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Research Article

GENETIC ANALYSIS AND MOLECULAR TAGGING OF BLAST RESISTANT GENE IN RICE (*Oryza sativa* L.) CULTIVARS

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Abstract- The inheritance investigation uncovered that blast resistance in R 1013-2307-1-1, R 1124-91-2-73, R 1518-762-3-564-1, R 1558-2423-3-1445-1 and R 1559-2425-2-1449-1 was controlled by a single dominant gene, while two independent dominant genes governed resistance in R 1519-781-5-598-1 and R 1540-1888-1278-1. The allelic studies revealed that genes for resistance present in R 1013-2307-1-1, R 1518-762-3-564-1 and R 1558-2423-3-1445-1 was allelic to *Pi-z*^{5} (IRBL 10 and 5173). Among the blast differential genes (monogenic lines) tested, only *'Pi-z*^{5}*'* gene consistently imparted complete resistance against the blast population in the Northern Hilly Region of Chhattisgarh, *Pi-z, Pi-9* and *Pi-k*^{h} provided variable level of resistance. On the other hand four genes *Pi-z*^{5}, *Pi-z, Pi-9* and *Pi-k*^{h} functional in Bastar Plateau (Jagdalpur). The severity of blast disease was considerably higher at Ambikapur station than at Jagdalpur so only one center (Ambikapur) could be reliably used to conduct screening trials. The race of the fungus at these two sites seems to be different. Eight strains *viz*., R 1518-762-3-564-1, R 1519-781-5-598-1, R 1540-1888-1278-1, R 1558-2423-3-1445-1, B 6441-FMR-6-0-0, F 7-10, IR42221-145-2-3-2 and 5173 showed consistently stable resistant reaction over the years. The gene present in B 6441-FMR-6-0-0 *{Pi 48(t)}* is a new blast resistance gene. Its relative position in rice chromosome is not known but the primers used for molecular study were found monomorphic.

Keywords- Rice, Blast, Resistance, Genetics, Inheritance, Allelic, Molecular Tagging.

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Introduction

Rice is the staple food for more than half of the world's population. Demand for rice continues to increase due to the ever-increasing rice consumer base. However, the present rate of increase in rice production (2000-09) has slowed down (1.21%) compared with that of previous decades (2.49%) during 1970-79 and (1.70%) in 1990-2000, due to various biotic and abiotic stresses [7]. Among the biotic stresses, blast disease is the most devastating disease in rice cultivation by causing maximum up to 90% yield loss [19].Symptoms of the rice blast are sown in figure [Fig-1]. It is considered as a major constraint in rice production in different rice ecosystems ranging from irrigated (40-100%) to rainfed (70%) and upland rice area (63%) in major rice growing countries of the world, except in Australia [25].

With a view to manage the disease, the use of resistant cultivars with major resistance (R) genes still remains one of the most reliable methods. Identification and incorporation of different blast resistance genes with overlapping resistance spectra have long been main objectives of rice breeding program worldwide [26]. However, because of either the rapid evolution of new pathogen races or the selection of a rare component of the pathogen population that is already virulent, resistance is rendered ineffective in many cultivars. Thus, breeding for more durable resistant cultivars therefore has become a priority in rice improvement.

Chhattisgarh state of India, considered as the 'rice bowl', has 3.61 million hectare under rice cultivation and a production of about 5.47 million tonnes [2]. The prevailing environment in some areas of Chhattisgarh such as Bastar Plateau and Northern Hilly Region favors the development of blast to epidemic proportions and has been considered as "hot spots" for the blast. Severe blast (S, >50%) was recorded in plateaus of Jharkhand and Chhattisgarh (Production –oriented survey report, 1994-2006) and that was higher than the plains in the same region [24]. Though in Chhattisgarh some rice varieties and breeding lines, as sources of blast resistance, were identified [18]. However, a proper understanding of this disease is of utmost importance, thus the study was carried out to identify the functional resistance conferring genes, detection of variability in the pathogen population, inheritance-allelic pattern and molecular tagging of the blast resistant gene.

Materials and Methods

The research work was carried out in the Department of Plant Breeding and Genetics, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur, Chhattisgarh, India. Collaboration was made with College of Agriculture and Research Station Ambikapur and Jagdalpur to facilitate screening against blast. The studies were extended over a period of five cropping seasons *viz*., wet season (*kharif*) 2007, 2008, 2009, and dry season (*rabi*) 2008, 2009. The experimental materials consisted of (a) a set of thirty one blast monogenic/differential lines along with seventy nine other genotypes including breeding lines, resistant and susceptible checks, were tested at blast 'hot spots' Ambikapur for three years (2007-2009) and Jagdalpur in 2007, (b) F_1, F_2, and F_3 populations of the 64 crosses attempted for the genetic dissection (28 for inheritance, 35 for allelic studies and 1 for molecular studies) were screened

against the blast population at Ambikapur to ascertain the genetic ratios. The experiment was conducted under field conditions. All the standard agronomic practices were followed during cultivation of the crop. Severity of the blast disease can be seen by figures [Fig-2,3&4]. Screening techniques employed as Uniform Blast Nursery (UBN) test procedure [13]. Evaluation was done about 30-35 days after seeding, when susceptible check reached 9 score, using the Standard Evaluation System (SES) based on a 0-9 scale as given by International Network for Genetic Evaluation of Rice, INGER [4]. For the genetic studies score 4 and 5 were clubbed with susceptible. In F_1 and F_2, plants were individually scored. The F_3 progenies were classified as breeding true for resistance (all plants in the line being resistant), segregating (both resistant and susceptible were observed) or breeding true for susceptibility (all plants in the line being susceptible). For the genetic studies score up-to 3 were kept as resistant while score 4 and 5 were clubbed with susceptible. The Chi-Square (χ^2) test was employed to test the significance of deviation of an observed segregation ratio from a theoretical one for the purpose of working out the genetic ratios in F_2 and F_3.

