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Morphological and Molecular Evaluation of Medium Slender (MS) Rice Genotypes for Leaf Blast Disease Resistance

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ABSTRACT: Over half of the world's population consumes rice as a staple food. *Magnaporthe oryzae* causes rice blast, which is one among the foremost destructive disease causing enormous yield losses to rice in several rice growing regions. Blast resistance is highly unreliable, with resistance frequently failing or weakening in field conditions, prompting a constant search for resistant donors/lines. The current research was carried out at Agricultural Research Station (ARS) Gangavati, to identify resistance among 22 medium slender rice genotypes alongside susceptible check HR-12 in uniform blast nursery (UBN). The disease reactions were recorded one week after inoculation, with Standard Evaluation Scale (SES) for leaf blast ranging 0-9, when the susceptible check (HR 12) was completely killed. None of the MS varieties shown resistant but three MS varieties *viz.* IET-26241, IET-25520 and Rp Bio-226 were shown moderately resistant reaction against blast. The five major blast resistant genes genetic frequencies varied from 28.57% (*Piz*-t) to 85.71%(*Pi*2) in the molecular evaluation of promising genotypes for major blast resistant genes using three STS and two SSR markers.

Keywords: Rice, Blast, *Magnaporthe oryzae*, MS, Genetic Frequency.

INTRODUCTION

Rice blast, caused by *Magnaporthe oryzae*, is one of the most common diseases of rice, with a global distribution and high destructive potential under favorable conditions. Rice blast disease was reported in China for the first time in 1637 (Rao, 1994). In South Carolina disease was recorded by the Metcalf and was the first to call the disease as early as in the year 1876 (Rao, 1994). Blast disease was recorded in India at Thanjavur delta of South India for the first time in 1918. However, when a devastating epidemic occurred in 1919 it attracted the attention (Padmanabhan, 1965).

Rice blast has been documented in nearly every rice-producing region of the world (Bonman, 1992; Ou, 1985) and this disease has been recorded from as many as 85 countries (Flores, 2008). Annually, rice blast causes about 10–30 per cent yield losses (Jiang *et al.* 2015). Infection of blast pathogen at seedling stage causes death of the whole plant, while in older plants it spreads to stem, nodes and panicle which may account for total loss (Talbot, 2003). Each year enough quantity of rice to feed 60 million people is being destroyed by rice blast disease (Zeigler *et al*., 1994). Between 1975 and1990, it is estimated that 157 million tonnes of rice were lost worldwide due to blast (Baker *et al*., 1997).

The severity of biotic stresses in rice production is increasing at a startling pace of late because of rapid changes in climate (Jamaloddin *et al.*, 2020). Hence, the use of resistant varieties is believed to be one of the most environmentally and economically efficient ways of crop protection. Fungicides used to combat rice blast result in higher production costs as well as toxic contamination of the environment and food (Sharma *et al*., 2012). Blast resistance was highly unreliable in the field, with resistance frequently failing or breaking down and is not long lasting because single resistance gene break down after three to five years of cultivar release due to strong pathogen plasticity (Lang *et al*., 2009).

Even though numerous resistant cultivars have been developed, resistance is not long lasting in the field (Devi *et al*., 2015). As a result, developing long-lasting blast-resistant cultivars is vital for addressing this

disease. By artificial inoculation, 22 MS rice genotypes were screened for blast resistance, along with a susceptible control (HR 12) in Uniform blast nursery (UBN) using Standard Evaluation Scale (SES) for leaf blast disease scoring, followed by molecular evaluation of promising genotypes for major blast resistant genes using three STS and two SSR markers.

MATERIALS AND METHODS

Plant Materials. A set of 22 medium slender rice genotypes and susceptible check (HR 12) were obtained from AICRIP- Rice Breeding, Agricultural Research Station, Gangavati and were screened phenotypically for blast resistance in uniform blast nursery (UBN) at Rice Pathology Laboratory.

Morphological evaluation for blast resistance. Each test entry was sown in a single 50cm long row with a 10cm row to row spacing respectively following uniform blast nursery (UBN) method (Fig. 1). To establish a strong disease pressure, the nursery was flanked on all sides by rows of susceptible check variety (Fig. 1). Blast pathogen isolates with mycelia were macerated in 5 mL distilled water before being plated onto sporulation medium. The plates were rinsed with 10ml of distilled water after 8 to 10 days of incubation at 25 ± 1°C to make a spore suspension. Spore suspension of pathogen (*Magnaporthe oryzae*) was adjusted to concentration of 1×10^5 spores/ml. A glass atomizer was used to spray 30-40 ml of spore solution with gelatin (0.1%) and Tween-20 (0.02%) onto 21-day-old seedlings. Blast disease symptoms were observed on inoculated plants one week after inoculation and scored for disease resistance and susceptibility when the typical blast lesions developed on each line using following standard 0-9 scale (IRRI - SES, 2013).

