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Molecular and field level screening for blast resistance among various rice varieties in two blast hotspots of Chhattisgarh

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Abstract
Rice blast, caused by *Magnaporthe oryzae*, is becoming one of the most ruinous ailments of rice (*Oryza sativa* L.) in Chhattisgarh. Utilization of resistant rice varieties had been demonstrated to be the most effective and sustainable way to command this disease. In the present study, the existences of blast resistance genes were identified in some of the rice genotypes in field level screening. The selected genotypes for screening were confirmed for blast resistance genes in some of the lines. The material was consisted of rice lines containing germplasm lines, advanced breeding lines, land races and blast resistant varieties. These lines were identified using the field level screening respectively and further confirmed through SSR markers RM224, RM 72 and RM206. Results showed the presence of gene *Pi 1* in nine varieties, Pi33 in three varieties and *Pi kh* in five varieties, some varities were with combination of genes against blast resistance. Infection index analysis after field level screening also showed that ten varieties had low infection index. Landraces carrying the genes *Pi 1* and *Pi kh* imparted moderate resistance under Chhattisgarh conditions which implied that pyramiding of these genes could improve the disease resistance in rice varieties of Chhattisgarh. Among the selected varieties IR64 and Tetep having Pi33, *Pi 1* and *Pi kh* genes could be used as gene donors in further breeding programmes.

Keywords: Disease resistance, field screening, rice blast, SSR markers, traditional varieties

Introduction
Rice (*Oryza sativa* L.) is one of the most necessary foods for half of the world's population (Von Braun, 2007) [20]. India is one of the biggest consumer and producer of rice. However, the world's largest food crop is damaged by a large number of organisms, which under epidemic conditions, cause serious yield loss. Among these, rice blast caused by *Magnaporthe oryzae* is one of the most calamitous diseases, often resulting in loss of yield about 70 to 80 per cent during an epidemic (Ou, 1985) [18]. On comparing with other district of Chhattisgarh, Ambikapur and Jagdalpur are disease prone area for rice blast. The weather conditions of Ambikapur and Jagdalpur provide a very favorable climatic condition for the blast pathogen to expand and make the area blast disease hotspot. Several ongoing survey and research work in the stations reveals that blast disease incidence of rice are in the range of 35 to 80 per cent in different hotspots of Chhattisgarh.
In spite of the availability of effectual fungicides, self reliance of the system of rice-growing area, resistant breeding is must to control the disease (Ballini *et al*., 2008) [6]. Chhattisgarh state is considered as one among the centers of diversity for rice, and rice cultivation is done here from ages. It is estimated that many traditional varieties were cultivated in Chhattisgarh, across the three agro-climatic zones in the state. Worldwide known eminent IR64 rice variety worked as a great source for resistance to several rice varieties (Mackill and Khush, 2018)
Many studies have been done for the identification and introgression of the blast resistance genes in rice (Miah *et al*., 2017; Khan *et al*., 2018) [17, 15]. However, no prominent study has been done to detect these resistance genes in case of blast in Chhattisgarh rice varieties. On surveying the underutilized resource of resistant genes in the varieties of Chhattisgarh, the present study aimed to identify traditional rice varieties with blast resistance genes *Pi 1, Pi 2* and *Pi kh*, using associated functional markers, following a field level screening under disease stress condition.

Materials and Methods
The present study was conducted in the blast nursery of SG College of Agriculture and

Research Station, Jagdalpur and RMD College of Agriculture and Research Station, Ambikapur by using 73 rice genotypes which included germplasm lines, advanced breeding lines, land races and blast resistant lines from Agriculture Research station of Raipur under Indira Gandhi Agriculture University.

Field level Screening

Field level screening of all the rice genotypes including resistant varieties and susceptible checks were performed in the Blast screening nursery of two major blast hotspots of Chhattisgarh. i.e. SG College of Agriculture and Research Station, Jagdalpur and RMD College of Agriculture and Research Station, Ambikapur. The experiment was carried out in the rainy season of 2019. i.e. in the month of August, 2019.