Fig-1 Rice blast symptoms (a), (b) leaf blast (c) collar blast (d) node blast (e) neck blast (f) panicle blast (Scardaci *et al*., 2003; IRRI 2004)

Fig-2 View of Blast Nursery *Kharif 2007*, Jagdalpur (C.G.)

Result and Discussion

Rice Blast Screening: -Blast monogenic lines and new rice genotypes were screened along with eight susceptible checks (Mahisugandha, Dubraj, Poornima, Danteshwari, Swarna, Mahamaya, Cheptigurmatia, and HR12) against blast population over the years 2007-2009 at Ambikapur and at Jagdalpur in 2007 only. The primary aim was to identify effective resistance conferring blast genes in Chhattisgarh. The reaction of these genes over the years and the different locations are given in Tables [Table-1&2]. Highly susceptible reaction (score 9) was consistently observed for all four checks over the years and locations. This served as a benchmark for the reliability of reaction of the test entries.

Fig-3 View of Blast Nursery *Kharif 2007*, Ambikapur (C.G.)

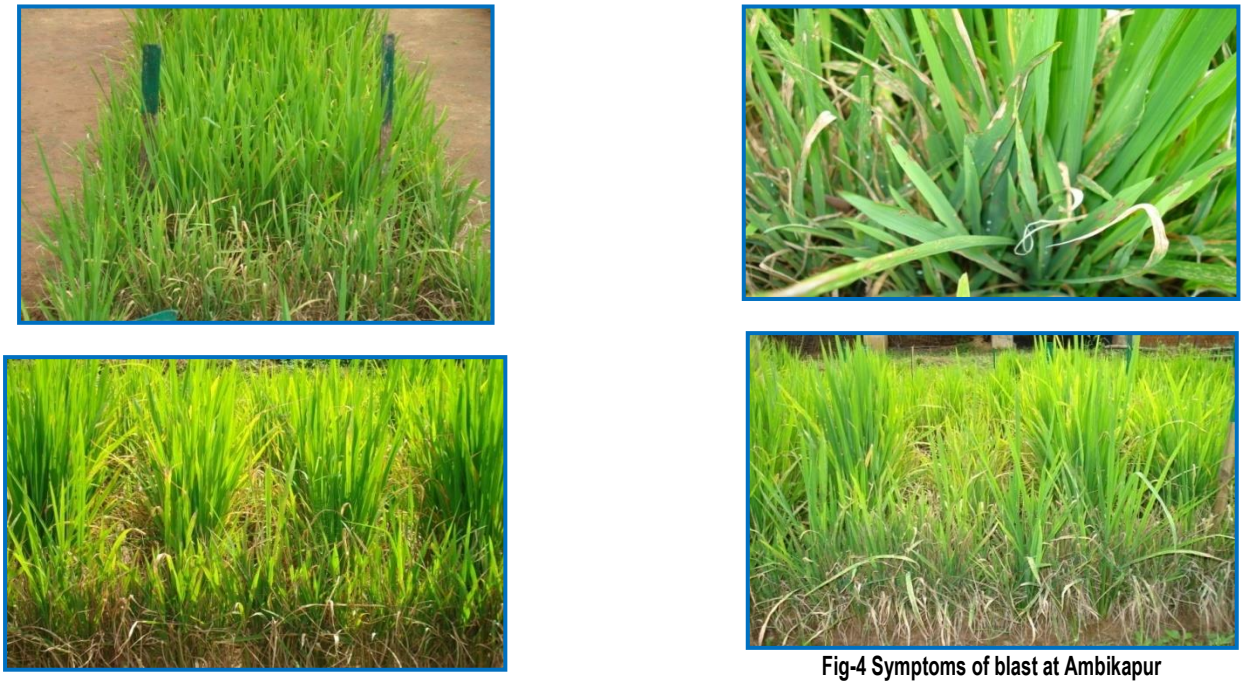

Fig-4 Symptoms of blast at Ambikapur

Table-1 *Reaction of blast in monogenic lines at Ambikapur and Jagdalpur*

S.N.	Entry No.	Designation	Target gene	Blast score			
				Ambikapur			Jagdalpur
				*kh.2007	*kh.2008	*kh.2009	*kh.2007
1.	IRBL 1	IRBLa-A	*Pi-a*	9	9	9	9
2.	IRBL 2	IRBLa-C	*Pi-a*	9	9	9	9
3.	IRBL 3	IRBLi-F5	*Pi-i*	9	9	9	9
4.	IRBL 4	IRBLks-F5	*Pi-k^{s}*	9	9	9	9
5.	IRBL 5	IRBLks-S	*Pi-k^{s}*	9	9	9	9
6.	IRBL 6	IRBLk-ka	*Pi-k*	9	9	9	9
7.	IRBL 7	IRBLkp-K60	*Pi-k^{p}*	9	9	9	9
8.	IRBL 8	IRBLkh-K3	*Pi-k^{h}*	3	5	5	3
9.	IRBL 9	IRBLz-Fu	*Pi-z*	1	3	5	1
10.	IRBL 10	IRBLz5-CA	*Pi-z^{5} = Pi-2(t)*	1	1	3	1
11.	IRBL 11	IRBLzt-T	*Pi-z^{t}*	9	9	9	9
12.	IRBL 12	IRBLta-K1	*Pi-ta = Pi-4(t)*	9	9	9	9
13.	IRBL 13	IRBLta-CT2	*Pi-ta*	9	9	9	9
14.	IRBL 14	IRBLb-B	*Pi-b*	9	9	9	9
15.	IRBL 15	IRBLt-K59	*Pi-t*	9	9	9	9
16.	IRBL 16	IRBLsh-S	*Pi-sh*	9	9	9	9
17.	IRBL 17	IRBLsh-B	*Pi-sh*	9	9	9	9
18.	IRBL 18	IRBL1-CL	*Pi-1*	9	9	9	9
19.	IRBL 19	IRBL3-CP4	*Pi-3*	9	9	9	9
20.	IRBL 20	IRBL5-M	*Pi-5(t)*	9	9	9	9
21.	IRBL 21	IRBL7-M	*Pi-7(t)*	9	9	9	9
22.	IRBL 22	IRBL9-W	*Pi-9*	1	3	5	1
23.	IRBL 23	IRBL12-M	*Pi-12(t)*	9	9	9	9
24.	IRBL 24	IRBL19-A	*Pi-19*	9	9	9	9
25.	IRBL 25	IRBLkm-Ts	*Pi-k^{m}*	9	9	9	9
26.	IRBL 26	IRBL20-IR24	*Pi-20*	9	9	9	9
27.	IRBL 27	IRBLta2-Pi	*Pi-ta^{2}*	9	9	9	9
28.	IRBL 28	IRBLta2-Re	*Pi-ta^{2}*	9	9	9	9
29.	IRBL 29	IRBLta-CP1	*Pi-ta*	9	9	9	9
30.	IRBL 30	IRBL11-Zh	*Pi-11(t)*	9	9	9	9
31.	IRBL 31	IRBLz5-CA(R)	*Pi-z^{5}*	1	1	3	1
32.	Mahisugandha	Susceptible Check	-	9	9	9	9
33.	Dubraj	Susceptible Check	-	9	9	9	9
