

Evaluation of DNA Markers Linked to Blast Resistant Genes, *Pikh*, *Pit(p)*, and *Pita*, for Parental Selection in Sri Lankan Rice Breeding

W.A.D. Jayawardana, G.A.U. Jayasekera^{1*}, R.L.C. Wijesundera¹
D.M.N. Dissanayake², S. D. S. Sooriyapathirana³, C.K. Weebadde⁴, K.L.N.S. Perera⁵
K.R.D. Gunapala⁶ and P. Hettige¹

Department of Plant Sciences
Faculty of Science
University of Colombo
Colombo 03, Sri Lanka

ABSTRACT: *Rice blast, is a serious disease affecting rice, caused by a fungal pathogen Magnaporthe grisea (anamorph - Pyricularia grisea). This paper summarizes the progress and the achievements made in detection of blast resistance genes in phenotypically evaluated, selected rice cultivars in Sri Lanka based on simple sequence repeats (SSR) DNA markers. In the present study blast pathogen was identified by morphological characters, molecular techniques which confirmed the identity as M. grisea. Total of 34 rice varieties including several Sri Lankan rice varieties and IRRI recommended rice varieties were evaluated for blast resistance. Total of 17 varieties were susceptible to the fungal infection and 17 varieties remained resistant. Tetep, Bg359, Bg304, IR64, Bg352, Bg357 were found to be highly resistant to the rice blast disease, while LD125, Bg94/1, Bw267-3, Bw451, Bw400, H7, Bg34-6, H10, IRR119 were highly susceptible. In marker genotyping, most of the highly resistant and resistant varieties demonstrated the presence of blast resistant Pita gene of variety Tetep and Pita gene was absent in the highly susceptible varieties. But Pita gene was absent in some of the resistant varieties like Bg300, Bg348 and Ptb33 and present in susceptible varieties H10, Bg276-5 and IR07F291. For polymorphism of Pikh gene most of the resistant varieties depicted the band lengths of 161-171bp. Susceptible varieties gave the band length of 151bp. According to results of marker genotyping with Pit(p) blast resistant gene, the resistant varieties gave a band length of 117bp while susceptible varieties produced the band length of 111bp. Hence, resulted polymorphism findings can be used in future studies to differentiate the resistant and susceptible varieties.*

Keywords: F2 population, *Magnaporthe grisea* (anamorph - *Pyricularia grisea*), marker genotyping, phenotypic screening, rice blast resistance.

¹ Department of Plant Science, Faculty of Science, University of Colombo, Colombo 03, Sri Lanka.

² Ministry of Agriculture, Battaramulla, Colombo, Sri Lanka

³ Department of Molecular Biology and Biotechnology, Faculty of Science, University of Peradeniya, Sri Lanka.

⁴ Department of Horticulture and Institute of International Agriculture, Michigan State University, United State of America.

⁵ Genetech molecular diagnostics and school of gene technology, Colombo 8, Sri Lanka.

⁶ Rice Research and Development Institute, Batalagoda, Ibbagamuwa, Kurunagala, Sri Lanka

* Corresponding author: jayasekeraanil@yahoo.com

INTRODUCTION

Rice blast, caused by the fungal pathogen *Magnaporthe grisea* (anamorph- *Pyricularia grisea*) is a serious disease affecting rice growing regions in Sri Lanka and in the world (Rossman *et al.*, 1990). The disease has been reported in over 85 countries across the world. Blast generally considered as the most destructive disease of rice due to its widespread distribution and potential to cause up to 50% yield loss when conditions are favourable for its occurrence (Skamnioti and Gurr, 2009). Rice blast is estimated to cause production losses amounting to US\$ 55 million each year in South and Southeast Asia. The fungus causes lesions on leaves, stems, peduncles, panicles, seeds, and even roots (Mithrasena *et al.*, 2012). The fungus can infect the rice from seedling stage through maturity. Other grasses, including crabgrass, are infected with closely related fungi of *Magnaporthe* family (*Magnaporthe grisea*, *Magnaporthe poae*, *Magnaporthe rhizophila* and *Magnaporthe salvinii* etc.), which causes nearly identical symptoms on their respective hosts (Sharma *et al.*, 2002). Rice blast is controlled usually by use of fungicides that results in high production costs and environmental pollution (Ghazanfar *et al.*, 2009).