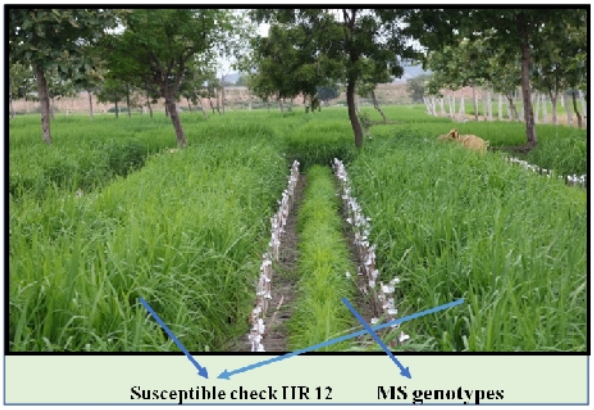


Fig. 1. View of uniform blast nursery (UBN) for screening of medium slender genotypes against leaf blast.

Table 1: Leaf blast disease resistance scoring system (IRRI, 2013).

Score	Disease reaction	Description
0	Highly resistant	No lesions observed
1	Resistant	Small brown specks of pin-point size or larger brown specks without sporulating centre.
2	Moderately resistant	Small roundish to slightly elongated, necrotic gray spots, about 1-2 mm in diameter, with a distinct brown margin.
3	Moderately resistant	Lesion type is the same as in scale 2, but a significant number of lesions are on the upper leaves.
4	Moderately susceptible	Typical susceptible blast lesions 3 mm or longer, infecting less than 4% of the leaf area.
5	Moderately susceptible	Typical blast lesions infecting 4-10% of the leaf area.
6	Moderately susceptible	Typical blast lesions infection 11-25% of the leaf area.
7	Susceptible	Typical blast lesions infection 26-50% of the leaf area.
8	Highly susceptible	Typical blast lesions infection 51-75% of the leaf area and many leaves are dead.
9	Highly susceptible	More than 75% leaf area affected.

Disease scoring for Rice Blast. After 25-30 DAS (1 week after inoculation) The test entries were evaluated on the severity of the leaf blasts using the SES scale (Table 1). Based on the blast severity the reactions of the lines are categorized into different categories of resistance and susceptibility (Table 2).

DNA Isolation. Leaf samples were taken from seedlings that were 20 to 25 days old and were stored immediately at –20°C till DNA was isolated. Genomic DNA was isolated from fresh, healthy and young leaves from 7 promising rice varieties following method of CTAB (Cetyl-Tri Methyl Ammonium Bromide) (Murray and Thompson, 1980). Genomic DNA samples were tested on 0.8% agarose gels to determine the quality and amount of DNA. This additional step would give us an idea on the extent of DNA shearing.

PCR and marker analysis. Two SSR and three STS markers were used for molecular validation of 7 MS rice varieties for rice blast resistance (Table 2). The primer sequences were derived from www.graminae.org and other previously published research on blast resistance genes and markers. The primer sequences were used and the oligos were synthesized from commercial facility (Eurofins, Bengaluru, India). Each polymerase chain reaction (PCR) was carried out in 10 µL reaction volume. A thermal cycler from Applied Biosystems was used to maintain the following temperature profiles and cycles 1 cycle at 95°C for 5 min (initial denaturation), followed by 34 cycles of at 95°C for 30 sec (Denaturation), annealing at 55-64°C (depending on primers) for 30 sec, extension at 72°C for 1 min, 1 cycle of final extension at 72°C for 10 min, and storage at

4°C. After completion of PCR, products were run on 3% agarose gel, prepared using 1X TE buffer and ethidium bromide. The DNA profile was documented using a gel documentation equipment after the electrophoresis was completed. (Essential V6, USA). Gel pictures were scored on the basis of expected bp for resistant allele, as 1 for presence and 0 for absence of resistant allele.

Table 2: Details of markers used for detection of respective R genes for leaf blast disease in PCR.