34.	Swarna	Susceptible Check	-	9	9	9	9
35.	Poornima.	Susceptible Check	-	9	9	9	9
36.	HR 12	Susceptible Check	-	9	9	9	9
37.	Mahamaya	Susceptible Check	-	9	9	9	9
38.	Cheptigurmatia	Susceptible Check	-	9	9	9	9
39.	Danteshwari	Susceptible Check	-	9	9	9	9

Table-2 *Reaction of genotypes screened over the years to monitor resistance and detecting variability*

SN	Genotype	Blast score			
		Ambikapur			Jagdalpur
		*kh. 2007	*kh. 2008	*kh. 2009	*kh. 2007
1.	IR-64	1	3	3	1
2.	MTU 1065	7	7	7	5
3.	MTU 1075	7	7	9	5
4.	OR 1898-18	9	9	9	7
5.	R 714-5-55-2-1	3	5	5	5
6.	R 979-67-2-44-1	5	7	7	5
7.	R 979-1528-2-1	3	5	5	7
8.	R 1013-2307-1-1	1	3	3	3
9.	R 1022-1803-1-1	3	5	5	5
10.	R 1027-2238-3-1	3	5	5	7
11.	R 1060-30-2-41-1	3	5	5	3
12.	R 1124-69-1-45-1	3	5	5	5
13.	R 1124-91-2-73	3	3	3	3
14.	R 1130-80-1-52-1	3	7	7	5
15.	R 1207-257-5-1	3	3	5	7
16.	R 1219-650-2-314-1	5	7	7	7
17.	R 1238-692-820-1-1	3	7	7	5
18.	R 1238-1820-1-1	3	5	7	5
17.	R 1240-913-2-1031-1	3	3	7	3
20.	R 1240-927-3-1056-1	5	7	5	5
21.	R 1247-1936-1-1	1	5	5	3
22.	R 1248-1489-2-822-1	9	9	9	7
23.	R 1250-1557-1-895-1	1	3	3	3
24.	R 1262-1667-1-1	1	5	5	3
25.	R 1262-1668-2-1	1	5	5	5
26.	R 1264-1670-1-1	3	3	5	5
27.	R 1327-483-1-1	7	7	7	3
28.	R 1448-153-65-2-1	9	7	7	3
29.	R 1448-578-2-473-1	3	1	3	3
30.	R 1454-87-50-4-1	7	7	7	5
31.	R 1454-171-96-1	7	7	9	7
32.	R 1456-199-3-180-1	5	3	5	3
33.	R 1462-243-100-7-1-1	5	7	7	7
34.	R 1470-345-338-2-1	3	3	3	1
35.	R 1473-529-249-4-1	1	1	3	7
36.	R 1475-468-564-2-1	3	5	5	5
37.	R 1493-625-3-499-1	3	5	3	3
38.	R 1502-643-784-1-1	3	3	5	3
39.	R 1518-762-3-564-1	1	1	3	1
40.	R 1518-767-4-569-1	1	5	5	3
41.	R 1519-769-2-574-1	1	3	3	1
42.	R 1519-773-5-583-1	3	3	3	7
43.	R 1519-778-2-590-1	1	3	3	3
44.	R 1519-781-5-598-1	1	1	3	1
45.	R 1519-784-1-599-1	1	1	3	3
46.	R 1520-936-1-811-1	9	7	9	3
47.	R 1528-1139-3-1003-1	3	5	5	7
48.	R 1529-1166-1-1020-1	3	3	3	7
49.	R 1529-1183-1-1041-1	1	1	3	5
50.	R 1529-1183-3-1043-1	1	1	3	5
51.	R 1530-1194-2-1061-1	1	3	5	5
52.	R 1537-1566-1-1210-1	3	5	5	7
53.	R 1538-1614-1-1221-1	3	5	5	9
54.	R 1539-1785-1-1263-1	1	3	3	3
55.	R 1540-1888-1278-1	1	1	1	3
56.	R 1543-1966-1-1290-1	3	3	3	3
57.	R 1551-2169-1-1354-1	3	3	3	3
58.	R 1558-2419-2-1442-1	3	3	3	3
59.	R 1558-2423-3-1445-1	1	1	1	3
60.	R 1559-2425-2-1449-1	1	1	3	3
61.	R 1559-2427-1-1450-1	1	1	3	3
62.	R 1559-2427-2-1451-1	1	3	3	3
63.	R 1560-2442-1-1456-1	1	3	3	3
64.	R 1723-2271-1-1404-1	1	3	3	3
65.	B 6441-FMR-6-0-0	1	1	1	1
66.	F 7-10	1	1	1	1
67.	IR 42221-145-2-3-2	1	1	1	1
68.	5173	1	1	1	1
69.	Abhaya	1	3	3	1
70.	G 95-02	1	3	3	1
71.	BR 240	1	3	3	1