Breeding of rice varieties with effective and sustained resistance to blast is considered as the most economical and environmentally sound strategy for management of rice blast (Mackill and Bonman, 1992). In Sri Lanka traditional rice varieties grown prior to 1950' s were highly susceptible to blast disease. Therefore, incorporation of blast resistance into new varieties through rice hybridization was incepted and resistant varieties were released. However the rapid breakdown of varietal resistance to blast has been possible and appears to be a result of emergence of virulent races within pathogen population and it indicates the narrow genetic diversity for blast resistance within these varieties that have been cultivated over the years in Sri Lanka (Dissanayake, 1995). Therefore, rice breeders have directed their efforts, towards enhancing durable blast resistance in new improved rice varieties via use of genetically diverse blast resistance sources. To breed rice varieties with more durable blast resistance, multiple resistance genes with qualitative and quantitative gene effect must be incorporated into individual rice varieties. Conventional methods currently deployed by rice breeders in Sri Lanka for identification blast resistance from rice germplasm and progenies in breeding programs are based on phenotypic screening (Jayawardana *et al.*, 2013). However phenotypic screening is influenced by environmental conditions and is cumbersome to detect and expensive to practice, because it requires the use of large field spaces. This necessitates the exploration of more efficient selection and breeding strategies than those currently in practice. Identifying blast resistant genes with DNA markers that are tightly linked to them, which can act as molecular tags will save money and time (Joshi *et al.*, 2009). Upon identification of polymorphic microsatellite markers closely linked to desirable characters marker aided selection (MAS) can be performed for selection of sources of resistance in early segregating generations and at early stages of plant development. These DNA marker tags can be used for direct identification of resistant genes when they are transferred from one varietal background to another during breeding programs (Fijellstorm *et al.*, 2002). The recent achievements made in DNA marker aided technology may provide new ways for developing durable blast resistant varieties with desirable traits in rice.

This paper summarize the progress and achievement made in detection of blast resistance genes in phenotypically evaluated, selected rice cultivars in Sri Lanka based on simple sequence repeats (SSR) DNA markers which help in developing durable blast resistance cultivars that can overcome destructive rice blast disease in Sri Lanka. In this research, to identify polymorphism in Sri Lankan rice germplasm, simple sequence repeats markers (*RM206*, *RM246*) and gene marker (*YL87/YL155*) were incorporated based on a genealogy

study that was conducted on the highly blast resistant variety *Tetep* by us prior to this study. It identified several IR varieties such as IR64, IR24, IR8, IR36 and mainly a Vietnamese variety *Tetep* (Sharma *et al.*, 2012) contributed blast resistant genes to Sri Lankan germplasm. The markers *RM206*, *RM246* and *YL87/YL155* are linked to the blast resistant genes, *Pita*, *Pit (p)* and *Pikh* respectively in variety *Tetep* (McCouch *et al.*, 1997). The ultimate objective of present study was to report the genetic basis of Sri Lankan rice germplasm using these genetic markers that will enable the rice breeders to have a better overview on blast resistance when transferring genes from one varietal background to another in future marker aided breeding program in Sri Lanka.

MATERIALS AND METHODS

Isolation and identification of rice blast fungus

The infected leaves of rice plants were cut in to small pieces (0.5-1 cm) and kept on a moist filter paper in petri plates and incubated for one day at room temperature. Blast lesions were carefully observed under a light microscope and fungal spores were identified and transferred into a specific medium called Rice Polish Agar (RPA) that enhances the fungal sporulation (Mithrasena *et al.*, 2012). The fungus was purified by sub culturing on PDA and RPA medium. Morphological characteristics of the fungus were observed under the light microscope.

