Genetic Studies on Enhanced Disease Reaction of Biparental Progenies (BIPs) to Blast Disease of Rice (*Oryza sativa* L.)

A. Mahalingam[1*], S. Robin[1] and R. Rabindran[2]

[1]*Department of Rice,* [2]*Department of Plant Pathology, Centre for Plant Protection Studies*
Tamil Nadu Agricultural University, Coimbatore- 641 003

Abstract

The relative effectiveness of intermating or biparental mating in generating superior segregants resistant to blast (*Pyricularia grisea*) in rice was studied with JGL 384 x Rasi cross combination. Under this investigation, parental lines (JGL 384, Rasi), 70 F_3 families and 32 intermated progenies (BIPs) were phenotypicaly screened for disease resistance along with checks (TN 1 and IR 50) by sandwich method. The donor parent Rasi was resistant (3.0) and recipient parent JGL 384 (7.0) was susceptible. Among 32 biparental progenies, 22 BIPs were found to be resistant (1.0 and 3.0) and 5 BIPs were moderately resistant to blast and BIPs had a mean score 3.19. In 70 F_3 families nearly 25 per cent were susceptible to blast and have a mean score of 4.02. None of the BIPs families had score 9. It showed that resistance was highly improved in BIPs than their F_3 families. BIPs exhibited superior mean performance than their parents, F_2 and F_3 generation for most of the economically important characters *viz.,* panicle length, number of productive tillers per plant, 1000 grain weight and single plant yield. Most of the intermated progenies were high yielder accompanied by early flowering and resistant to blast disease. Intermating in early segregating generation is an effective approach to generate transgressive segregants with high yielding ability, early flowering and resistance to blast disease by breaking undesirable linkages between yield, grain quality and blast disease.

Blast disease of rice caused by the filamentous fungus *Magnaporthe grisea* has been one of the most damaging diseases of rice and remains most difficult crop diseases to manage (Khush and Jena, 2007). Resistance to blast in rice is controlled by monogenic dominant, monogenic recessive, two dominant independent genes, two dominant complementary genes or partial resistance controlled by minor genes. Over 80 complete major resistance *Pi* genes have been described in rice germplasm worldwide (Ballini *et al.,* 2008). To date, *Pi-ta, Pi 2 / Piz-t, Pi 5, Pi 9, Pikm, Pi-b, Pi 36, Pi 37,* $Pi\text{-}d_2$, and *Pit* (Ashikawa *et al.,* 2008; Hayashi and Yoshida, 2009) have been characterized molecularly and their gene structure and resistance functions have been extensively investigated. In spite of the wide distribution of many known genes in rice varieties grown in different countries, genetic studies on blast resistance are limited in tropics. This is partly attributed to the extremely variable nature of blast pathogen, lack of a suitable differential system for the efficient identification of the genes and presence of several resistance genes in *indica* type varieties may account for the complex nature of genetics of blast resistance (Mackill *et al., 1985*). However, varieties released as resistant became susceptible after only few seasons or few years of cultivation due to evolution of the pathogen and its adaptation to cultivated resistant varieties. Thus, breeding for disease resistance is a continuous challenge to rice breeders and pathologists. Biparental mating is one of the simplest random mating design available to enforce recombinations and breaking down undesirable linkages as pointed out by Comstock and Robinson (1952). F_2 are the critical generation in rice breeding and they determine the eventual success or failure of hybridization programme (Jennings *et al.*, 1979). Frederickson and Kronstad (1985) stressed that in autogamous crops, intermating among early segregants could open vistas to new levels of genetic variability by breaking up the linkage and genetic recombination within group. The present investigation was carried out with the objective of studying the effectiveness of biparental mating on releasing superior transgressive segregants with high yield and blast resistance.