Sr. No.	Gene	Marker	Marker type	Annealing temperature	Forward primer	Reverse primer
1.	*Piz*-t	Zt56591	STS	58°C	TTGCTGAGCCATTGTTAAACA	ATCTCTTCATATATATGAAGGCCAC
2.	*Pi*2	*Pi*2-i	STS	60°C	CAGCGATGGTATGAGCACAA	CGTTCCTATACTGCCACATCG
3.	*Pi*9	*Pi*9-i	STS	60°C	GCTGTGCTCCAAATGAGGAT	GCGATCTCACATCCTTTGCT
4.	*Pi*33	RM72	SSR	62°C	CCGGCGATAAAACAATGAG	GCATCGGTCCTAACTAAGGG
5.	*Pi*37	RM212	SSR	60°C	CCACTTTCAGCTACTACCAG	CACCCATTTGTCTCTCATTATG

RESULTS AND DISCUSSION

A total of 22 MS rice cultivars, as well as a susceptible check, were tested in this investigation for their reaction to leaf blast disease resistance, none of the test entries shown to be highly resistant with a score of 0. Hence, none of the MS rice varieties evaluated under the present investigation were found to be either highly resistant or resistant. However, the least scores of 3 was recorded by three MS genotypes *viz.* IET-26241, IET-25520 and Rp-Bio 226 corresponded to moderate resistance. Further, ten medium slender rice genotypes namely GNV-1905, IET-27904, IET-27438, BPT mutant 1801, BPT mutant 1802, BPT mutant 1806, RNR-15048, Gangavati sanna, GNV 10-89 and GGV-05-01 were found to be moderately susceptible against leaf blast disease with phenotypic scores of 4 to 6. About five MSVs viz., GNV-1907, IET-27870, BPT mutant 1804, BPT mutant 1811 and BPT-5204 were found to show susceptible reaction to leaf blast disease with phenotypic scores of 7. Four medium slender rice types and susceptible check HR 12 had the highest susceptibility with phenotypic scores of 8 and 9 (Table 3). Similar method was followed by Sowmya *et al.,* (2014) for blast resistance testing of several landraces and found that HR 12 has a highly susceptible reaction to blast, which is consistent with our findings. Devi *et al.,* (2015), screened 326 ILs (Introgression lines) 50 ILs showed resistant reaction with a mean score of 0 to 3 and 276 were susceptible with a mean score of 4 to 9 for leaf blast resistance at DRR in three seasons (2010 to 2011), whereas both recipient parents showed high susceptibility. Yan, *et al.* (2017) screened set of 32 germplasm by artificial inoculation with *M. oryzae* under UBN (Uniform Blast Nursey at Zhejiang Province in China in the year 2012–2014. Disease reactions were scored from 0 to 9 at 35 days after sowing, when the susceptible checks CO39, Yuanfengzao, and LTH were completely killed. He discovered that one germplasm was susceptible, one was moderately resistant (N11, score = 4) and thirty germplasms were resistant (score 0–3) among 32 germplasms.

Table 3: Phenotypic scoring of medium slender genotypes for leaf blast disease resistance.

Sr. No.	Genotypes	Phenotypic score	Blast Reaction
1.	GNV-1905	6	MS
2.	GNV-1906	8	HS
3.	GNV-1907	7	S
4.	IET-27904	6	MS
5.	IET-27416	8	HS
6.	IET-27870	7	S
7.	IET-26241	3	MR
8.	IET-27438	4	MS
9.	IET-25520	3	MR
10.	BPT mutant 1801	6	MS
11.	BPT mutant 1802	5	MS
12.	BPT mutant 1804	7	S
13.	BPT mutant 1805	8	HS
14.	BPT mutant 1806	6	MS
15.	BPT mutant 1809	8	HS
16.	BPT mutant 1811	7	S
17.	RNR - 15048	6	MS
18.	Gangavati sanna	6	MS
19.	Rp-Bio 226	3	MR
20.	GNV 10-89	4	MS
21.	GGV-05-01	5	MS
22.	BPT-5204	7	S
	Susceptible check		
	HR12	9	HS