A total of 73 rice genotypes were planted in both the research stations, one in the North of the state and second in the south of the state. In this experiment IR64 and HR 12 were used as resistant and check. Scanty rainfall along with sunshine creates a good humid weather to developed condia of the pathogen available in the air. To create a stress condition of Blast disease over the planted genotypes a continuous spraying of rotten infected leaf of blast were done in the interval of 20-30 days 2-3times in the whole screening season. This is done to create such an environment to check which line can survive in the extreme conditions of blast and on the basis of these susceptible tolerant and resistant lines can be easily identified.

Table 1: 0-9 grade disease rating scale used for screening of blast nursery

Grade	Disease severity	Host response
0	No lesion is observed	Highly Resistant
1	Small brown spots of pin point size. Typically susceptible blast lesions, 3mm or longer infecting 4-10% of the leaf.	Resistant
2	Small roundish to slightly elongated, necrotic gray spots, about 1-2 mm in diameter, with a distinct brown margin. Lesions are present in lower leaf surface.	Moderately Resistant
3	Lesion type same as in 2, but significant number of lesions are found on upper surface.	Moderately Resistant
4	Typically susceptible blast lesions, 3mm or longer infecting less than 4% of leaf.	Moderately susceptible
5	Typically susceptible blast lesions, 3mm or longer infecting 4-10% of the leaf.	Moderately susceptible
6	Typically susceptible blast lesions, 3mm or longer infecting 11-25% of the leaf.	Susceptible
7	Typically susceptible blast lesions, 3mm or longer infecting 26-50% of the leaf.	Susceptible
8	Typically susceptible blast lesions, 3mm or longer infecting 51-75% of the leaf affected.	Highly Susceptible
9	Typically susceptible blast lesions, 3mm or longer infecting more than 75% of the leaf affected.	Highly susceptible

Molecular level screening

DNA was extracted from the leaves of varieties following the procedure of Regowsky *et al.* (1991) [19] with minor modifications. Reported simple sequence repeat (SSR) markers specific to genes under study were used for Polymerase Chain Reaction (PCR) following Cordeiro *et al.* (2002) [8]. The reaction was carried out in 25 µl reaction mixture containing 25 ng genomic DNA, 1.5 Mm MgCl2, 200 µM total dNTPs, 1 unit of Taq DNA polymerase, 1x PCR buffer and 0.2 µM each of forward and reverse primer. Amplification was done in programmable thermo cycler programmed as follows: An initial denaturation at 94 ºC for 5 minutes followed by 35 cycles of 94 ºC for 1 minute, at annealing temperature for 1 minute and 72 ºC for 2 minutes; followed by a final elongation at 72 ºC for 5 minutes and a 4 ºC hold. Amplified products were separated by agarose gel electrophoresis using 1.8 percent gel and photographed using a gel documentation system.

With the progressing work of Blast Screening simultaneously some varieties were selected on the basis of prior knowledge and continuous blast screening observations also for molecular analysis.

1. IR64 (Resistant Check)
2. Meherdhan
3. Tetep
4. IR112301
5. R 2048-186-2-1251
6. SRD55
7. Safri 17-2-mutant
8. NK 17508
9. Til Komal mutant
10. Jaya
11. R- 2304-404-2315
12. HR12 (Susceptible check)

