Electronic Journal of Plant Breeding,
ISSN 0975-928X

DOI: 10.5958/0975-928X.2016.00003.X

# Identification of blast resistant rice (*oryza sativa* l.) genotypes in indigenous and exotic germplasm and validation of *pi* gene linked molecular markers

**Thippeswamy. S*, Y. Chandramohan, Zameema Samreen, B. Srinivas, D. Padmaja and K. Pravalika**
Rice Research Scheme, Regional Agricultural Research Station,
Professor Jayashankar Telangana State Agricultural University,
Polasa-505 529, Jagtial, Karimnagar (Dist.), Telangana, India.
* Email: thippeswamys@yahoo.com

(Received: June 2015; Accepted: June 2015)

**Abstract**
Rice Blast, a most devastating disease of Rice caused by *Magnaporthe grisea* can be effectively managed by use of resistant rice genotypes. Availability of resistant donors and validated molecular markers are essential to develop resistant cultivars against different races and to pyramid the resistant genes, In the present investigation, 312 indigenous and 65 exotic germplasm lines were evaluated against blast resistance at RARS, Jagtial. More percentage (83%) of exotic germplasm showed resistance to rice blast disease compared to indigenous germplasm (46%). Three genotypes (JGL23710, JGL23713 and JGL23714) in indigenous germplasm and two genotypes (IR09N500 and IR12M101) in exotic germplasm were immune to rice blast disease. "These can be used as donor genetic stock for development of highly resistant rice cultivars with high yields". Among five linked markers studied for *Pi-1* gene, one marker RM6094 was able to identify resistant genotypes at allelic level and for *Pi-2* gene, RM527 was validated in four genotypes out of six genotypes used. This information will help rice breeders to improve the resistance to rice blast by marker assisted selection.
**Key words:** Rice, Blast resistant germplasm, marker validation, Marker Assisted Selection.

## Introduction

Rice is the most important food security crop and staple food of half of the world's population. Major increase in rice production occurred during the past four decades as a result of adoption of green revolution technology. Any decline in its acreage and production will have a perceivable impact on the state's economy and food security. However, the rate of increase in rice production has slowed down. It is estimated that we will have to produce 30% more rice in 2030. For this purpose, we need rice varieties with higher yield potential and greater yield stability. Breakdown of blast resistance is the major cause of yield instability in several rice growing areas. Further, chemicals are pollutants and hazardous to the environment. Exploitation of host plant resistance is the most reliable and environment friendly method of disease management. In this direction, a number of resistance genes have been identified from different sources. Resistance is considered durable when it remains effective in a cultivar despite wide spread cultivation in an environment favoring the disease. Durable resistance may be controlled by a single gene, multiple genes with cumulative effects or poly genes, and the resistance produced may be either complete or incomplete (partial). Development and use of blast-resistant varieties is the most practical and economical approach to control blast. The use of resistant cultivars is the most economically viable and effective way of controlling rice blast but the useful life span of many cultivars is only a few years in disease conducive environments because of the breakdown of resistance due to high pathogenic variability and the breeding of cultivars with more durable resistance has become a priority in rice improvement programs. Incorporating different resistance genes into new varieties besides efficient deployment strategies using varieties with different resistance genes are the most efficient and cost effective approaches to manage these rice diseases.

Blast resistance in rice has been targeted at international and national levels but a lot has to be done at regional level especially in the rice growing areas of South India. Being a traditional crop in south India, modern varieties in rice mainly include fine and super fine varieties. Unfortunately, most of the commercial varieties like BPT5204 and Swarna are

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vulnerable to blast. There is a need to identify effective resistant donors for local isolates. Use of molecular marker helps in pyramiding of resistant genes. Till date, over 80 major resistance *Pi* genes have been identified in rice germplasm worldwide (Ballini et al. 2008, Jia and Moldenhauer, 2010). Among these, *Pi-1* and *Pi-2* are known to give durable resistance (Hittalmani *et al.* 2000). Though there are many linked markers identified, there is need to validate them in the donors. This experiment aims at identification of resistant genotypes by screening 312 indigenous and 65 exotic genotypes and validating six SSR markers linked to two blast resistant genes (*Pi1* and *Pi2*). This helps in improving popular varieties with genetic resistance against the blast pathogens using precision selection tools of MAS in conjunction with conventional convergent breeding techniques.

