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# Molecular and field level screening for blast resistance gene donors among traditional rice varieties of Kerala

Henry Nickolas[1*], V.G . Jayalekshmy[2], C.K. Yamini Varma[3] and Veena Vighneswaran[4]

[1]*University of Tasmania, Hobart, Tasmania - 7001, Australia*
[2]*College of Agriculture, Kerala Agricultural University, Thiruvananthapuram - 695522, Kerala, India*
[3]*Pepper Research Station, Kerala Agricultural University,Panniyur - 670 142, Kerala, India*
[4] *Rice Research Station, Kerala Agricultural University,Vyttila, Ernakulam - 682028, Kerala, India*

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**Abstract**

Rice blast, caused by *Magnaporthe oryzae*, is becoming one of the most devastating diseases of rice (*Oryza sativa* L.) in Kerala. Use of resistant rice varieties had been proved to be the most effective and sustainable way to control this disease. In the present study, the existence of blast resistance genes *Pi 1, Pi 2* and *Pi kh* in selected landraces of Kerala were identified using the SSR markers RM224, RM527 and RM206 respectively, and further confirmed through a field level screening. Results showed the presence of gene *Pi 1* in nine varieties, *Pi 2* in twenty varieties and *Pi kh* in six varieties. Infection index analysis after field level screening showed that nine varieties had low infection index. Landraces carrying the genes *Pi 1* and *Pi kh* imparted moderate resistance under Kerala conditions which implied that pyramiding of these genes could improve the disease resistance in rice varieties of Kerala. Among the selected varieties, *Parambuvattan* and *Kavunginpoothala*, having both *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 the staple food for more than half of the world's population (Von Braun, 2007). India is a major consumer and producer of rice. However, the world's largest food crop is infested by a large number of pests and pathogens, which under epidemic conditions, cause serious yield loss. Among these, rice blast caused by *Magnaporthe oryzae* is one of the most destructive diseases, often resulting in yield loss as high as 70 to 80 per cent during an epidemic (Ou, 1985). Compared to other districts of Kerala state, the northern district of Palakkad is a disease prone area for rice blast. A survey conducted in 2012 revealed that blast disease incidence of rice was in the range of 34 to 80 per cent, and severity was in the range of 11.7 to 65.13 per cent in different rice growing areas of Palakkad district (Yamini, 2012). Krishnaveni et al. (2012) have also reported that Pattambi region in Palakkad district is a hot spot for rice blast disease.

Despite the availability of effective fungicides, independent of the rice-growing system or area, breeding for resistance is needed to control the disease (Ballini et al., 2008). Kerala is considered as one among the centres of diversity for rice, and rice cultivation here dates to 3000 BC (Manilal, 1990). It is estimated that nearly 2000 traditional varieties were cultivated in Kerala, across the eight agro-ecosystems in the state. Globaly known prominent Pattambi rice varieties acted as sources for resistance to many rice varieties (Kalode and Krishna, 1979).

*Author for correspondence: Phone: +61406620410, Email: henrynickolas@gmail.com

Various studies have been done on the identification and introgression of the blast resistance genes in rice (Miah et al., 2017; Khan et al., 2018). However, no pertinent study has been done to detect these resistance genes against blast in Kerala rice varieties. Exploring the underutilized resource of resistant genes in the varieties of Kerala, 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

Thirty traditional varieties were collected from various agricultural research stations of Kerala under Kerala Agricultural University including Regional Agricultural Research Station (RARS) Pattambi, Farming Systems Research Station (FSRS), Kottarakkara and Regional Agricultural Research Station (RARS), Kayamkulam. Four control varieties were also selected, including three check varieties for resistant genes and one for susceptible genes (Table 1).

*Molecular level screening*

DNA was extracted from the leaves of thirty-four varieties following the procedure of Regowsky et al. (1991) 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). The reaction was carried out in 25 µl reaction mixture containing 25 ng genomic DNA, 1.5 Mm $MgCl_2$, 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 thermocycler programmed as follows: An initial denaturation at 94 °C for 5 minutes followed by 35 cycles of 94 °C for 1 minute, at annealing temperature for 1 minute and 72 °C for 2 minutes; followed by a final elongation at 72 °C for 5 minutes and a 4 °C hold. Amplified products were separated by agarose gel electrophoresis using 1.8 per cent gel and photographed using a gel documentation system.