72.	Mahisugandha ch**	9	9	9	9
73.	Dubraj ch**	9	9	9	9
74.	Swarna ch**	9	9	9	9
75.	Poornima ch**	9	9	9	9
76.	HR 12 ch**	9	9	9	9
77.	Mahamaya ch**	9	9	9	9
78.	Cheptigurmatia ch**	9	9	9	9
79.	Danteshwari ch**	9	9	9	9

kh = kharif season, **ch=susceptible check

Of the thirty-one monogenic lines tested at Ambikapur during *kharif* 2007 only IRBL 9, IRBL 10, IRBL 22, IRBL 31 and IRBL 8 possessing the genes *Pi-z, Pi-z^5, Pi-9, Pi-z^5* and *Pi-k^h* respectively provided resistance (score 1 & 3), while the remaining 26 lines / genes proved highly susceptible and same as the checks. During *kharif* 2008, resistant reaction was recorded for four entries *viz*., IRBL 10, IRBL 31 (both possessing *Pi-z^5* gene), IRBL 22 *(Pi-9)* and IRBL 9 *(Pi-z)* (score 1 & 3), while IRBL 8 (*Pi-k^h*) was moderately resistant and all other entries highly susceptible (score 9). But only two blast monogenic lines *viz*., IRBL 10, IRBL 31 (both possessing *Pi-z^5* gene) were recorded resistant reaction (score 3) and other three monogenic lines IRBL 9 *(Pi-z),* IRBL 22 *(Pi-9)* and IRBL 8 *(Pi-k^h)* were found moderately resistant (score 5) at Ambikapur during the *kharif* 2009. Thus, the *Pi-z^5* gene should be utilized in developing blast resistant varieties for the Chhattisgarh state. This gene is providing durable and stable resistance in the region. Identification of functional blast resistance gene (s) for a particular region is a prerequisite for their meaningful deployment [22].

Overall, twenty nine new genotypes*viz*., IR 64, R 1013-2307-1-1, R 1124-91-2-73, R 1250-1557-1-895-1, R 1448-578-2-473-1, R 1470-345-338-2-1, R 1518-762-3-564-1, R 1519-769-2-574-1, R 1519-778-2-590-1, R 1519-781-5-598-1, R 1519-784-1-599-1, R 1539-1785-1-1263-1, R 1540-1888-1278-1, R 1543-1966-1-1290-1, R 1551-2169-1-1354-1, R 1558-2419-2-1442-1, R 1558-2423-3-1445-1, R 1559-2425-2-1449-1, R 1559-2427-1-1450-1, R 1559-2427-2-1451-1, R 1560-2442-1-1456-1, R 1723-2271-1-1404-1, B 6441-FMR-6-0-0, F 7-10, IR 42221-145-2-3-2, 5173, Abhaya, G 95-02 and BR 240 proved to be resistant over the years (2007-2009) at Ambikapur and in 2007 at Jagdalpur.

The Colombian cultivar 5173 has *Pi-z^5*gene [10], proved highly resistant with scores of 1 over the three years testing at Ambikapur. Also score of 1 and 3 was observed right through from 2007-2009 for monogenic lines IRBL 10 and IRBL 31 that are representatives of *Pi-z^5* gene and both the lines were derived from C101 A51. The reason why 5173 showed better (less) score than all these NIL's is possibly due to additional effective minor genes / QTL's that may be present in cultivar 5173 which supported the resistance of gene *Pi-z^5*. The same may be the case with IR42221-145-2-3-2 that possess *Pi-z^5* gene.

The gene present in Guyanese strains B 6441-F-MR-6-0-0 (*Pi-48*), F 7-10 (*Pi-49*) were reported to be new blast resistant gene [18]. Both showed highly resistant score of 1, so they can be used as new donors for the blast resistant gene. F 7-10 has extra-long slender grain and high production potential. The other two Guyanese strains BR 240 and G 95-02 were also imparting resistance of variable level.

Genetical study: -Sixty-three crosses were made to analyze the inheritance and allelic relationships of the genes involved in the resistant parents. The F_1, F_2, and F_3, populations of the crosses were screened against the blast population prevailing at Ambikapur for classification of the plants / progenies to fit the appropriate genetic ratios. The reactions of the various populations are presented in Tables [Table-3&4].