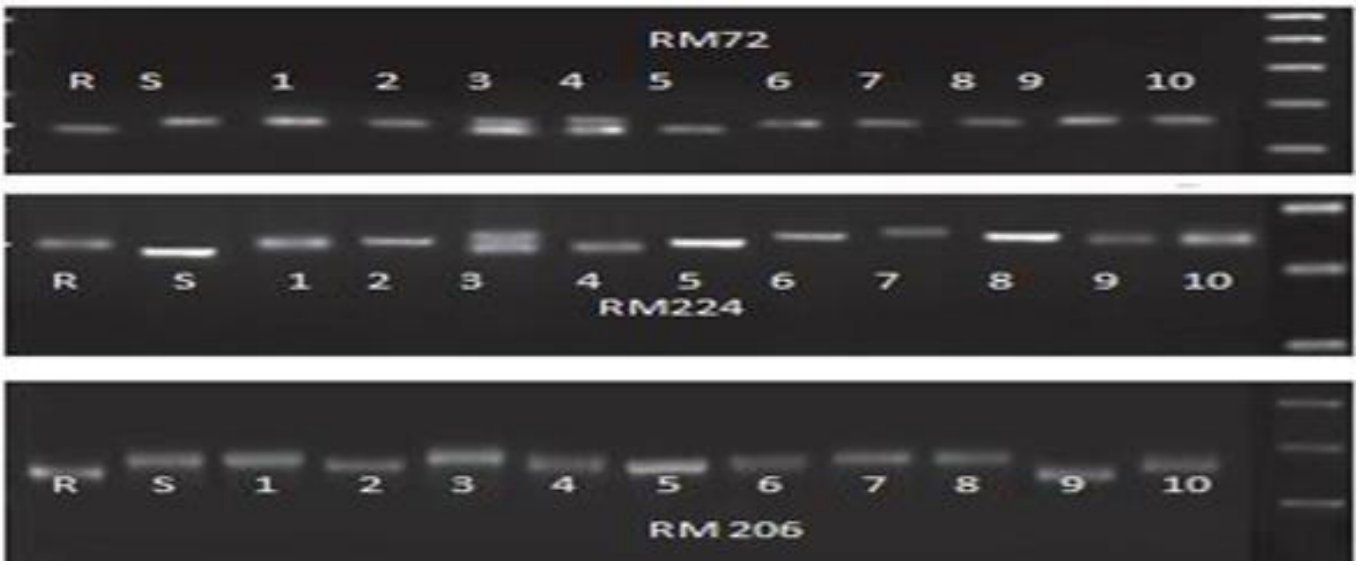
Table 2: Various genes under study, primers used for each gene and their amplification

Genes	Check variety	SSR Primer	Amplification base pair	References
Pi 1	Tetep	RM 224	157	Fuentes *et al*., 2008
Pi 33	IR64	RM 72	166	Singh *et al*., 2015
Pikh	Tetep	RM 206	147	Sharma *et al*., 2010; Prasad *et al*., 2012

Susceptible check variety was commonly used for all three genes
References are for the respective SSR primers and amplification base pair

Result and Discussion

In the present study, varieties with the presence of three blast resistance genes, *Pi 1*, *Pi33*and *Pi kh* were identified, which were reported to give broad-spectrum resistance to different races of *Magnaporthe oryzae* (Liu *et al*., 2002; Khan *et al*., 2018) [16, 15] and which have been widely used for the breeding of resistant cultivars (Madhavi *et al*., 2016). In this study, some varieties were found to be heterozygotes since seeds were collected from the plants grown in open condition. Result of molecular level screening for identification of different gene, Gene Pi1 - (IR64, IR112301, Tetep, Meher dhan, SRD55, R-2304-404-2315-1, Jaya, NK17508). (R 2048-186-2-1251). Gene Pi33 - (SRD55, IR64). (R 2304-404-2315, Safri-17-2- mutant). Gene Pikh – (Tetep, NK17508, IR11230, Tilkomal mutant, SRD55, R-2304-404-2315, Jaya. Gene combinations such as Pi1+Pikh+Pi33 were also found in sum varieties (SRD55, R- 2304-404-2315, IR11230) and combination of Pi1+Pi33 were also found (Tetep, Jaya).