*Field level Screening*

The experiment was conducted at Pattambi, Kerala, a hotspot of rice blast disease with thirty traditional varieties and two check varieties (one susceptible variety *Jyothi* [KAU, 2011] and one resistant variety *Tetep*), in an area of 5 cents during *Kharif* season, 2013. All agronomic practices on the main field were carried out on time as per the package of practices recommendations of the Kerala Agricultural University (KAU, 2011). The nursery was prepared and seedlings were uprooted 30 days after sowing and transplanted to the main field. Randomized Block Design was used for the field level screening of the varieties. Thirty-two varieties were taken and arranged in lines, with each line containing 20 hills in 3 replications. Layout of uniform blast nursery (UBN) method, approved by International Rice Research Institute (IRRI), was used for screening of rice germplasm against *Magnaporthe oryzae* in the main field.

Isolation and inoculation of the pathogen were done following Yamini (2012). The blast pathogen was isolated from paddy leaf samples collected from hot spot areas of farmer's field in Pattambi during December 2012 following standard procedures using Potato Dextrose Agar (PDA) medium (Tuite, 1969). The isolation of the pathogen was done from the leaves showing typical symptoms after keeping in a moist chamber for 24 hours. The tissue isolation technique was done following standard procedure. On the third day when mycelial growth was observed, the mycelial bits were aseptically transferred to PDA slants. The cultures were periodically subcultured in PDA slants. The pathogenicity test of the isolate was conducted and Koch's postulates proved. The spore suspension was prepared from 7-day old blast culture grown on PDA and the mycelia was scraped into 10 ml of distilled water and the solution was filtered through two-fold cheesecloth to remove the fungal debris. The spore concentration was adjusted to 1 x $10^5$

*Table 1.* Various genes under study, primers used for each gene and their amplification base pair

| Genes | Check variety | SSR Primer | Amplification base pair | [b]References |
|---|---|---|---|---|
| *Pi 1* | C101LAC | RM224 | 146 bp | (Fuentes et al., 2008) |
| *Pi 2* | C101A5 | RM527 | 233 bp | (Arunakanthi et al., 2008; Prasad et al., 2011) |
| *Pi kh* | *Tetep* | RM206 | 145 bp | (Sharma et al., 2010; Prasad et al., 2011) |
| [a]Susceptible geneBPT5204 | | | | |

[a]susceptible check variety was commonly used for all three genes

[b]references are for the respective SSR primers and amplification base pair

spores per ml using hemocytometer as described by Aneja (2009). The spore suspension containing Tween – 20 (0.2 per cent) was sprayed uniformly over one-month-old plants. The inoculum was sprayed in the evening till the entire plant surface become wet with spore suspension and left overnight. Scoring was done after 15 days of post-infection using Standard Evaluation System (SES) (Chaudhary, 1996). In the field screening, periodical scorings (disease intensity) were made for the development of symptoms on leaves (Krishnaveni et al., 2012) using the 0-9 SES scale of IRRI at 47 days after transplantation (DAT) and 79 DAT.

*Infection Index and statistical analysis*

Infection index (ID) of the disease was calculated (Table 2) using scores obtained from the field screening following McKinney (1923).

$$ID = \frac{\text{Sum of all numerical ratings}}{\text{Total no. of plants}} \times \frac{100}{\text{Maximum disease category}}$$

where, sum of all numerical ratings is the disease score. All analyses were done using MS-Excel. Data generated from the laboratory study as well as the field study were subjected to the analysis of variance (ANOVA) after appropriate transformation where needed. The correlation coefficient between the number of genes and disease scores was calculated following Cochran and Cox (1957).