For the molecular identification, fungal DNA was extracted using Promega Wizard DNA extraction kit and subjected to PCR using forward and reverse primers for internally transcribed spacers (*ITS1*) and (*ITS4*) respectively (Guillamon *et al.*, 1998). PCR products were size separated in 2 % agarose gel. DNA bands were excised and purified using Promega PCR product agarose gel purification kit. Purified DNA was sequenced using a Big Dye terminator v3.1 cycle sequencing kit and an ABI-310 Genetic Analyzer. The obtained sequence was entered to BLAST program and BLASTn analysis was carried out.

Phenotypic screening of rice varieties

Total 34 rice varieties including Sri Lankan rice varieties and International Rice Research Institute (IRRI) recommended rice varieties were obtained from Rice Research and Development Institute (RRDI), *Bathalagoda*. The selected rice varieties were established in the field of upland blast nursery at *Bathalagoda* according to the complete randomized design Fig. 1. Three replicates for each rice variety were maintained. A total of 144 rice lines including *Pachchaiperumal* rice lines were included. All these rice lines were surrounded by a highly susceptible *Pachchaiperumal* rice line in order to enhance the level of infection and better scoring. Prior to the infection, nitrogen level of the field was increased by adding nitrogen fertilizer to the established field for the proper establishment of the disease (Dissanayake, 1995).



Fig. 1. Established rice field for scoring blast disease

After two weeks of the field establishment of rice varieties, infection was conducted by using both dry leaf spore suspension of fungus *M. grisea* and blast infected leaves of *Pachchaiperumal* plants. Infection was carried out two times per day. The required high humidity and moisture level were maintained by covering the entire field at night to create moisture chambers which favours the fungal growth. At the day time, the covers were removed and the entire field was exposed to the sunlight. The infection was continued till the typical blast lesions appeared in the rice leaves. All rice lines were scored by using IRRI stipulated standard scoring system. Based on the scoring system the rice varieties were grouped in to six major classes such as highly resistant, resistant, mildly resistant, highly susceptible, susceptible, mildly susceptible (IRRI, 1996). Lesion shape, colour, size, number of lesions, percentage of the lesions per leaves were taken as measurements indicated in Table 1. The mean values obtained for each variety were used to determine the susceptibility and resistivity of rice varieties.

Table 1. Standard evaluation system for rice blast, IRRI, 1996

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

Marker genotyping of phenotypically evaluated rice varieties

Rice varieties which were phenotypically evaluated (except H4 and Bg96-3 rice varieties) were used in the marker genotyping. All varieties were grown in pots and maintained for a period of 21 days at plant house in the Department of Plant Science, University of Colombo. Prior to DNA extractions leaves of all varieties were collected, preserved in liquid N₂ and stored at -80 °C temperature in Genetech Institute, Colombo (McCouch *et al.*, 1997). DNA was extracted using CTAB method (Doyle and Doyle, 1987). Two SSR markers linked to the blast resistant genes in variety *Tetep*; *RM206* (linked to the gene *Pikh*) and *RM246* (linked to the gene *Pit(p)*) and one gene specific marker *YL155/YL87* (linked to the gene *Pita*) were used for genotyping. Agarose and polyacrylamide gel electrophoresis were incorporated for the size separation of PCR products (Joshi *et al.*, 2009).

PCR amplification

PCR was performed in 25 µl reaction volumes in with Gene Amp PCR system 9600, Perkin Elmer, USA. PCR mix was contained 2.5 µl of 10X buffer containing MgCl₂ (Thermo Scientific 10X Dream Taq Buffer), 2.5 µl of 3.5 mM dNTP, 3.0 µl from 2.5 µM of primer mixture Table 2, 0.2 µl of 5U/µl Taq DNA polymerase and 12.8 µl of sterile water. Each master mixture was prepared by using multiples of above recorded reagent volumes relevantly to the number of PCR. 21µl of the PCR master mixture was added to 4µl of DNA in PCR tube and the reaction was performed.