Resistant Check - IR64, Tetep
Susceptible Check - HR12

1. IR112301
2. R 2048-186-2-1251
3. R-RF 105
4. SRD55
5. R 2304-404-2315-1
6. Safri 17-2-mutant2
7. Tilkomal mutant
8. Jaya
9. NK 17508

References

1. Ahn SN, Kim YK, Hong HC, Han SS, Kwon SJ, Choi HC, *et al*. Molecular mapping of new gene for resistance to rice blast (Pyricularia grisea) Euphytica1. 2000;16: 17-22.
2. Ahn SW, Ou SH. Quantitative resistance of rice to blast disease. Phytopathology. 1982;72:279-282.
3. AICRP. (All India Coordinated rice improvement programme). 2011. Varietal improvement. Progress report Directorate of rice research, Hyderabad. 2011;1:607p.
4. Aneja KR. Experiments in Microbiology Plant Pathology and Biotechnology. New age international publishers, Hyderabad, 2009, 607p.
5. Arunakanthi B, Srinivasprasad M, Madhanmohan K, Balachandran SM, Madhav MS, Reddy CS, *et al*. Introgression of major blast resistance genes Pi-1, Pi-2 and Pi-khin indica rice cultivars Samba Mahsuri and Swarna. J Mycol. Plant Pathol. 2008;38:625-630.
6. Ballini E, Morel JB, Droc G, Price A, Courtois B, Notteghem JL, *et al*. 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 Interaction. 2008;21:859-868.
7. Bonman JM, Mackill DJ. Durable resistance to rice blast disease. Oryza. 1998;25:103-110.
8. Cordeiro GM, Christopher MJ, Henry RJ, Reinke RF. Identification of microsatellite Markers for fragrance in rice by analysis of the rice genome sequence. Mol. Breed. 2002;9:245-250.
9. Costanzo S, Jia Y. Sequence variation at the rice blast resistance gene Pi-km locus: implications for the development of allele specific markers. Plant Sci. 2010;178:523-530.
10. Divya B, Robin S, Rabindran R, Senthil S, Raveendran M, Joel AJ. Marker assisted backcross breeding approach to improve blast resistance in Indian rice (*Oryza sativa*) variety ADT43.Euphytica. 2014;200:61-77. DOI: 10.1007/s10681-014-1182-5.
11. Eizenga GC, Agrama HA, Lee FN, Yan W, Jia Y. Identifying novel resistance genes in newly introduced blast resistant rice germplasm. Crop Sci. 2006;46:1870-1878.
12. Ghazanfar MU, Habib A, Sahi ST. Screening of rice germplasm against pyricularia oryzae the cause of rice blast disease. Pak. J. Phytopathol. 2009;21(1):41-44.
13. Hittalmani S, Mew T, Foolad MR, Huang N. Identification of blast resistance gene Pi-2(t) in a segregating population of Rice. Theor. Appl. Genet. 1995;91:9-14.
14. Hittalmani S, Parco A, Mew TV, Zeigler RS, Huang N. Fine mapping and DNA marker-assisted pyramiding of the three major genes for blast resistance in rice. Theor. Appl. Genet. 2000;100:1121-1128.
15. Khan GH, Shikari AB, Vaishnavi R, Najeeb S, Padder BA, Bhat ZA, *et al*. Marker – assisted introgression of three dominant blast resistance genes into an aromatic rice cultivar Mushk Budji. Sci. Rep. 2018;8(1):4091.
16. Liu G, Lu G, Zeng L, Wang GL. Two broad- spectrum blast resistance genes, Pi9(t) and Pi2(t), are physically linked on rice chromosome 6. Mol. Genet. Genomics. 2002;267:472-480.
17. Miah G, Rafii MY, Ismail MR, Pteh AB, Rahim HA, Latif MA. Marker assisted introgression of broad specrum blast resistance genes into the cultivated MR219 rice variety. J. Sci. food Agric. 2017;97:2810-2818.
18. Ou SH. Rice Disease. (2nd Ed.) Common Wealth Agricultural Bureaux, Central sales, Farnham Royal, Slough, U.K, 1985, pp.109-150.
19. Regowsky I, Mamnig S, Lin J, Langridge P. Small scale isolation of total DNA from cereals. Genome. 1991;34:88- 95.
20. Von Braun J. The world food situation: new driving forces and required actions. International Food Policy Research Institute, Washington DC, 2007, 18p.