**Results and discussion**

This study identified the donors for blast resistant genes among the selected varieties of Kerala. Traditional rice varieties of Kerala carry unique resistant genes (Rekha et al., 2011), that lower the risk of disease attack, compared to the high yielding varieties (Devi et al., 2017). In the present study, varieties with the presence of three blast resistance genes, *Pi 1, Pi 2* 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) 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. Gene *Pi 1* was identified in nine varieties, in which six varieties were in heterozygous condition (*Thekkancheera, Thekkanchitteni, Njavara* from Kunnathoor, *Parambuvattan, Kavunginpoothala,* and *Karutha Modan)* and three in homozygous condition (*Jedduhalliga, Chuvanna Modan, Njavara* from Karipra) (Plate 1a). *Gene Pi 2* was found in twenty varieties, wherein except one (*Thekkancheera)*, all varieties were in homozygous condition (*Aryan, Velutharikayama, Jedduhalliga, Vadakkanchitteni, Thekkan, Veluthavattan, Kodiyan, Kattamodan, Chuvanna Modan, Oorumundakan, Palakkadan Njavara* from Elampalloor, *Nadan Njavara* from Elampalloor, *Njavara* from Karipra, *Ponnaryan, Vellari, Thavalakannan, Velutharithavala-kkannan, Kayama* and *Karutha Modan)* (Plate 1b).

*Table 2.* Infection index of various treatments calculated using scores obtained 79 DAT

| Rice Varieties | Infection index mean* | |
|---|---|---|
| *Tetep* | 7.34 | (15.72) |
| *Thekkanchitteni* | 25.15 | (30.1) |
| *Kavunginpoothala* | 27.01 | (31.31) |
| *Njavara* from Karipra | 32.73 | (34.9) |
| *Thekkancheera* | 35.15 | (36.36) |
| *Karutha Modan* | 35.52 | (36.58) |
| *Parambuvattan* | 37.00 | (37.46) |
| *Cheradi* from Kunnathoor | 37.37 | (37.68) |
| *Chuvanna Modan* | 37.93 | (38.02) |
| *Oorumundakan* | 38.10 | (38.12) |
| *Njavara* from Kunnathoor | 38.48 | (38.34) |
| *Thekkan* | 40.76 | (39.68) |
| *Nadan Njavara* from Elampalloor | 41.21 | (39.94) |
| *Kodiyan* | 42.15 | (40.48) |
| *Palakkadan Njavara* from Elampalloor | 42.25 | (40.54) |
| *Aryan* | 44.71 | (41.96) |
| *Kayama* | 45.14 | (42.21) |
| *Vadakkanchitteni* | 45.51 | (42.42) |
| *Velutharithavalakkannan* | 45.52 | (42.43) |
| *Jedduhalliga* | 46.24 | (42.84) |
| *Velutharikayama* | 51.51 | (45.87) |
| *Vellari* | 53.31 | (46.9) |
| *Thavalakkannan* | 58.41 | (49.84) |
| *Cheriya aryan* | 59.27 | (50.34) |
| *Ponnaryan* | 59.90 | (50.71) |
| *Veluthavattan* | 60.46 | (51.04) |
| *Maskathi* | 61.09 | (51.41) |
| *Kumpacheradi* from Puthoor | 61.46 | (51.63) |
| *Jyothi* | 61.85 | (51.85) |
| *Athikkiraya* | 65.53 | (54.05) |
| *Kattamodan* | 66.08 | (54.38) |
| *Eravapandi* | 73.69 | (59.14) |
| CD (0.05) | | 8.142 |