Table 2. Primer sequences

| Gene | Linked marker | Forward primer | Reverse primer |
|---------------|-------------------|--------------------------------|-------------------------------|
| <i>Pikh</i> | <i>RM206</i> | CCCATGCGTTTAACTATTCT | CGTTCCATCGATCCGTATGG |
| <i>Pit(p)</i> | <i>RM246</i> | GAGCTCCATCAGCCATTCAG | CTGAGTGCTGTGCGACT |
| <i>Pita</i> | <i>YL155/YL87</i> | YL155:AGCAGGTTATAAGCTAG GCC | YL87:CTACCAACAAGTTCATC AAA |

RESULTS AND DISCUSSION

Fungal isolation and molecular identification

Rice blast fungus was isolated and purified using RPA medium. Fungus appeared gray ash colour mycelia and concentric rings on PDA and RPA medium as shown in Fig. 2. Typical hyaline, three septate fungal spores were observed as shown in Fig. 3. The results obtained in this study are in accordance with previous research reports published on the isolation of *Magnaporthe* (Mithrasena *et al.*, 2012).

Analysis of DNA sequences using (ITS1 and ITS4 primers using amplified DNA sequences) BLAST confirmed the identity of blast fungus which was 99% identical to *M. gisea* and it was used for the inoculation process.