## Materials and Methods

The present investigation was conducted at Rice Research Scheme, Regional Agricultural Research Station, Polasa, Jagtial, Karimnagar, India during late *Kharif* 2013 and rabi 2013-14. The experimental material comprised of 312 indigenous germplasm lines (most of them developed in Rice Research Scheme, Jagtial and popular varieties) and 65 exotic rice accessions developed at International Rice Research Institute (IRRI, Manila Philippines).

### Blast disease screening

A total of 312 indigenous Jagtial rice germplasm lines were dry sown on 9$^{th}$ August 2013 in blast screening nursery. Each genotype was sown in single row of 2m length with 25cm row to row spacing and spreader rows (seed mixture of susceptible entries like HR12, JGL3844, JGL11118 and Swarna) were sown all along the border and after every 20 entries. Artificial inoculation of the blast isolates was performed in nursery to ensure the disease incidence. Disease scores were recorded after 18 to 21 days of inoculation, using standard evaluation system for rice with 0–9 scale (IRRI, 2002). Genotypes were scored as **0** = no evidence of infection/lesions, **1** = small brown specks of pin-point size or larger brown specks without sporulating (indicative of hypersensitive reaction); **2** = small roundish to slightly elongated, necrotic grey spots, about 1-2 mm in diameter, with a distinct brown margin; **3** = significant number of grey spots on upper leaves; **4** = typical susceptible blast lesions 3 mm or longer, infecting less than 4% of the leaf area; **5** = typical symptoms infecting 4-10% leaf area; **6** = typical blast symptoms on 11 - 25% leaf area; **7** = spindle-shaped lesions with a grey centre covering 26-50% leaf area; **8** = blast symptoms covering 51 - 75% of leaf area; **9** = lesions covering >75% leaf area and one or more leaves killed by coalescence of lesions.

The genotypes that were scored with a reaction of <3 were classified as resistant (R), whereas genotypes with a reaction of >3 – 5 were classified as moderately resistant (MR) and genotypes scored with a reaction of >5 were classified as susceptible (S) types.

Sixty five exotic genotypes were screened during rabi2013-14 at Rice Research Scheme. These were directly sown on raised beds on 10$^{th}$ December 2013 and spreader rows were sown all along the border and after every 20 entries. Resistant and susceptible entries (IRBN64 and 65, local resistant and susceptible checks) were sown at regular interval. Screening and scoring was done as explained earlier.

### Marker validation

Six molecular markers reported to be linked to two blast resistance genes (*Pi-1* and *Pi-2*) were studied using six identified (four resistant and two susceptible) exotic genotypes (IRBLA-C, B40, IR09N500, IR10A228, IR12M101 and IRBL5-M) during rabi, 2013-14. Based on the centi-Morgan (cM) distance between gene and the marker, closely linked markers were identified and used for polymerase chain reaction (PCR) analysis (Table 1). DNA was isolated using mini-preparation method (Thippeswamy *et. al.,* 2014) by collecting young leaves. The integrity of DNA was judged through gel analysis by casting 0.8% agarose gel in 1X TBE (Tris Borate EDTA) buffer containing 3µl of Ethidium Bromide at 100 Volts.

PCR amplification was carried out in 20 µl reaction volume containing 20 ng genomic DNA, 1X PCR buffer (Tris with 1.5 mM $MgCl_2$), 50 µM dNTP (2.5mM each dNTP), 5pM of each forward and reverse primer, 0.5 units of *taq* polymerase enzyme. Amplification was performed in a thermal cycler (Eppendorf, USA) with a program of initial denaturation at 94°C for 5 minutes, cyclic denaturation at 94°C for 2 minute, primer annealing at 50 - 54°C (vary from marker to marker) for 1 minute and primer extension at 72°C for 2 minute. The cycle was repeated 40 times and ended with the final extension at 72°C for 10 minutes. The amplified PCR products were resolved in gel electrophoresis on 3.0% agarose gel (Lonza, USA) along with 50 bp molecular marker (Bangalore Genie, India), stained with ethidium bromide and documented using gel

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documentation system (Alpha Innotech, USA). The genotypic dataset was generated based on the PCR amplification profile by scoring presence and absence of specific allele with specific base pair (bp) size for all the samples and markers.

**Results and discussion**

Among the most devastating diseases that constrain rice production, Rice Blast ranks first because of its wide distribution and high incidence under favourable conditions. Although many resistant varieties have been developed, due to genetic plasticity in the pathogen genome, there is a continuous threat to the effectiveness of the developed cultivars (Patil *et al.*, 2013). To breed rice varieties with more durable blast resistance, multiple resistance utilizing both qualitative and quantitative genes must be incorporated into individual varieties (Joshi *et al*., 2009). Identification of new donors is very important in development of resistant cultivars.

