, NKB = Neck blast per plant, NB = Node blast per plant, CLB = Collar blast per plant, DPP = Death panicle per plant, YPP = Yield per plant.

Number of successful and unsuccessful grain in crosses plant

The synchronization in flowering for crossing between donor and recipient parents were done at 76 DAT, 78 DAT and 81 DAT among Radhunipagol, Kataribhog and BRRI dhan 34, respectively. BRRI dhan 34 was an elite parent and it was crossed with the donor parents Kataribhog and Radhunipagol. In case of crossing between BRRI dhan 34 and Kataribhog, the total crossed plants were fifteen where successful pollinated panicle were three whereas the unsuccessful were twelve. On the other hand, crossing between BRRI dhan 34 and Radhunipagol, the total crossed plants were fifteen and successful pollinated panicle were four whereas the unsuccessful were eleven. From the above result it can be revealed that number of unsuccessful pollinated panicle were higher than the number of successful pollinated panicle (Table 5). It might be reason due to some genetic gap, genetic complex or multiple gene factors between recipient and donor plants.

Table 5: Number of successful and unsuccessful grain in crosses plant.

Cross	Total Number of Crosse Plant	Successful	Unsuccessful	Crosses percentage	
				Successful	Unsuccessful
BRRI dhan 34 × Kataribhog	15	3	1 2	20%	80%
BRRI dhan 34 × Radhunipagol	15	4	11	26%	74%

Discussion

The mean squares against three cultivars revealed significant differences for all the disease parameters (data not shown). The significant variations indicated ample variability in the characters and breeders could drive the breeding methods either selection or hybridization for the improvement of present yield status and degree of pathogenicity of the blast selected fine rice cultivars. Development of high yielding disease resistant varieties in almost every year by the rice breeders and commercial agencies is being culminated pertaining exploitable variability in the popular fine aromatic rice landraces that leads to erosion of these valuable rice germplasm. Research results concerning that the incidence of number of leaf spots per plant, percent leaf blast severity, number of death leaf, neck blast, node blast, collar blast and death panicle per plant were not only higher but also statistically different in BRRI dhan 34 than Kataribhog and Radhunipagol where Kataribhog and Radhunipagol were statistically identical. The results also suggested the occurrence of higher death leaf and higher number of spots significant association to susceptibility of the variety. It is absolutely clear that BRRI dhan 34 is highly susceptible to blast disease whereas Kataribhog and Radhunipagol are resistant to moderately resistant to this disease. The finding is also supported the results of Begum et al. [16] as they opined that BRRI dhan 34 is highly susceptible to blast disease.

In case of analysis of variance, significant variations were observed among the genotypes for all the characters studied except number of death leaf per plant. The mean squares of three replications were higher under number of spots per plant and percent leaf blast severity whereas lower under death panicle per plant. The genotypic mean squares of number of spots per plant, percent leaf blast severity, neck blast per plant, node blast per plant, collar blast per plant, number of death panicle per plant and yield per plant showed significant variations at 0.1% level of probability whereas number of death leaf per plant showed insignificant variation.

The phenotypic variances were greater than the genotypic variances and the estimated PCV values were also higher than corresponding GCV values. Very high phenotypic and genotypic coefficients of variation were found for collar blast per plant and second highest phenotypic and genotypic coefficients of variation were found for number of spots per plant whereas the lowest was found in number of death leaf per plant. High PCV and GCV were reported by Begum et al. [16] for number of spots per plant. The highest heritability value (99.49%) was estimated for collar blast per plant whereas the lowest heritability value (40.08%) was estimated for number of death leaf per plant. After all, the highest genetic progress was 812.51% for collar blast per plant and 796.64% for number of spots per plant. Begum et al. [16] estimated the highest heritability value ($h2$ = 99.95%) for number of spots per plant.

Number of spots per plant along with all other disease parameters asserted significant and positive correlation with percent leaf blast severity, neck blast, node blast, collar blast, number of death panicle per plant and yield per plant. The results from the correlation coefficient analysis of two variables, the disease components and yield indicated that they have statistically significant and positive relationship. Both variables run some extent positively which is interesting to note the erroneous conclusions. It may be hypothesized that, genetic uniformity in selected varieties corresponds against blast pathogen becoming a disadvantage or horizontal resistance conferred in complete resistance. A plant with horizontal resistance is never completely resistant or completely susceptible. Despite these, it was also indicated that, yield components along with many morphological as well as physiological characters with minor gene effect are affected when exposed to natural environment which can be resulted positive correlation.

In path co-efficient analysis, number of spots per plant showed negative direct effect with yield per plant. With the increasing of number of spots per plant, the amount of photosynthetic region will be gradually reduced. Ultimately, the amount of stored food for grain formation will also be reduced. Percent leaf blast severity exhibited positive direct effect with yield per plant. Begum et al. [16] also found positive direct effect on yield per plant. Number of death leaf per plant had highly negative direct effect with yield per plant. It showed positive indirect effect via neck blast per plant, node blast per plant, collar blast per plant and percent leaf blast severity. This trait exhibited negligible negative indirect effect with number of death panicle and number of spots per plant. Begum et al. [16] found positive direct effect on yield and positive indirect effect via number of spots per plant, percent leaf blast severity. On the other hand, node blast, collar blast and neck blast per plant exhibited positive direct effect on yield per plant.

These traits had positive indirect effect via number of death panicle, percent leaf blast severity and also via each other whereas negative indirect effect with number of death leaf per plant, number of spots per plant. The last trait i.e. number of death panicle per plant exhibited negative direct effect on yield per plant. It had positive indirect effect via neck blast per plant, node blast per plant and collar blast per plant but had negligible negative indirect effect with number of death leaf per plant, number of spots per plant and percent leaf blast severity. The interaction of the fine aromatic

rice- Pyricularia system indeed difficult to reveal that pathogenesis processes, generally at higher level of complexity is directly causal in determining yield. These disease effects on yield and their relations may limit application of genetic engineering techniques to breeding for production. However, sustainable higher yields in BRRI dhan 34 under Pyricularia stress is emphasized here and measurements of disease severity were conducted which would be further utilized in breeding program. Moreover, two pathological traits, number of blast spot and leaf blast severity look bemusing and require further investigation and protocol development for fine aromatic rice if they are to be incorporated into breeding programs.

From the output of crossing, it can be revealed that number of unsuccessful fertilized floret was higher than the number of successful fertilized floret due to lack of synchronization, unskilled improper error and some environmental factor. Failure of successful grain setting after crossing may be overcome through management of crossing as well. This is the first time of breeding studies, however it is postulated that fine aromatic rice breeding program against blast disease has had a great impact on national rice production.

Conclusion

Blast is a worldwide important disease of rice. Many researchers have been trying to manage the blast of rice with different tactics. The transmission of blast disease resistant genes to the susceptible cultivars may provide opportunities for breeders to develop high-yield, blast resistance fine aromatic rice landraces. The breeding program is the best route for identifying sources of resistance features of blast disease resistant variety development in agriculture sector.

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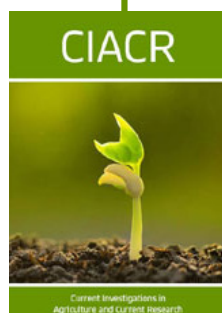
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