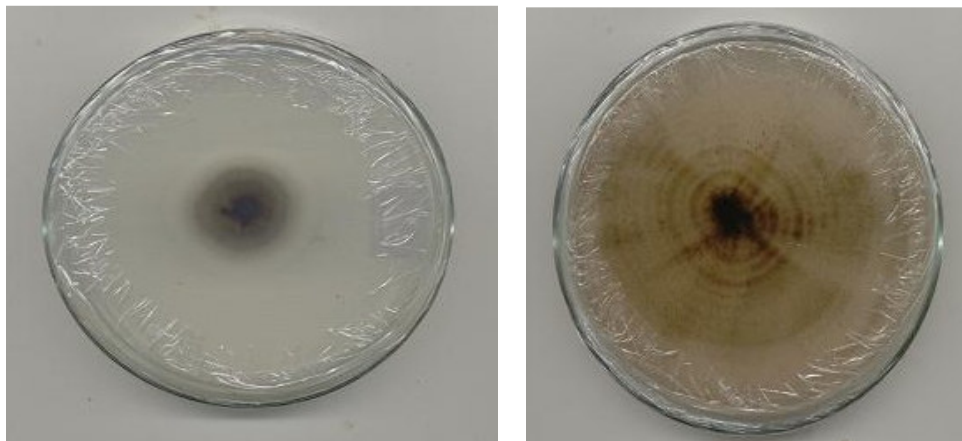


Fig. 2. Morphology of *M. grisea* in PDA and RPA

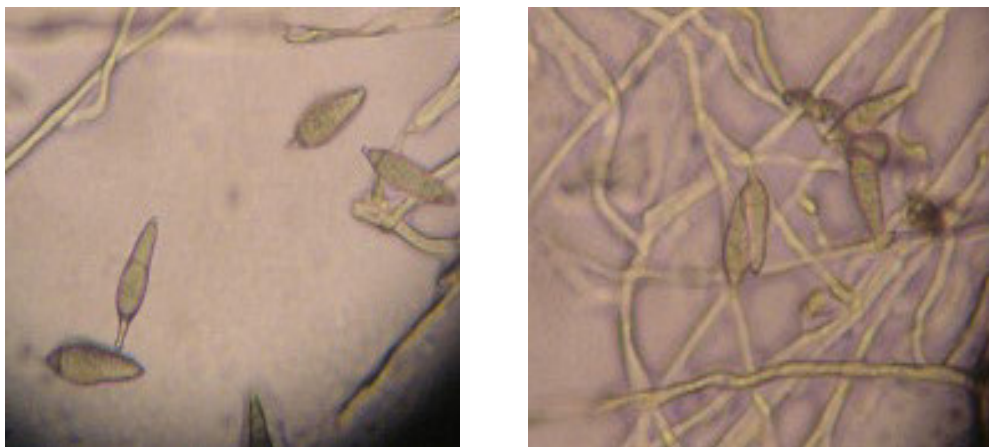


Fig. 3. Typical three septate, fusiform, hyaline spores of *M. grisea*

Phenotypic screening of rice varieties

Out of 34 varieties screened 17 varieties were susceptible to the fungal infection, while rest of the varieties remained resistant to the rice blast. *Tetep*, Bg359, Bg304, IR64, Bg352 and Bg357 varieties were highly resistant to the rice blast disease. LD125, Bg94/1, Bw267-3, Bw451, Bw400, H7, Bg34-6, H10 and IRRI119 varieties were highly susceptible as indicated in Table 3. Most of the resistant varieties have retained their resistance over the years while some of them (H7, H10, Bw451, Bw78, Bw400, Bw267-3) have lost their resistance in comparison with the previous study (Rajapakse *et al.*, 2000).

All 34 varieties were clustered in to six groups (highly resistant, resistant, mildly resistant, mildly susceptible, susceptible and highly susceptible). *Tetep* was found to be highly resistant and *Pachchaiperumal* found be highly susceptible as shown in Fig. 4.

Table 3. Rice varietal groups based on blast resistance

| Group | Rice varieties |
|------------------------|---|
| Highly resistant | <i>Tetep</i> , Bg304, Bg359, IR64, Bg352, Bg357 |
| Resistant | Bg360, H4, <i>Swarna sub</i> , Bg300, Bg96-3, Bg403, Ptb33, Bg407, Bg34-8, Bg96-741 |
| Moderately resistant | <i>Engatek</i> |
| Moderately susceptible | None |
| Susceptible | Bg450, Bg276-5, Ld66, IR07F290, Bw78, IR07F291 <i>Sambamashuri</i> |
| Highly susceptible | <i>Pachchaiperumal</i> , LD125, Bg94-1, Bw267-3, Bw451, Bw400, H7, Bg34-6, H10, IRR1119 |



Fig. 4. Highly susceptible *Pachchaiperumal* (A), Highly resistant *Tetep* (B)

The blast resistance scores of all the rice varieties are indicated in Fig. 5.

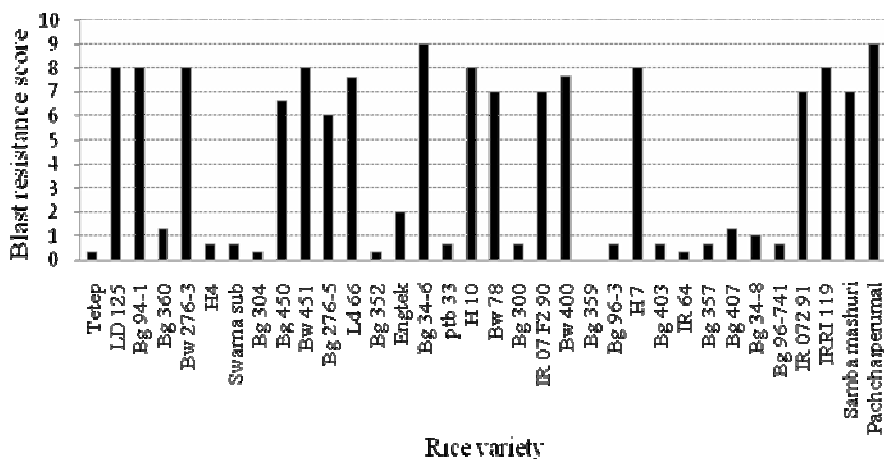


Fig. 5. Blast resistance scores of rice varieties

DNA marker analysis

RM206 marker polymorphism linked to the gene *Pikh*

The expected band length observed for SSR marker *RM206* was 147bp. Most of the resistant varieties depicted the band lengths of (161-171bp) for *RM206* and susceptible varieties given the band length of 151bp. The marker polymorphisms which was prominent for *Pikh* blast resistant gene clearly differentiated some of the resistant and susceptible varieties Table 4. Hence this information can be used in future studies to identify these resistant and susceptible varieties based on above polymorphism. The other resistant varieties like Bg352, Bg360, Bg300 and Bg357 were given average band length of 151-155bp and the varieties *Swarnasub-1* and Bg407 did not produce clear bands. While rest of the susceptible varieties Ld66, IR07F290, Bw78, IR07F291, *Sambamashuri*, Bg94/1, H10 and Bw267-3 yielded band lengths of (169-177bp) and the varieties Bw451 and Ld125 did not produce any band.