1. Inheritance of resistance: Seven resistant parents *viz*., R 1013-2307-1-1, R 1124-91-2-73, R 1518-762-3-564-1, R 1519-781-5-598-1, R 1540-1888-1278-1, R 1558-2423-3-1445-1 and R 1559-2425-2-1449-1were crossed with four susceptible parents (HR 12, Swarna, Mahamaya and Cheptigurmatia).The F_1 populations of all the crosses showed resistance reaction against the blast population. This indicated the dominant nature of the resistance gene(s) involved. The F_2 population of the crosses of R 1013-2307-1-1, R 1124-91-2-73, R 1518-762-3-564-1, R 1558-2423-3-1445-1 and R 1559-2425-2-1449-1 with susceptible parents segregated in a frequency of three resistant plants: one susceptible plant (3R:1S). This suggested the presence of one single dominant gene in the resistant parent. Further, the F_3 progenies of these crosses for each resistant parent were analyzed. A segregation pattern of one homozygous resistant: two segregating (heterozygous): one homozygous susceptible, (1R:2Sg:1S) was observed for these crosses as expected following simple Mendelian inheritance. This confirmed the inheritance of a single dominant gene present in these resistant parents, while the segregation behavior of F_2 population of the crosses of R 1519-781-5-598-1 and R 1540-1888-1278-1 with susceptible parents fit well in fifteen resistant plants: one susceptible plant ratio (15R:1S) signifying the possibility of two independent dominant genes controlling resistance. Further, the F_3 progenies of these crosses were evaluated and classified into seven homozygous resistant: eight segregating (heterozygous): one homozygous susceptible (7R:8Sg:1S) ratio ratifying the existence of two independent dominant genes in these resistant parents [Table-3].

Resistant parents R 1013-2307-1-1, R 1124-91-2-73, R 1518-762-3-564-1, R 1558-2423-3-1445-1 and R 1559-2425-2-1449-1 possess only one gene for resistance, which is dominant. In many of the earlier studies resistance has been reported to be governed by one dominant gene [3, 8, 10, 12, 14, 17, 18 and 21], although resistance to blast has also been reported to be controlled by recessive genes [11].

The strains R 1519-781-5-598-1 and R 1540-1888-1278-1 have two dominant genes for resistance. Resistance to blast has been noted by several workers to be governed by two dominant genes [12, 15, 16, 17, 18 and 20]. Even three dominant genes have been found to control resistance [28].

Control of a trait by a dominant gene is considered to be an advantage to the breeder as it makes identification of the resistant plants easier, which is also expressed, in heterozygous condition. In-depth understanding of the inheritance of the resistance gene greatly enhances the breeder's ability to plan an appropriate breeding strategy to exploit / transfer the target gene(s). Since, the resistance genes in the parents studied are inherited independently they are expected to be transferred quite easily.

2. Allelic test: The segregation behavior of the F_2 populations of the cross between unknown resistant parents R 1013-2307-1-1, R 1518-762-3-564-1, and R 1558-2423-3-1445-1 with known resistant donors B 6441-FMR-6-0-0 *(Pi-48 t),* F 7-10 *(Pi-49 t)* and IRBL 22 *(Pi -9)* showed a 15R:1S ratio pointing out that two independently dominant gene were involved in each of these crosses. The reaction of the F_3 progenies of all these crosses tested were partitioned into 7R:8Sg:1S segregation classes. This corroborate that the gene identified in R 1013-2307-1-1, R 1518-762-3-564-1 and R 1558-2423-3-1445-1 were different from those found in B 6441-FMR-6-0-0 (*Pi-48 t*), F 7-10 *(Pi-49 t)* and IRBL 22 *(Pi-9).* The F_2 and F_3 populations of the crosses involving R 1013-2307-1-1, R 1518-762-3-564-1 and R 1558-2423-3-1445-1 with IRBL 10 *(Pi-z^5)* and 5173 *(Pi-z^5)* did not segregate for blast resistance. This signified that the gene(s) involved in R 1013-2307-1-1, R 1518-762-3-564-1, and R 1558-2423-3-1445-1were allelic to that of IRBL 10 *(Pi-z^5)* and 5173 *(Pi-z^5)*. This indicates the presence of the gene *Pi-z^5* gene in these parents (R 1013-2307-1-1, R 1518-762-3-564-1 and R 1558-2423-3-1445-1).

Unknown resistant parents R 1124-91-2-73 and R 1559-2425-2-1449-1were tested for their allelic relationship with B 6441-FMR-6-0-0 *(Pi-48 t),* F 7-10 *(Pi-49 t),* IRBL 10 *(Pi-z^5)* and 5173 *(Pi-z^5)*. The F_2 reactions of these crosses were classified into 15R:1S segregation ratio demonstrating that the single gene present in these parents were inherited independently and were non-allelic to the

Table-3 *Reaction of F1, F_2 and F_3 population to Magnapor the griseain crosses of rice.*