*Corresponding author:
mahalingamcpbg2008@gmail.com
Supplementary Tables only in online edition

Materials and Methods

Development of Biparental progenies : The F_2 generation seeds of JGL 384 × Rasi cross and their parents were obtained and experiments were conducted at Paddy Breeding Station, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore. F_2 generation which comprised the biparental mating block was raised during *kharif* 2008-2009 and raised in non – replicated rows of 800 single plants. In F_2 population, four plants as male and four plants as female parents were selected at random. Each male parent was crossed with each of the female parents as per the North Carolina Design II of biparental mating suggested by Comstock and Robinson (1948). Simultaneously, respective male and female parents were also selfed to generate eight F_3 families. Thus sixteen biparental progenies (BIPs) were made which would constitute one set and like wise two sets were made. A total of thirty two BIPs and sixteen F_3 families were produced. For crossing wet cloth method suggested by Chaisang *et al*. (1967) was followed. The parents, F_1s, F_3 families and biparental progenies were raised during *rabi* 2008-2009 in a Randomized Block design with two replications adopting a spacing of 20 cm between rows and 10 cm between plants and for F_2 populations 200 plants were raised as a non replicated row. Observations were recorded on days to 50% flowering, plant height, panicle length, number of productive tillers per plant, 1000 grain weight and single plant yield.

Maintenance and mass multiplication of blast pathogen : Ten days old cultures of the local isolate were collected from Department of Plant Pathology. These isolates were used as inoculum for mass multiplication. The spores were mass cultured by inoculating the pathogens in flasks containing 30 days old sterile meristem pith of maize (*Zea mays* L.). After inoculation, the flasks were incubated at room temperature (25 to 28^0 C) for a period of 10 days. The spore suspension was isolated from the flasks and used for spraying.

Evaluation of biparental progenies and F_3 families for blast resistance : Parents (JGL 384 and Rasi), 70 F_3 families (including 16 parents of BIPs) and 32 biparental or intermated progenies were phenotypicaly screened for resistance against blast disease by sandwich method. Screening was done in artificial condition favoring disease incidence. The susceptible checks (IR 50 and TN 1 mixtures) were sown in raised beds of glasshouse enabling high humid condition. Seedlings of 1-day-old age were inoculated. After the appearance of blast incidence in susceptible checks, the experimental materials (Parents, F_3 families, BIPs) were raised. For every single row of test entries one row of susceptible check was planted to enhance the natural inoculums. The spore suspension was sprayed during evening hours after 15 days of sowing. The disease reaction of each line was scored according to the Standard Evaluation System (SES, 1996). The segregants were classified based on disease score as follows, lines with score 1 as highly resistant, 3 as resistant, 5 as moderately resistant, 7 as susceptible and lines with score 9 as highly susceptible.

Results and Discussion

In the present investigation BIPs exhibited numerically higher mean performance for most of the traits studied *viz.,* panicle length (22.14 cm), number of productive tillers per plant (16.39), 1000 grain weight (20.49 g) and single plant yield (27.90 g) over F_2 and F_3 generations. A comparison of range values for biparental progenies and their F_1, F_2 and F_3 generation revealed that it was in general higher in biparental progenies for the traits *viz.,* days to 50 percent flowering (73.91 – 100.09 days), panicle length (18.51 – 23.82 cm) and 1000 grain weight (16.84 – 23.05 g) (Table 1). A comparison of value range for the BIPs, F_2 and F_3 generations revealed that the values of range were in general higher in BIPs than selfed generations. The lower limit of the range was foreshortened for days to 50% flowering, plant height and 1000 grain weight. More over the upperlimit of certain characters particularly for number of productive tillers per plant and single plant yield increased in the desired direction in case of intermated progenies. Thus, it was evident that intermating in early segregating generation was an effective method to promote transgressive segregants with early maturity. Enhancement in the trait mean value might be due to pooling of favorable alleles through recombination which was possible because of intermating. Superior mean performance of intermated progenies appeared to be due to creation of more of the genetic variability by breakage of undesirable linkages which otherwise conceal the genetic variation in the small size F_2 generation. Non randomness in crossing of segregants which is unavoidable for certain characters like number of productive tillers per plant, due to more tillers required for crossing and selfing purpose and days to flowering due to synchrony in flowering time would also contribute towards higher mean performance of BIPs. Mean performance is a basic and an important criterion in selecting superior segregants. According to Finkner *et al.* (1973), progenies with highest mean were relatively effective in selecting the superior segregants. Joshi (1979) explained that intermating of F_2 population increased the population mean in BIPs. This is of immense value to the breeder, since usually populations mean decreases with advancement of generations.