Blast screening during both the seasons was most effective which was indicated by blast disease score of 9 in spreader rows and susceptible genotypes (Swarna and HR12) (Fig.1). Blast disease ranged from 0 (JGL23710, JGL23713 and JGL23714) to 9 (JGL20995, JGL15324, JGL15644, JGL17970, JGL18080, JGL18079, JGL1834, JGL17670, JGL19610, JGL19612, JGL20779, JGL21831, JGL23179, JGL23181, JGL23770, JGL23773, JGL23815, JGL22297, JGL23178, JGL23763, JGL22268, JGL22277, JGL23634, JGL22284, JGL22285, JGL21828, JGL22281 and JGL23832) in indigenous germplasm and 0 (IR 09N500 and IR 12M101) to 7 (IRBL5-M and IR 1552) in exotic germplasm (Supplementary Table 1 and 2). In indigenous germplasm, out of 312 genotypes, 46 per cent (143) of genotypes were resistant, 26 per cent (82) were moderately resistant and 28 per cent (87) of them were susceptible. Whereas in exotic (IRRI) germplasm most of the genotypes showed resistance to local race of blast fungus (83%, 54 genotypes), 14 per cent of them showed moderately resistant and remaining four percent (2 genotypes) were susceptible (Table 2). These results indicate potentiality of exotic germplasm for blast resistance breeding. More percentage of resistant genotypes in exotic germplasm may be due to the fact that these were the part of International rice blast screening nursery. Strengthening of breeding programs and use of International Network of Genetic Evaluation of Rice (INGER) material has been reported in Egypt by Badawi and Draz (2004).

Three genotypes (JGL23710, JGL23713 and JGL23714) in indigenous germplasm and two genotypes (IR09N500 and IR12M101) in exotic germplasm were immune to rice blast disease (Data not shown). The resistant genotypes can be utilised as donor genetic stock for development of highly resistant rice cultivars with high yields. Screening of germplasm against the rice blast pathogen led to identification of potential donors in Nepal (Chaudhary *et al.*, 2004).

Three immune genotypes in indigenous germplasm were developed from the same pedigree (JGL13595xVD62) and many lines were utilized in development of 33 highly resistant indigenous genotypes. Lines showed resistance though they were derived from moderately resistant and susceptible genotypes. This may be due to involvement of multiple genes and interaction between them.

Use of molecular marker helps in pyramiding of resistant genes into a high yielding cultivar. Most of the 80 blast genes (*Pi*) identified in rice germplasm worldwide are dominant in nature (Fjellstrom *et. al.*, 2004; Ballini *et al*., 2008 and Jia and Moldenhauer, 2010). Among these *Pi-1* and *Pi-2* are known to give durable resistance (Hittalmani *et al*., 2000 and Thippeswamy *et. al.*, 2006). Though there are many linked markers identified, there is need to validate in the donors. In the present investigation, one SSR marker RM6094, out of five linked markers (RM5926, RM7654-H, RM7654-2, RM6094) to *Pi-1* gene is able to detect resistant and susceptible genotypes with high precision (Table 3 and Fig. 2). Another marker RM5926 was able to identify all four resistant genotypes but failed to identify susceptible genotypes, because resistant allele of RM5926 was present in the one of the two susceptible genotypes. RM7654-2 allele linked to *Pi-1* gene was present in three out of four resistant genotypes and was absent in one resistant (IR 12M101) and two susceptible genotypes. Among five linked markers to *Pi-1* gene, RM7654-H was not able to identify resistant and susceptible genotypes as the linked allele was present in two resistant and two susceptible genotypes.

Only one marker (RM527) linked to *Pi-2* blast resistant gene was used for validation with susceptible and resistant genotypes. Resistant allele of RM527 was present in two resistant genotypes (IRBLA-C and IR 09N500), while it was absent in two other resistant genotypes (IR 10A228 and IR 12M101). Resistance in these two genotypes may be due to the presence of *Pi-1* or other resistance genes

DOI: 10.5958/0975-928X.2016.00003.X

other than *Pi-2*. Two markers validated in the present investigation RM6094 for *Pi-1* gene and RM527 for *Pi-2* gene will be more useful for marker assisted pyramiding of resistant genes in developing durable blast resistant cultivars.

**Acknowledgement**

The authors wish to acknowledge the International Rice Research Institute (IRRI, Manila), for providing material as part of International Rice Blast Nursery.