SN	Cross combination	F_1 Reac-tion	Reaction of F_2 plants						Reaction of F_3 progenies						
			No. of Plants			Expected Ratio	χ^2 value	P-value	No. of progenies				Expected Ratio	χ^2 value	P-value
			R	S	Total	R:S			R	Sg	S	Total	R:Sg:S		
1.	R 1013-2307-1-1 x Swarna	R	331	94	425	3:1	1.883	0.20-0.10	29	63	39	131	1:2:1	1.718	0.50-0.30
2.	R 1013-2307-1-1 x HR 12	R	287	78	365	3:1	2.565	0.20-0.10	37	62	26	125	1:2:1	1.944	0.50-0.30
3.	R 1013-2307-1-1 x Mahamaya	R	315	113	428	3:1	0.449	0.50-0.30	41	72	33	146	1:2:1	0.904	0.70-0.50
4.	R 1013-2307-1-1 x Cheptigurmatia	R	362	114	476	3:1	0.280	0.70-0.50	35	78	41	154	1:2:1	0.494	0.80-0.70
5.	R 1124-91-2-73 x Swarna	R	405	118	523	3:1	1.658	0.20-0.10	37	71	30	138	1:2:1	0.826	0.70-0.50
6.	R 1124-91-2-73 x HR 12	R	253	102	355	3:1	2.638	0.20-0.10	35	68	26	129	1:2:1	1.636	0.50-0.30
7.	R 1124-91-2-73 x Mahamaya	R	288	81	369	3:1	1.829	0.20-0.10	29	77	35	141	1:2:1	1.709	0.50-0.30
8.	R 1124-91-2-73 x Cheptigurmatia	R	262	86	348	3:1	0.015	0.95-0.90	37	80	35	152	1:2:1	0.474	0.80-0.70
9.	R 1518-762-3-564-1 x Swarna	R	322	90	412	3:1	2.188	0.20-0.10	26	59	23	108	1:2:1	1.093	0.70-0.50
10.	R 1518-762-3-564-1 x HR 12	R	299	104	403	3:1	0.140	0.80-0.70	24	59	32	115	1:2:1	1.191	0.70-0.50
11.	R 1518-762-3-564-1 x Mahamaya	R	313	95	408	3:1	0.641	0.50-0.30	31	79	32	142	1:2:1	1.817	0.50-0.30
12.	R 1518-762-3-564-1 x Cheptigurmatia	R	280	79	359	3:1	1.717	0.20-0.10	29	58	22	109	1:2:1	1.349	0.70-0.50
13.	R 1519-781-5-598-1 x Swarna	R	352	24	376	15:1	0.011	0.95-0.90	49	53	8	110	7:8:1	0.273	0.90-0.80
14.	R 1519-781-5-598-1 x HR 12	R	372	17	389	15:1	2.346	0.20-0.10	58	78	7	143	7:8:1	1.344	0.70-0.50
15.	R 1519-781-5-598-1 x Mahamaya	R	369	27	396	15:1	0.218	0.70-0.50	48	58	6	112	7:8:1	0.235	0.90-0.80
16.	R 1519-781-5-598-1 x Cheptigurmatia	R	345	21	366	15:1	0.164	0.70-0.50	47	55	7	109	7:8:1	0.020	0.99-0.98
17.	R 1540-1888-1278-1 x Swarna	R	371	32	403	15:1	1.965	0.20-0.10	53	67	6	126	7:8:1	0.782	0.70-0.50
18.	R 1540-1888-1278-1 x HR 12	R	470	41	511	15:1	2.743	0.10-0.05	60	78	7	145	7:8:1	1.073	0.70-0.50
19.	R 1540-1888-1278-1 x Mahamaya	R	443	33	476	15:1	0.379	0.70-0.50	58	75	8	141	7:8:1	0.583	0.80-0.70
20.	R 1540-1888-1278-1 x Cheptigurmatia	R	433	22	455	15:1	1.554	0.30-0.20	60	66	8	134	7:8:1	0.064	0.98-0.95
21.	R 1558-2423-3-1445-1 x Swarna	R	328	106	434	3:1	0.077	0.80-0.70	33	73	30	136	1:2:1	0.868	0.70-0.50
22.	R 1558-2423-3-1445-1 x HR 12	R	281	75	356	3:1	2.936	0.10-0.05	33	76	25	134	1:2:1	3.373	0.20-0.10
23.	R 1558-2423-3-1445-1 x Mahamaya	R	294	104	398	3:1	0.271	0.70-0.50	34	70	28	132	1:2:1	1.030	0.70-0.50
24.	R 1558-2423-3-1445-1 x Cheptigurmatia	R	263	84	347	3:1	0.116	0.80-0.70	32	81	35	148	1:2:1	1.446	0.50-0.30
25.	R 1559-2425-2-1449-1 x Swarna	R	265	75	340	3:1	1.569	0.30-0.20	28	62	22	112	1:2:1	1.929	0.50-0.30
26.	R 1559-2425-2-1449-1 x HR 12	R	290	79	369	3:1	2.537	0.20-0.10	33	78	28	139	1:2:1	2.439	0.30-0.20
27.	R 1559-2425-2-1449-1 x Mahamaya	R	298	103	401	3:1	0.101	0.80-0.70	38	69	30	137	1:2:1	0.942	0.70-0.50-
28.	R 1559-2425-2-1449-1 x Cheptigurmatia	R	275	109	384	3:1	2.347	0.10-0.05	36	68	31	135	1:2:1	0.378	0.90-0.80

In F_2: R = Resistant, S = Susceptible.
In F_3: R = Breeding true for resistance, Sg = Segregating, S = Breeding true for susceptibility.

B 6441-FMR-6-0-0 *(Pi-48 t),* F 7-10 *(Pi-49 t),* IRBL 10 *(Pi-z^5)* and 5173 *(Pi-z^5)*. However, the F_2 and F_3 populations of the crosses of R 1124-91-2-73 and R 1559-2425-2-1449-1 with IRBL 22 *(Pi 9)* did not segregate for blast resistance. This confirmed that the gene present in R 1124-91-2-73 and R 1559-2425-2-1449-1 was allelic to *Pi 9* gene of IRBL 22.