Based on average score for thirty plants per family in F_3 generation and 10 plants per family in BIPs blast disease reaction was considered. A comparative performance of F_3s and BIPs against blast disease is given in Table 2**.** The mean score of F_3 families was 4.02. Among 70 F_3 families screened, 53 families had a disease score of 5 or less to be considered as resistant. Only 25 per cent were susceptible. In contrast, mean score of BIPs was 3.19. It indicated that blast resistance improved in BIPs than in F_3 progenies. The range values were 1 to 9 in F_3s and 1 to 7 in BIPs. The results were in accordance with the findings of Ram *et al.* (2007) who reported for introgression of agronomicaly important characters from closely related wild species into cultivated rice, a better approach would be one or two back cross followed by selective inter mating in segregating generation to break undesirable linkages for superior recombination.

In the present investigation it was interesting to find that, the intermated progenies *viz.,* BIPs3, BIPs 5, BIPs 7, BIPs 8, BIPs 21, BIPs 24, BIPs 29 and BIPs 32 had higher yield accompanied with early flowering and highly or moderately resistant to blast disease. The progenies like BIPs 13, BIPs 14 were late in flowering with higher yield and highly resistant to blast disease Promising intermated progenies *viz.,* BIPs 13, BIPs 14, BIPs 32 may be selected and forwarded to next generation and may be used for further evaluation and experimental study. It was concluded that, biparental or intermating in early segregating generation is an effective method releasing superior transgressive segregants with early in flowering and resistance to blast disease of rice.

References

Ashikawa, I., Hayashi, N., Yamane, H., Kanamori, H., Wu, J., Matsumoto, T., Ono, K. and Yano, M. 2008. Two adjacent nucleotidebinding site-leucine-rich repeat class genes are required to confer *Pikm*-specific rice blast resistance. *Genetics* 180:2267–2276.

Ballini, E., Morel, J. B., Droc, G., Price, A., Courtois, B., Notteghem, J. L. and Tharreau, D. 2008. A genome-wide meta-analysis of rice blast resistance genes and quantitative trait loci provides new insights into partial and complete resistance. *Mol. Plant-Microbe Interact*. 21: 859–868.

Chaisang, K., Ponnaiya, B. W. X. and Balasubramanian, K. M. 1967. Studies on anthesis, pollination and hybridization technique in rice (*Oryza sativa L.). Madras Agric. J.,* 54: 118-123.

Comstock, R. E. and Robinson, H. F. 1948. Estimation of average dominance of genes. *In:* Heterosi*s*, Gowen, J.W. (Ed), Lowa State College Press-Ames, pp: 494-515.

Fredrickson, L. J. and Kronsrad, W. E. 1985. A comparison of intermating and selfing following selection for heading date in two diverse winter wheat crosses. *Crop Sci.* 25: 555-560.

Hayashi, K., and Yoshida, H. 2009. Refunctionalization of the ancient rice blast disease resistance gene *Pit* by the recruitment of a retrotransposon as a promoter**.** *Plant J.* 57: 413–425.

Jennings, P. R., Coffman, W. R. and Kauffman, H.E. 1979. Rice Improvement. IRRI, Philippines, pp: 52-74.

Khush, G. S., and Jena, K. K. 2007. Current status and future prospects of research on blast disease in rice (*Oryza sativa*). *In:* Proceedings of the 4th International Rice Blast Conference, Changsa, Hunan, China. P: 1

Mackill, D. J., Bonman, J. M., Suh, H. S. and Srilingam. R. 1985. Genes fro resistance to Philippine isolates of the rice blast pathogen. Rice Genet. Newsl. 2: 80 – 81.

Silue, D., Notteghem, J. L. and Tharreau, D. L. 1992. Evidence for a gene for gene relationship in the *Oryza sativa* - *Magnaporthe grisea* pathosystem. *Phytopathology* 82:577–580.

Table 1: Mean performance of parents, F_1s, F_2s, F_3s and BIPs of JGL 384 × Rasi cross combination