**References**

Badawi, A. T. and Draz, A. E. 2004. A report on the role of the International Network for Genetic Evaluation of Rice (INGER) in rice breeding in Egypt. *Egyptian J. Agri. Res*., 82 (1): 57 - 72.

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.

Chaudhary, B., Shrestha, S. M. and Sharma, R. C. 2004. Pathogenic variability of rice blast isolates in Nepal. *Int. Rice Res. Notes*., 29 (2): 47 - 49.

Conaway-Bormans, C.A., Marchetti, M.A., Johnson, C.W. McClung, A.M and Park, W.D. 2003. Molecular markers linked to the blast resistance gene *Pi-z* in rice for use in marker-assisted selection. *Theor. Appl. Genet*., 107: 1014–1020.

Fjellstrom, R., Conaway-Bormans, C.A., McClung, A., Marchetti, M.A., Shank, A.R., Park, W.D. 2004. Development of DNA markers suitable for marker assisted selection of three Pi genes conferring resistance to multiple *Pyricularia grisae* pathotypes. *Crop Sci*., 44:1790–1798.

Fuentes, J. L., Correa-Victoria, F. J., Escobar, F., Prado, G., Aricapa, G., Duque, M. C. and Tohme. J. 2008. Identification of microsatellite markers linked to the blast resistance gene Pi-1(t) in rice. *Euphytica*, 160: 295—304.

Hittalmani, S., Parco, A., Mew, T. V., Zeigler, R. S. and Huang, N. 2000. Fine mapping and DNA marker-assisted pyramiding of the three major genes for blast resistance in rice. *Theor. Appl. Genet.*, 100: 1121—1128.

IRRI (International Rice Research Institute). 2002. Leaf blast. In. Standard Evaluation System for Rice, 5th edn. International Rice Research Institute, Manila, Philippines.pp.15.

Jia, Y. and Moldenhauer, K. 2010. Development of mono and digenic rice lines of rice blast blast resistance gene Pi-ta, Pi-k (s/h). *Plant Reg.*, 4: 163- 166.

Joshi, B. K., H. P. Bimb, G. Parajuli, and B. Chaudhary. 2009. Molecular tagging, allele mining and marker aided breeding for blast resistance in rice. *BSN e-Bulletin*, 1: 1 - 23.

Patil K G., Surapaneni Saikumar, Varma, C. M. K., Kancharla Nagesh, Sanka Thippeswamy, Vinay Shenoy, Ramesha, M. S. and Shashidhar, H. E. 2013. Marker-assisted breeding of *Pi-1* and *Piz-5* genes imparting resistance to rice blast in PRR78, restorer line of Pusa RH-10 Basmati rice hybrid, *Plant Breed.*, 132: 61–69.

Thippeswamy, S., Nagesh, K. and Reddy, C. S. 2006. Identification of effective blast resistance genes and validation of linked molecular markers in rice. In proceedings of "International Symposium on Frontiers in Genetics and Biotechnology-Retrospect and Prospect, Osmania University, Hyderabad, India. P-III-18.

Thippeswamy, S., Chandramohan, Y., Madhavilatha, B., Pravalika, K., Zameema Samreen, Vinod, G. and Kalpana. E. 2014. Identification of gall midge resistant parental lines and validation of fertility restoration linked markers for hybrid rice technology. *Electron. j. plant Breed.* **5**(3): 415-427.

Electronic Journal of Plant Breeding,
ISSN 0975-928X

DOI: 10.5958/0975-928X.2016.00003.X

**Table 1:** List of markers linked to *Pi-1* and *Pi-2* genes used for validation
*Note: + presence of allele*

| Sl. No | Marker | Linked gene | Chromosome No. | Forward Sequence (5'----------3') | Reverse Sequence (5'-------3') | Linked allele | References |
|---|---|---|---|---|---|---|---|
| 1 | RM5926 | *Pi-1* | 11 | ATATACTGTAGGTCCATCCA | AGATAGTATAGCGTAGCAGC | 176 | Thippeswamy *et al.*, ,2006 |
| 2 | RM 6094 | *Pi-1* | 11 | TGCTTGATCTGTGTTCGTCC | TAGCAGCACCAGCATGAAAG | 182 | Fuentes *et al.*, 2008 |
| 3 | RM7654-A | *Pi-1* | 11 | CAAAAGTCTGACCGTTTACC | CTCATGGTTGTGTCGTGGTC | 193 | Fuentes *et al.*, 2008 |
| 4 | RM7654-H | *Pi-1* | 11 | CTCATGGTTGTGTCGTGGTC | GTGCAGTGCCAGTGGTACG | 173 | Fuentes *et al.*, 2008 |
| 5 | RM7654-2 | *Pi-1* | 11 | GTGTCGTGGTCGTAACTTG | TAAGAGACGGAAGAGTGAGC | 216 | Fuentes *et al.*, 2008 |
| 6 | RM527 | *Pi-2* | 6 | GGCTCGATCTAGAAAATCCG | TTGCACAGGTTGCGATAGAG | 233 | Conaway-Bormans *et al.*, 2003 |