RM246 marker polymorphism linked to the gene *Pit (p)*

Expected band length observed for SSR marker *RM246* was 115bp. The resistant varieties produced a band having a length of 117bp while susceptible varieties gave a band of 111bp as indicated in Table 4. Rest of the resistant varieties such as Tetep, Bg357, IR64, *Swarnasub1*, Bg300, Bg359 and Bg96-741 did not produce clear bands and the varieties Ptb33 and Bg352 produced a band of 111bp. The other susceptible varieties such as Ld125, IRR119 and Bw267-3 produced a 117bp band while susceptible varieties IR07F291, Bw78, IR07F290, Bg94/1, Bg34-6 and H7) did not produce any band.

YL87/YL155 Marker Polymorphism Linked to the Gene *Pita*

Most of the highly resistant and resistant varieties demonstrated the presence of blast resistant *Pita* gene of variety *Tetep* as indicated in Table 4. The *Pita* gene was absent in the highly susceptible and susceptible varieties. However *Pita* gene was also absent in some of the resistant varieties like Bg300, Bg348 and Ptb33, which indicates the idea of presence of

some other blast resistant gene contributing to their blast resistance. Some of the susceptible varieties were shown the presence of *Pita* gene such as H10, Bg276-5 and IR07F291. Since this marker has been designed to amplify a specific region within the sequence of dominant *Pita* gene, presence of 1000bp long *YL155/YL87* amplicon may directly provide evidence to prove that the respective cultivar holds the gene of interest.

According to previous reports the resistance of these varieties were broken recently (Dissanayake, 1995), which indicates the presence of single gene cannot be overcome virulent pathogens and their narrow genetic diversity made them susceptible to blast races (varieties H10, IR07F291 and Bg276-5). The same research findings described Bg276-5 was a blast resistant variety by the time of release and lost such resistance by 1989/1990. Similarly H10 was also blast resistant in 1958, at the time of first release and later subjected to a resistant breakdown. Hence even with the presence of *Pita* gene in the genomes the cultivars may be susceptible to *M. grisea* pathotypes due to environmental or physiological conditions (Jayawardana *et al.*, 2013). This would clarify the conflict of having dominant *Pita* gene in currently susceptible varieties Bg276-5, IR07F291 and H10. In addition locating *Pita* and *Pita*² genes in the centromeric region of the 12th chromosome of the rice genome have been suppressed the recombination between two genes. According to Bryan *et al.* (2000) and Shikari *et al.* (2013), *Pita* gene may be tightly linked with or allelic to *Pita*² and share same set of YL markers. The only difference of these genes is the performance of blast resistance towards different *M. grisea* isolates. Thus, there may be a significant chance for the existence of *Pita*² gene by *YL155/YL87* marker instead of *Pita*. So, this might also be a possible cause for amplification of 1000bp fragment in Bg276-5, H10 and IR07F291 which had been identified as blast susceptible varieties. Furthermore, the isolates used to screen the blast resistance or susceptibility status of cultivars may be virulent against the particular gene thus the cultivars may have been categorized as susceptible. And Shikari *et al.* (2013) states, there is a possibility of inhibiting the blast resistance triggered by resistance genes with the actions of other genic regions. Therefore these reasons might be possible facts for above mentioned varieties to be blast susceptible even they demonstrated positive amplification with *Pita* gene marker.

Overall suggestion is that the phenotypic data on blast resistance of evaluated Sri Lankan rice varieties were verified with these DNA marker data. It is important to use these marker data for identification of blast resistance lines in Sri Lankan rice breeding programs. It is also significantly important in direct selection of resistant genes when they are transferred from one varietal background to another for producing durable blast resistant varieties.