*Figures in parentheses are transformed data

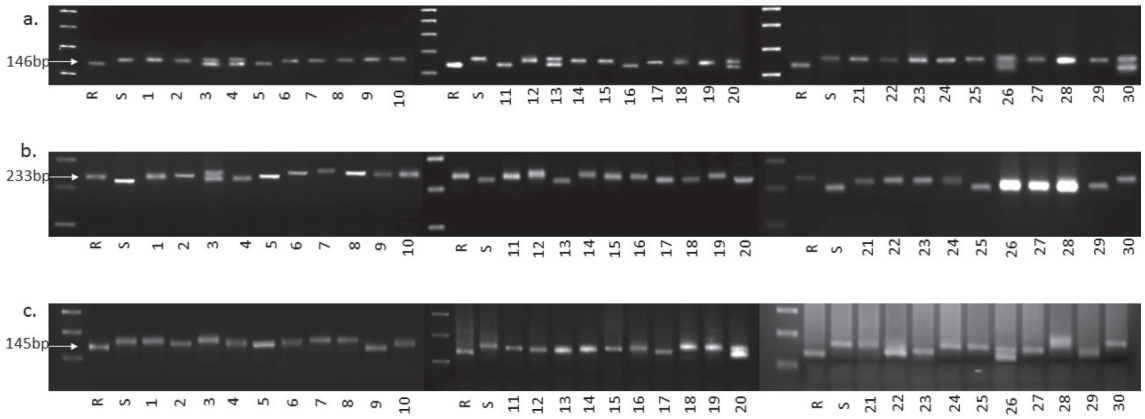


Plate 1. Amplification profile of the DNA of thirty traditional varieties and two check varieties using three different primers. a. *Pi 1* screening, where R is C101Lac, b. *Pi 2* screening, where R is C101A51, c. *Pi kh* screening, where R is *Tetep*. In a, b and c: S-BPT5204, *1-Aryan, 2-Velutharikayama, 3-Thekkancheera, 4-Thekkanchitteni, 5-Jedduhalliga, 6-Vadakkanchitteni, 7- Thekkan, 8-Veluthavatta, 9-Kodiyan, 10-Kattamodan, 11-Chuvanna Modan, 12-Oorumundakan, 13-Njavara* from Kunnathoor*, 14-Palakkadan Njavara* from Elampalloor*, 15-Nadan Njavara* from Elampalloor*, 16-Njavara* from Karipra*, 17-Cheradi* from Kunnathoor*, 18-Kumpacheradi* from Puthoor*, 19-Ponnaryan, 20-Parambuvattan, 21-Vellari, 22-Thavalakkannan, 23-Velutharithavalakkannan, 24-Kayama, 25-Maskathi, 26-Kavunginpoothala, 27-Eravapandi, 28- Athikkiraya, 29-Cheriya aryan, 30-Karutha Modan.*

Further more, g*ene Pi kh* was found in six varieties, in which two were heterozygotes (*Parambuvattan* and *Kavunginpoothal*a) and four were homozygotes (*Oorumundakan*, *Thavalakkannan*, *Velutharithavala-kkannan,* and *Cheraidi* from Kunnathoor) (Plate 1c).

Gene combinations such as *Pi 1 + Pi 2, Pi 2 + Pi kh* and *Pi 1 + Pi kh* were also found in a few varieties. *Chuvanna Modan, Njavara* from Karipra, *Thekkancheera, Jedduhalliga* and *Karutha Modan* were found with the presence of resistant genes *Pi 1* and *Pi 2*. *Thavalakkannan, Velutharithavalakkannan* and *Oorumundakan* had both *Pi 2* and *Pi kh*. Genes *Pi 1* and *Pi kh* were present in two varieties - *Parambuvattan* and *Kavunginpoothala*.

Based on the scores obtained during the field screening, ten varieties were classified as moderately resistant (*Thekkanchitteni, Njavara* from Kunnathoor*, Cheradi* from Kunnathoor*, Chuvanna Modan, Njavara* from Karipra*, Thekkancheera, Oorumundakan, Parambuvattan, Kavunginpoothala* and *Karutha Modan)*. Fifteen varieties were grouped under the section moderately susceptible (*Cheriya aryan, Aryan, Ponnaryan, Vellari, Velutharikayama, Kayama, Jedduhalliga, Vadakkanchitteni, Thekkan, Veluthavattan, Kodiyan, Palakkadan Njavara* from Elampalloor*, Nadan Njavara* from Elampalloor*, Thavalakkannan and Velutharithavalakkannan)*, and the remaining six varieties were included in the group susceptible (*Maskathi, Eravapandi, Athikkiraya, Kumpacheradi* from Puthoor*, Kattamodan* and *Jyothi)*. None of the selected varieties was found immune (except the check variety *Tetep*), or highly susceptible. In this screening, the check variety *Jyothi* was confirmed as a susceptible variety supporting the previous report (KAU, 2011).