The resistance to rice blast involving the parents R 1519-781-5-598-1, R 1540-1888-1278-1 found to possess two independent dominant genes did not segregate in F_2 and F_3 populations of its crosses with parents having only one resistant gene *(Pi-z^5)* in 5173 and IRBL 10. This pointed out that one of the gene present in R 1519-781-5-598-1, R 1540-1888-1278-1 was allelic to (i.e. same as) the gene *Pi-z^5*. Furthermore, the F_2 population of the crosses of these two unknown resistant parents with other known resistant parents B 6441-FMR-6-0-0

(Pi-48 t), F 7-10 *(Pi-49 t)* and IRBL 22 *(Pi-9)* segregate in a ratio of 63:1, indicating that the genes in those unknown parents were non allelic to the gene present in B 6441-FMR-6-0-0 *(Pi-48 t),* F 7-10 *(Pi-49 t)* and IRBL 22 *(Pi-9).*

Table-4 *Reaction of F1, F_2 and F_3 population to Magnaporthe griseain crosses of rice.*

SN	Cross combination	F_1 Reaction	Reaction of F_2 plants						Reaction of F_3 progenies						
			No. of Plants			Expected Ratio	χ^2 value	P-value	No. of progenies				Expected Ratio	χ^2 value	P-value
			R	S	Total	R:S			R	Sg	S	Total	R:Sg:S		
1.	R 1013-2307-1-1 x B 6441-FMR-6-0-0	R	329	25	354	15:1	0.398	0.70-0.50	58	70	6	134	7:8:1	0.814	0.70-0.50
2.	R 1013-2307-1-1 x F 7-10	R	301	25	326	15:1	1.120	0.30-0.20	60	73	9	142	7:8:1	0.131	0.95-0.90
3.	R 1013-2307-1-1 x 5173	R	387	0	387	-	-	-	145	0	0	145	-	-	-
4.	R 1013-2307-1-1 x IRBL 10	R	364	0	364	-	-	-	138	0	0	138	-	-	-
5.	R 1013-2307-1-1 x IRBL 22	R	375	28	403	15:1	0.335	0.70-0.50	56	68	12	136	7:8:1	1.647	0.50-0.30
6.	R 1124-91-2-73 x B 6441-FMR-6-0-0	R	380	32	412	15:1	1.618	0.30-0.20	59	71	5	135	7:8:1	1.582	0.50-0.30
7.	R 1124-91-2-73 x F 7-10	R	385	30	415	15:1	0.679	0.50-0.30	62	70	5	137	7:8:1	1.586	0.50-0.30
8.	R 1124-91-2-73 x 5173	R	376	34	410	15:1	2.920	0.10-0.05	56	67	6	129	7:8:1	0.628	0.80-0.70
9.	R 1124-91-2-73 x IRBL 10	R	287	24	311	15:1	1.142	0.30-0.20	56	65	7	128	7:8:1	0.141	0.95-0.90
10.	R 1124-91-2-73 x IRBL 22	R	461	0	461	-	-	-	140	0	0	140	-	-	-
11.	R 1518-762-3-564-1x B 6441-FMR-6-0-0	R	397	34	431	15:1	1.975	0.20-0.10	56	74	6	136	7:8:1	1.471	0.50-0.30
12.	R 1518-762-3-564-1x F 7-10	R	484	41	525	15:1	2.179	0.20-0.10	54	67	7	128	7:8:1	0.337	0.90-0.80
13.	R 1518-762-3-564-1x 5173	R	418	0	418	-	-	-	129	0	0	129	-	-	-
14.	R 1518-762-3-564-1x IRBL 10	R	569	0	569	-	-	-	138	0	0	138	-	-	-
15.	R 1518-762-3-564-1x IRBL 22	R	333	30	369	15:1	2.226	0.20-0.10	52	64	5	121	7:8:1	1.087	0.70-0.50
16.	R 1519-781-5-598-1 x B 6441-FMR-6-0-0	R	477	12	489	63:1	2.527	0.20-0.10	-	-	-	-	-	-	-
17.	R 1519-781-5-598-1x F 7-10	R	417	9	426	63:1	0.838	0.50-0.30	-	-	-	-	-	-	-
18.	R 1519-781-5-598-1x 5173	R	411	0	411	-	-		135	0	0	135	-	-	
19.	R 1519-781-5-598-1x IRBL 10	R	385	0	385	-	-		134	0	0	134	-	-	
20.	R 1519-781-5-598-1x IRBL 22	R	356	9	365	63:1	1.936	0.20-0.10	-	-	-	-	-	-	-
21.	R 1540-1888-1278-1 x B 6441-FMR-6-0-0	R	337	8	345	63:1	1.283	0.20-0.10	-	-	-	-	-	-	-
22.	R 1540-1888-1278-1 x F 7-10	R	447	11	458	63:1	2.097	0.20-0.10	-	-	-	-	-	-	-
23.	R 1540-1888-1278-1 x 5173	R	429	0	429	-	-		136	-	-	136	-	-	
24.	R 1540-1888-1278-1 x IRBL 10	R	416	0	416	-	-		138	-	-	138	-	-	
25.	R 1540-1888-1278-1x IRBL 22	R	488	10	498	63:1	0.643	0.50-0.30	-	-	-	-	-	-	-
26.	R 1558-2423-3-1445-1 x B 6441-FMR-6-0-0	R	534	44	578	15:1	1.831	0.20-0.10	55	78	9	142	7:8:1	1.509	0.70-0.50
27.	R 1558-2423-3-1445-1 x F 7-10	R	462	39	501	15:1	2.013	0.20-0.10	56	66	10	132	7:8:1	0.424	0.90-0.80
28.	R 1558-2423-3-1445-1 x 5173	R	376	0	376		-		131	0	0	131	-	-	
29.	R 1558-2423-3-1445-1 x IRBL 10	R	342	0	342	-	-		121	0	0	121	-	-	
30.	R 1558-2423-3-1445-1 x IRBL 22	R	393	33	426	15:1	1.628	0.30-0.20	54	63	5	122	7:8:1	0.977	0.70-0.50
31.	R 1559-2425-2-1449-1 x B 6441-FMR-6-0-0	R	367	22	389	15:1	0.235	0.70-0.50	57	64	5	126	7:8:1	1.129	0.70-0.50
32.	R 1559-2425-2-1449-1 x F 7-10	R	386	22	408	15:1	0.512	0.50-0.30	55	66	4	125	7:8:1	2.058	0.30-0.20
33.	R 1559-2425-2-1449-1 x 5173	R	337	28	365	15:1	1.258	0.30-0.20	54	64	5	123	7:8:1	1.042	0.70-0.50
34.	R 1559-2425-2-1449-1x IRBL 10	R	347	30	377	15:1	1.876	0.20-0.10	51	61	4	116	7:8:1	1.613	0.50-0.30
35.	R 1559-2425-2-1449-1 x IRBL 22	R	381	0	381	-	-	-	125	0	0	125	-	-	-