Table 4. Marker alleles for three genes, *Pikh*, *Pit(p)*, and *Pita*

| Variety | Disease reaction | RM206 (<i>Pikh</i>) (allele size in bp) | RM246 (<i>Pitp</i>) (allele size in bp) | YL155/YL87(<i>Pita</i>) *(Presence=P, Absence=A of allele) |
|--------------|------------------|---|---|---|
| <i>Tetep</i> | HR | 169 | — | P |
| Bg304 | HR | 171 | 117 | P |
| Bg359 | HR | 171 | — | P |
| IR64 | HR | 171 | — | P |
| Bg352 | HR | 151 | 111 | P |
| Bg357 | HR | 155 | — | P |

| Variety | Disease reaction | RM206 (<i>Pikh</i>) (allele size in bp) | RM246 (<i>Pitp</i>) (allele size in bp) | YL155/YL87(<i>Pita</i>) *(Presence=P, Absence=A of allele) |
|------------------------|------------------|---|---|---|
| Bg360 | R | 151 | 117 | P |
| <i>Swarnasub1</i> | R | — | — | P |
| Bg300 | R | 151 | — | A |
| Bg403 | R | 169 | 117 | P |
| Ptb33 | R | 169 | 111 | A |
| Bg407 | R | — | 117 | P |
| Bg34-8 | R | 169 | 117 | A |
| Bg96-741 | R | 169 | — | P |
| <i>Engatek</i> | R | 169 | 117 | P |
| Bg450 | S | 151 | 111 | A |
| Bg276-5 | S | 151 | 111 | P |
| Ld66 | S | 169 | 111 | A |
| IR07F290 | S | 169 | — | A |
| Bw78 | S | 177 | — | A |
| IR07F291 | S | 171 | — | P |
| <i>Sambamashuri</i> | S | 169 | 113 | A |
| LD125 | HS | — | 117 | A |
| Bg94/1 | HS | 169 | — | A |
| Bw267-3 | HS | 171 | 117 | A |
| Bw451 | HS | — | 111 | A |
| Bw400 | HS | 151 | 111 | A |
| H7 | HS | 151 | — | A |
| IRRI119 | HS | 155 | 117 | A |
| H10 | HS | 169 | 111 | P |
| Bg34-6 | HS | 151 | — | A |
| <i>Pachchaiperumal</i> | HS | 151 | 111 | A |

Note: P- Presence, A- Absence

CONCLUSIONS

The blast fungus *M. grisea* (anamorph- *Pyricularia grisea*) was identified using molecular based techniques and sequence alignments data showed that the strain used in the present study was 99% similar to *M. grisea*. Out of 34 rice varieties screened in the phenotypic screening, 17 varieties were found to be susceptible to the pathogen and rests of the varieties were remained resistant. While some of the varieties have lost their blast resistance after few years of cultivation in comparison with the previous study (varieties H7, H10, Bw451, Bw78, Bw400 and Bw267-3). This appears to be a result of emergence of virulent races within pathogen population and narrow genetic diversity of these varieties for blast disease. Genetic basis of blast resistance in Sri Lankan rice germplasm was identified by the marker genotyping process performed with the SSR markers belong to major blast resistance genes [*Pita*, *Pikh* and *Pit(p)*] which verified the phenotypic screening made prior to genotyping. Hence these polymorphism findings can be used in future studies to differentiate most of the resistant and susceptible varieties used for the phenotypic evaluation in the present study and

greatly important for rice breeding programs such as incorporation of blast resistant genes from one varietal back ground to an another to produce durable blast resistant varieties.

ACKNOWLEDGEMENTS

The authors thank the project Higher Education for Twenty First Century (HETC) for financial support and Plant Science Department, University of Colombo, Rice Research and Development Institute, Bathalagoda for providing necessary facilities to carry out the research work.

REFERENCES

Bryan, G.T., Jia, Y.L., Mcadams, S.A., Hershey, H.P. and Valent, B. (2000). Direct interaction of resistance genes and avirulence genes products confers rice blast resistance. *EMBO. J.* 19(15), 4000 - 4014.

Dissanayake, D.M.N. (1995). Genetic base of blast resistance in cultivated rice varieties in Sri Lanka and breeding strategies for durable resistance to blast. *Trop. Agriculturist.* 150, 1 - 15.