Generation	Days to 50 per cent lowering (days)	Plant height (cm)	Panicle length (cm)	Number of productive tillers per plant	1000 grain weight (gm)	Single plant yield (gm)	Blast disease score
JGL 384	**104.40**	**80.80**	**21.40**	**11.43**	**18.80**	**25.29**	**7**
Rasi	**84.60**	**79.20**	**22.200**	**10.80**	**20.71**	**20.84**	**3**
F_1	**94.20**	**80.00**	**23.20**	**17.40**	**20.25**	**28.98**	**-**
F_2 Mean	**86.54**	**79.12**	**20.76**	**13.29**	**19.10**	**23.88**	**-**
Range	78.0 – 100	67 – 90	19 – 23	7 –20	17.5 - 21.4	17.8 - 31.1	-
SD	4.82	3.32	0.97	3.31	1.01	3.18	-
F_3 Mean	**88.29**	**76.56**	**20.92**	**11.46**	**19.04**	**19.51**	**4.34**
Range	81.95 - 97.21	73.60 - 80.18	19.82 - 21.91	10.34 - 13.02	18.30 - 20.08	18.12 - 21.61	1-9
SD	4.12	2.78	0.72	0.91	0.63	0.97	2.42
BIPs Mean	**85.53**	**78.69**	**22.14**	**16.39**	**20.45**	**27.90**	**3.25**
Range	73.91 - 100.09	70.78 – 85.74	18.51 – 23.87	11.46 – 22.61	16.84 – 23.05	21.44 – 33.83	1-7
SD	6.22	4.15	1.23	3.46	1.89	3.09	2.0

Supplementary Table 1. Phenotypic reaction of different intermated progenies (BIPs) against blast disease in rice

Number of BIPs	DTF	PH	PL	NPT	1000 GW	SPY	Disease score	Disease reaction
JGL 384	104.40	80.80	21.40	11.43	18.80	25.29	7	S
Rasi	84.60	79.20	22.200	10.80	20.71	20.84	3	R
BIPs 1	79.50	81.78	22.35	14.37	21.40	26.58	5	MR
BIPs 2	80.18	76.86	22.48	16.61	20.37	27.66	3	R
BIPs 3	78.41	74.73	23.15	20.74	19.70	31.85	3	R
BIPs 4	80.09	77.14	20.61	13.53	19.69	24.24	7	S
BIPs 5	83.95	72.19	22.63	12.62	19.89	23.22	1	HR
BIPs 6	92.14	77.00	23.00	19.87	20.65	29.75	3	R
BIPs 7	**78.01**	78.36	21.96	14.64	18.60	26.93	3	R
BIPs 8	81.28	77.73	22.36	12.70	19.71	24.08	1	HR
BIPs 9	85.67	80.77	21.78	20.03	21.76	29.88	3	R
BIPs 10	84.41	83.11	22.49	22.61	18.93	**33.84**	3	R
BIPs 11	82.27	**70.37**	22.28	14.70	20.70	25.80	7	S
BIPs 12	80.30	72.14	22.52	14.07	21.42	24.91	3	R
BIPs 13	88.72	74.90	23.26	21.07	20.01	31.47	1	HR
BIPs 14	92.96	79.87	**23.99**	**22.71**	21.27	32.72	**1**	HR
BIPs 15	90.67	**86.10**	21.39	16.59	18.65	28.28	7	S
BIPs 16	99.39	76.38	**18.51**	12.46	21.70	24.46	3	R
BIPs 17	86.71	78.71	22.05	**11.47**	20.44	23.51	1	HR
BIPs 18	89.05	85.87	22.93	18.29	19.20	29.23	3	R
BIPs 19	86.74	83.17	23.28	14.63	20.15	26.58	1	HR
BIPs 20	89.10	79.22	19.78	14.35	19.88	25.61	3	R
BIPs 21	84.86	77.01	22.48	14.63	19.20	26.30	1	HR
BIPs 22	91.08	77.44	22.45	18.18	19.03	28.69	3	R
BIPs 23	93.06	76.88	22.49	12.42	21.66	24.10	7	S
BIPs 24	84.01	80.14	22.48	16.34	19.40	26.41	1	HR
BIPs 25	**100.09**	75.69	23.71	13.00	19.09	24.76	7	S
BIPs 26	96.55	81.44	22.73	12.47	22.35	24.62	**7**	S
BIPs 27	85.16	80.91	23.06	14.30	20.37	26.59	3	R
BIPs 28	98.93	84.93	23.80	12.43	21.08	23.56	3	R
BIPs 29	82.59	79.68	23.88	12.11	21.45	22.97	1	HR
BIPs 30	85.20	73.82	23.50	20.94	20.19	31.05	5	MR
BIPs 31	82.50	79.54	23.17	16.62	**17.93**	26.54	1	HR
BIPs 32	81.27	71.92	20.13	21.13	**23.42**	32.17	1	HR
Mean - BIPs	86.71	78.30	22.39	16.02	20.29	27.13	3.19	R