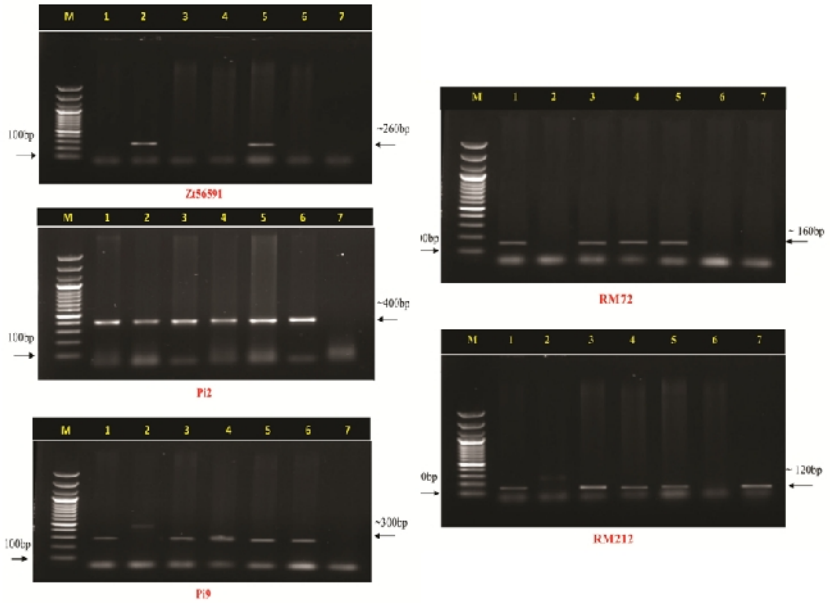
By genotyping accessions with allelic related markers, the major blast resistance genes from different origins can be identified. Rice blast resistance genes will be selected using markers to aid in the development of multi-disease resistant rice varieties. The results of genotypic screening of promising rice varieties for the presence or absence of five major rice blast resistance genes using three STS and two SSR markers shown in Table 4, and the electrophoresis pattern of each SSR and STS marker linked to the blast resistant gene presented in Fig. 2.

Table 4: Scores of promising genotypes for the presence of blast resistance genes following genotypic evaluation with markers.

Varieties	Marker, gene name with expected base pair size (bp) for resistance allele					Number of R genes present
	Zt56591	*Pi*2	*Pi*9	RM 72	RM 212	
	Piz-t	*Pi*2	*Pi*9	*Pi*33	*Pi*37	
	260	400	300	160	120	
IET 26241	0	1	1	1	1	4
IET 27438	1	1	0	0	0	2
GNV 10-89	0	1	1	1	1	4
IET 25520	0	1	1	1	1	4
Rp Bio 226	1	1	1	1	1	5
GGV-05-01	0	1	1	0	0	2
BPT 5204	0	0	0	0	1	1
Genetic frequency (%)	**28.57**	**85.71**	**71.42**	**57.14**	**71.42**	

The scores for the presence (1) and absence (0) of amplicon linked to three STS and two SSR markers

PCR results estimation for five genes governing blast resistance viz. *Piz*-t, *Pi*2, *Pi*9, *Pi*33 and *Pi*37 were resolved by visualization of amplicons. The primer Zt56591 was used to amplify the rice blast R gene and was observed by a product of 260-bp and was only detected in two rice genotypes. The primer *Pi*2 was used to amplify major rice blast R gene which yielded a 400-bp fragment and was detected in six genotypes. *Pi*9 primer was used to amplify *Pi*9 gene and observed as an amplicon of 300-bp and detected in five genotypes. *Pi*33 was detected with marker RM72 produced amplicon of 160-bp and observed in four rice genotypes. PCR-based screening of *Pi*37 showed that five genotypes produced band of 120-bp when amplified with RM212 primer.



M-Ladder (100bp), 1-IET 26241, 2- IET27438, 3-GNV 10-89, 4-IET-25520, 5-Rp Bio 226, 6-GGV-05-01, 7-BPT-5204

Fig. 2. Molecular profiling of promising genotypes resistant to leaf blast.

From comparative analysis of molecular identification and nursery screening for blast resistance, it was found that Rp Bio 226 gave moderately resistance reaction in phenotypic screening and has five resistance genes in molecular profiling. Similarly, IET-26241 and IET25520 gave moderately resistance reaction in phenotypic screening but has only four resistance genes. GNV 10-89 has four resistance genes for blast resistance but it showed moderately susceptible reaction in the phenotypic scoring. IET-27438 and GGV-05-01 showed moderately susceptible reaction and has two resistance genes. BPT-5204 has one resistant gene and

it showed susceptible reaction in phenotypic screening. Similar results were also reported previously by Yadav *et al.,* (2017), presence of several genes did not ensure resistance in some cases. This phenotypic variation could be due to the existence of a specific allele type in those lines, and the disparity between phenotypic and genotypic scoring could be attributable to environmental influences. Also, the genotypes with less number of genes showed moderately resistance reaction, this may be due to presence of genes conferring partial resistance which are more effective against blast pathogen. There may be cases of having some other resistance genes in the genotypes but not recognized with present set of primers.