Doyle, J.J. and Doyle, J.L. (1987). Isolation of plant DNA from fresh tissue. *J. Focus.* 12, 13 - 15.

Fjellstrom, R., McClung, A., Shank, A.R., Marchetti, M.A., Bormans, C. and Park, W.D. (2002). Progress on development of microsatellite markers associated with rice blast resistance genes. Proceedings of the 29th Rice Technical Working Group Meeting, Little Rock, AR, USA, 43-44.

Ghazanfar, M.K., Wakil, W. and Sahi, S.T. (2009). Influence of Various Fungicides on the Management of Rice Blast Disease. *Myco. Path.* 7, 29 - 34.

Guillamon, J.M., Sebate, J., Cano, J. and Querol, A. (1998). Rapid Identification of Wine Yeast species on RLFP analysis of Ribosomal ITS region. *Archives of Microbiology.* 169 (5), 387 - 389.

IRRI. (1996). Standard evaluation system for rice. 4th ed. IRRI, Manila, Philippines.

Jayawardana, W.A.D., Jayasekera, G.A.U., Wijesundera, R.L.C. and Dissanayake, D.M.N. (2013). The phenotypic screening of rice varieties for blast resistance towards developing DNA markers linked to resistance genes. Proceedings of 69th annual sessions of Sri Lanka Association for Advancement of Science. Part1 939/D, 69, 237.

Joshi, B.K., Parajuli, G. and Chaudhry, K. (2009). Molecular tagging, allele mining, marker breeding for blast resistance in rice. *BSN-E Bulletin* 1, 1 - 23.

Mackill, D.J. and Bonman, J.M. (1992). Inheritance of blast resistance in near isogenic lines of rice. *J. Phytopathology.* 82, 746 - 749.

McCouch, S.R, Chen, X., Panaud, O., Temnykh, S. and Xu, Y. (1997). Microsatellite marker development, mapping and applications in rice genetics and breeding. *Plant Mol. Biol.* *35*(1-2), 89 - 99.

Mithrasena, J.Y., Wijesundera, W.S.S., Wijesundera, R.L.C., Wimalasiri, D. C. and Priyanti, R.P. (2012). Pathogenic and genetic diversity of *Magnaporthe oryzae* population from Sri Lanka. *Rice Science.* *19*(3), 241 - 246.

Rajapakse, R.M.T., Sandanayake, C.A. and Pathinayake, B.D. (2000). Foot prints in rice varieties improvement and its impact on rice production in Sri Lanka. In proceedings of the annual symposium of the Department of Agriculture Edited by Kotagama, H.B., Wijesekera, G.A.W. and Wijesundara, D.S.R. Sri Lanka: Department of Agriculture.

Rossman, A.Y., Howard, R.J. and Valent, B. (1990). *Pyricularia grisea*, the correct name for the rice blast fungus. *Mycologia.* *82*, 509 - 512.

Sharma, T.R., Chauhan, R.S., Singh, B.M., Sagar, V., Paul, R. and Rathour, R. (2002). RAPD and virulence analyses of *Magnaporthe grisea* in rice populations from north-western Himalayan region of India. *J. Phytopathol.* *150*, 649 - 656.

Sharma, T.R., Rai, A.K., Guptha, S.K., Vijayanan, T., Devanna, B.N. and Ray ,S. (2012). Rice blast management through plant resistance: retrospect and prospect. *Rev.Agri Res.* DOI 10. 1007/S40003-011-0003-5.

Shikari, A.B., Khanna, A., Krishnan, S.G., Singh, U.D., Rathour, R., Tonapi, V., Sharma, T.R., Nagarajan, M., Prabhu, K.V. and Singh, A.K. (2013). Molecular analysis and phenotypic validation of blast resistance genes *Pita* and *Pita*² in landraces of rice (*Oryza sativa* L.). *Indian J. Genetic and Plant breeding.* *73*(2), 131 - 141.

Skamnioti, P. and Gurr, S.J. (2009). Against the grain-safe guarding rice from rice blast disease. *J. Trends Biotechnol.* *27*(3), 141 - 150.