Varieties were found significantly different from each other with respect to infection indices (ID) ($F_{31,93}$=2.11, $p<0.05$ at 47 DAT and $F_{31,93}$=2.22, $p<0.05$ at 79 DAT). Variety *Tetep* was found to be with minimum average ID (7.3) and it was significantly different from all the remaining varieties. Further lower ID was obtained for varieties *Thekkanchitteni* (25.2) and *Kavunginpoothala* (27.0), which were on par with *Njavara* from Karipra (32.7), *Thekkancheera* (35.2), *Karutha Modan* (35.5), *Parambuvattan* (37.0), *Cheradi* from Kunnathoor (37.4), *Chuvanna Modan* (37.9) and *Oorumundakan* (38.1). Infection indices of the varieties were found in concurrence with the classification obtained from these disease scores. All moderately resistant varieties had low infection index.

The significant negative correlation between the number

of genes and respective score averages (-0.52) indicates that the level of disease resistance increased with the number of resistant genes present, demonstrating the effect of gene pyramiding or gene stacking. Gene pyramiding results in the simultaneous expression of more than one gene in a variety, where with resistant genes, it can develop durable resistance (Kumari et al., 2017). The comparison of disease scores with the presence or absence of resistance genes clearly showed that the varieties having genes *Pi 1* and *Pi kh* had lower disease score supporting the previous findings about these genes in delivering broad-spectrum resistance against geographically diverse strains of *M. oryzae* (Kumari et al., 2017; Khan et al., 2018). This emphasizes that the pyramiding of genes *Pi 1* and *Pi kh* can undoubtedly impart better blast resistance under Kerala condition (Figure 1). Therefore, the varieties *Parambuvattan* and *Kavunginpoothala* which carry both genes *Pi 1* and *Pi kh* can be used as donors in the breeding programmes for rice blast resistance. Varieties *Chuvanna Modan* and *Njavara* from Karipra having the gene *Pi 1* in homozygous condition, and the varieties *Oorumundakan* and *Cheradi* from Kunnathoor with the gene *Pi kh* in homozygous condition can be used as

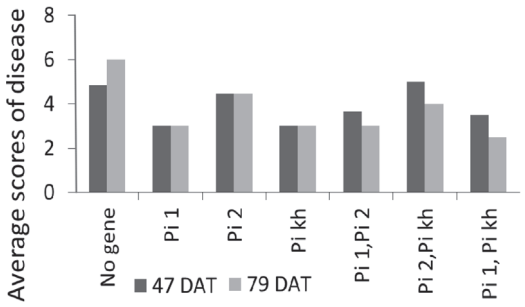


Figure 1. Average disease scores of varieties carrying genes as specified at two different scorings.

Varieties carrying specific gene and gene combinations are noted. *Pi 1 - Thekkanchitteni and Njavara* from Kunnathoor*; Pi 2 - Aryan, Ponnaryan, Vellari, Velutharikayama, Kayama, Vadakkanchitteni, Thekkan, Veluthavattan, Kodiyan, Kattamodan, Palakkadan Njavara* from Elampalloor*, Nadan Njavara* from Elampalloor*; Pi kh – Cheradi* from Kunnathoor*; Pi 1, Pi 2 – Thekkancheera, Jedduhalliga, Karutha Modan, Chuvanna Modan, Njavara* from Karipra*; Pi 2, Pi kh – Thavalakkannan, Velutharithavalakkannan, Oorumundakan; Pi 1, Pi kh – Parambuvattan, Kavunginpoothala* donors for respective genes. The varieties identified as donors are having specific grain qualities of Kerala varieties - *Parambuvattan* with red bold and *Kavunginpoothala* with white bold grains. Many prominent rice varieties popular in Kerala such as Jyothi, Uma etc. are susceptible to rice blast and need to be improved for blast disease resistance. Since the donors have been identified from the traditional varieties of Kerala with specific grain qualities, reconstitution of these varieties by maintaining the grain quality can easily be achieved.

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