CONCLUSION

In the current investigation a total of 22 varieties were tested along with susceptible check (HR 12) by artificial inoculation in uniform blast nursery (UBN) for blast disease resistance, results revealed that IET-26241, IET-25520 and Rp-Bio 226 were found to be moderately resistant and molecular identification of blast resistant genes in promising genotypes divulge that out of 5 primers, Rp-Bio 226 gave positive bands with all the primers. GNV 10-89, IET-25520 and IET-26241 gave positive bands for Pi2-i, Pi9-i, RM 72 and RM 212 primers. IET-27438 gave positive bands for Zt56591 and Pi2-i primers. GGV-05-01 gave positive bands for Pi2-i and Pi9-i. and BPT-5204 gave positive bands for RM212 primer.

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Conflicts of Interest. The results furnished in this paper were from my own research and there were no any conflicts from other research scholars or scientists.

REFERENCES

Baker, B. (1997). Signaling in plant-microbe interactions. *Science*, *276*(1): 726-733.

Bonman, J. M. (1992). Durable resistance to rice blast disease-environmental influences. *Euphytica*, *63*(3): 115-123.

Devi, S. J. R. S., Singh K., Umakanth B., Vishalakshi B., Renuka P., & Sudhakar K. V. (2015). Development and Identification of Novel Rice Blast Resistant Sources and Their Characterization Using Molecular Markers. *Rice Science*, *22*(6): 300-308.

Flores, A. (2008). New DNA markers for rice blast-resistance gene. *Agriculture Research*, *56*(8): 22-23.

Jamaloddin M., Durga Rani C. V., & Swathi, G. (2020) Marker assisted gene pyramiding (MAGP) for bacterial blight and blast resistance into mega rice variety "Tellahamsa". *PLoS One*, *15*: 0234088.

Jiang, J., Yang, D., Ali, J., & Mou, T. (2015). Molecular marker-assisted pyramiding of broad-spectrum disease resistance genes, *Pi2* and *Xa23*, into GZ63-4S, an elite thermo-sensitive genic male-sterile line in rice. *Moecular. Breeding*, *35*(1): 83.

Lang N. T., Luy T. T., Ha P. T. T., & Buu B. C. (2009). Monogenic lines resistance to blast disease in rice (*Oryza sativa* L.) in Vietnam. *International Journal of Genetics and Molecular Biology*, *1*(7): 127–136.

Murray, M. G., & Thompson, W. F. (1980) Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research*, *8*(1): 4321-4325.

Ou, S. H. (1985). *Rice Diseases. IRRI*.

Rao, M. K. (1994). Rice Blast Disease. *Daya Publishing house*, New Delhi, India, 1-10.

Sharma T. R., Rai A. K., Gupta S. K., Vijayan J, Devanna B. N., & Ray S. (2012). Rice Blast Management Through Host-Plant Resistance. *Retrospect and Prospects. Agriculture Research*, 1(1):37–52.

Padmanabhan, S. Y. (1965). Estimating losses from rice blast in India. The rice blast disease: *IRRI*, *3*(1): 203-221.

Sowmya, H. M., Prashanthi, S. K., & Hegde, Y. R. (2014). Traditional landraces of rice for blast (*Magnaporthe oryzae*) resistance and analysis of biochemical components involved in disease reaction. *Asian Journal of Bio Science*, *9*(2): 261-266.

Talbot, N. J. (2003). On the trail of a cereal killer: exploring the biology of *Magnaporthe grisea*. *Annual Review of. Microbiology*, *57*(2): 177-202.

Yadav, M. K., Ngangkham, U., Subudhi, H. N., Bag, M. K., Adak, T., Munda, S., Samantaray, S., & Jena, M. (2017). Correction: Use of molecular markers in identification and characterization of resistance to rice blast in India. *Plos one*, *12*(6): 179-184.

Yan, L., Yuan Y. B., Liang P. Y., Juan J. Z., Xiang Z. Y., & Lin, W. H. (2017). Molecular Screening of Blast Resistance Genes in Rice Germplasms Resistant to *Magnaporthe oryzae*. *Rice Science*, *24*(1): 41-47.

Zeigler, R. S., Leong, S. A., & Teng, P. S. (1994). *Rice Blast Disease*, *30*(1): 56-58.

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