In F_2: R = Resistant, S = Susceptible.
In F_3: R = Breeding true for resistance, Sg = Segregating, S = Breeding true for susceptibility.

3. Molecular study: Molecular markers for trait selection have numerous advantages over morphological markers used in conventional plant breeding. Of the 40 major blast resistance genes identified so far, about 30 genes have been mapped on different rice chromosomes, and tightly linked DNA markers have been developed [5]. Partial resistance gene to rice blast in the *Oryza sativa japonica* group cv. Chubu 32 is controlled by *Pi 34,* a major quantitative trait locus (QTL) on chromosome 11 and several uncharacterized QTLs were also mapped [27]. Field resistance to rice blast in cultivar Chubu 111 was controlled by a dominate gene, designated as *Pi 39(t),* that co-segregates with the single sequence repeat marker loci RM3843 and RM 5473 on chromosome 4 [23]. Rice blast resistance gene, *Pi 40* derived from the EE genome wild *Oryza australiensis*, has been localized on chromosome 6 and fine mapped using the e-landing approach [6]. Single Sequence Repeat markers have several advantages over other markers. SSR markers are reliable, co-dominant, multi-allelic, chromosome specific. The gene present in B 6441-FMR-6-0-0, {*Pi-48(t)*} is a new blast resistance gene; its relative position on rice chromosome is not known. A total of twenty eight SSR markers distributed all over the 12 chromosome of rice were selected and used to amplify parental and bulk DNA, with the objective to identify the parental polymorphism and possible co-segregation of these marker(s) with the gene of interest. However, on 2.5% agarose gel, none of the primer exhibited polymorphism. The obvious reason for not getting polymorphism might be the use of agarose gel, in which, only big differences in DNA size can be resolved. At present we do not have PAGE sequencing system, which can resolve small differences and secondly the cross was attempted between two closely related indica lines, which rarely shows polymorphism. Since, the primers used were monomorphic, so further co-segregation and linkage analysis was not done [Fig-5].

This study was intended in developing a comprehensive understanding of the mode of inheritance, the allelic relationships of the resistance conferring genes in donors in Chhattisgarh along with the functional resistance genes for the region are identified, the variation in the fungus population has been detected and an attempt was also made to tag the blast resistance gene using molecular markers. This study would enable the breeders and pathologist to have a greater insight into the nature of the genetic interactions between the blast fungus and its host.

The stability of resistance conferring genes in given rice cultivar is determined by how the blast pathogen changes and the way the resistance is deployed [1]. Thus, the ability of the breeders to develop varieties with effective durable blast resistance for the region is likely to be enhanced with the results obtained in this study.

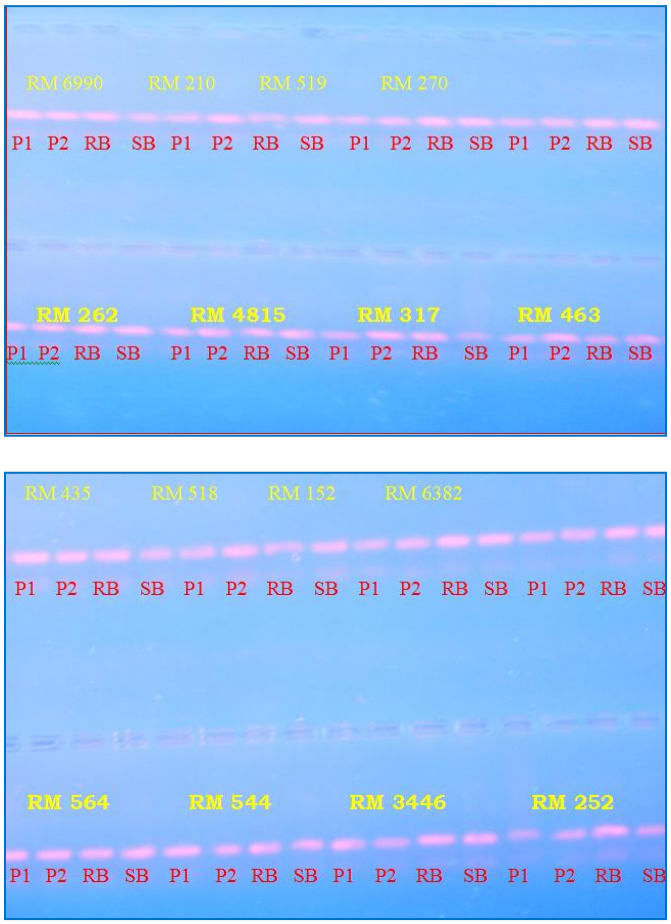

Fig-5 Bulk Segregant Analysis

Conflict of Interest: None declared

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