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ISSN 0975-928X

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**Table2.** Number of indigenous and exotic genotypes with different blast scores

| Blast score | Disease reaction | Indigenous | | Exotic | |
|---|---|---|---|---|---|
| | | No. of genotypes | % of total genotpes | No. of genotypes | % of total genotypes |
| 0 | R | 3 | 1 | 2 | 3 |
| 1 | R | 30 | 10 | 9 | 14 |
| 2 | R | 37 | 12 | 29 | 45 |
| 3 | R | 73 | 23 | 14 | 22 |
| 4 | MR | 43 | 14 | 4 | 6 |
| 5 | MR | 39 | 13 | 5 | 8 |
| 6 | S | 11 | 4 | 1 | 2 |
| 7 | S | 45 | 14 | 1 | 2 |
| 8 | S | 3 | 1 | - | - |
| 9 | S | 28 | 9 | - | - |

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ISSN 0975-928X

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**Table 3.** Screening of blast resistant and susceptible genotypes for presence of SSR markers alleles linked to the resistant *Pi-1* and *Pi-2* genes.

| Genotype | Blast Score | Disease Reaction | Loci | RM 5926 | RM7654-H | RM7654-2 | RM6094 | RM7654-A | RM527 |
|---|---|---|---|---|---|---|---|---|---|
| | | | Linked loci (bp) | 176 | 173 | 216 | 182 | 193 | 233 |
| | | | R-gene | *Pi-1* | *Pi-1* | *Pi-1* | *Pi-1* | *Pi-1* | *Pi-2* |
| IRBLA-C | 1 | R | | + | + | + | + | + | + |
| B40 | 9 | S | | | + | | | | |
| IR 09N500 | 0 | R | | + | | + | + | | + |
| IR 10A228 | 3 | R | | + | | + | + | | |
| IR 12M101 | 0 | R | | + | + | | + | | |
| IRBL5-M | 7 | S | | + | + | | | | |

Electronic Journal of Plant Breeding,
ISSN 0975-928X

DOI: 10.5958/0975-928X.2016.00003.X

**Figure 1**. Typical symptoms of Rice blast in susceptible genotypes

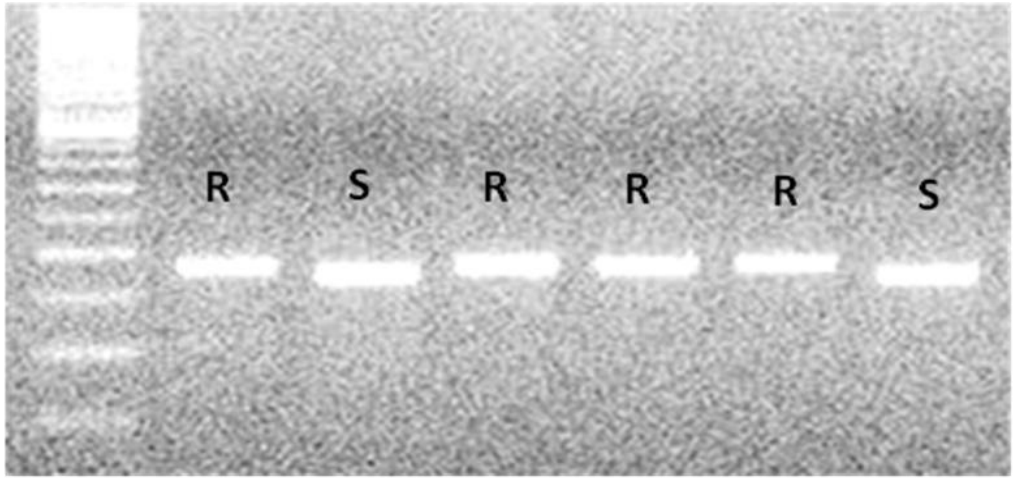


Figure 2. PCR amplification profile of RM6094 linked *Pi-1* gene (lane 1- 6 are genotypes shown in Table 3)

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