Evaluation of blast resistance genes \( Pi9 \) and \( Pi54 \) in rice against local isolates of Tamil Nadu

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Abstract

Rice production system is greatly affected by blast disease caused by \textit{Magnaporthe grisea} Barr which causes significant yield reduction throughout Asia and different parts of the globe. Developing resistant varieties has been most efficient and economical method for controlling blast disease in rice. Identification of broad spectrum resistance genes against prevailing isolates of the pathogen is a key determining success of developing resistant varieties. However, the blast resistance characterization and evaluation has not been well studied. In the current study, 3 parental lines namely CO 51 (\( Pi54 \)), 562-4 (a NIL of CO 43 harboring \( Pi 9 \)) and a susceptible genotype Improved White Ponni (IWP) were evaluated against local isolates of blast pathogen. Presence of \( Pi9 \) and \( Pi54 \) blast resistance genes in the parental lines was confirmed by using functional markers such as NBS4 and Pi54MAS respectively. All the three genotypes were evaluated against blast disease in a hot spot environment. Both CO51 and 562-4 carrying the \( Pi54 \) and \( Pi9 \) genes respectively showed moderate resistance against blast disease whereas IWP showed a susceptibility reaction against blast disease. Results indicated that pyramiding of these genes would improve resistance against blast disease. The present study has favored the selection of blast resistant parental lines which can be utilized as donor in breeding programs.

Key words

Rice, Biotic stress, Blast, Pi resistance genes, Blast screening.

INTRODUCTION

Blast is one of the most devastating fungal diseases affecting rice production system globally and this disease, accounts for 70 - 80\% yield losses in rice. Rice blast disease caused by an ascomycete fungus \textit{Magnaporthe grisea} Barr. (Miah \textit{et al}., 2013; Nasruddin and Amin, 2013). In India, blast disease is found wherever rice is cultivated but predominantly visible in the areas where high humidity and low temperature are present. It is a major disease which limits rice yield and also affects the grain quality. It is also referred as rice fever disease, and has been reported in approximately 85 countries throughout the world. It was majorly found in India, Korea and Phillipines (Shafaullah \textit{et al}., 2011; Bevitori and Ghini, 2014; Motlagh \textit{et al}., 2015; Irri.org).

For controlling the blast disease, use of chemicals is expensive, hazardous to the environment and may cause health problems. The use of R (resistant) genes is considered as most economical and environmentally friendly approach for control of this blast disease (Khanna \textit{et al}., 2015). Resistance is governed by either major R genes, which gives complete protection against few races of the pathogen or on the other hand minor genes, which provides partial protection against the pathogen (Wang \textit{et al}., 1994). So far, more than 350 QTLs (associated with blast resistance) and 100 blast resistance (R) genes have been identified for rice blast resistance, and 27 resistance genes viz \( Pib \), \( Pb1 \), \( Pita \), \( Pid3-A4 \), \( Pikh \), \( Pish \), \( Pik \), \( Pikp,Pi9 \), \( Pi2 \), \( Pizt \), \( Pid2 \), \( Pii \), \( Pi33 \), \( Pi36 \), \( Pi37 \),
**Study location:**
Genotypes were evaluated at Hybrid Rice Evaluation Centre (HREC), Gudalur located at latitude of 11° 30’N, longitude of 76° 30’E and an elevation of 1317.00 m above MSL, Nilgiris Tamil Nadu, which is a natural hot spot and most suitable area for leaf blast disease, whereas the presence of blast disease is seen throughout the year and during winter and rainy season higher blast disease incidence is observed (Selvaraj et al., 2011).

**Genotyping of parental lines**
Genomic DNA was extracted from all the parental lines by using modified CTAB protocol (Ausubel, 1994). DNA was measured by using Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) and used for PCR amplification using primers specific to two major blast resistance genes namely Pi9 (Qu et al., 2006) and Pi54 (Ramkumar et al., 2011).

**Table 1. Functional markers sequence used for screening of blast resistance genes in parental lines**

| S.No | Resistance Genes | Functional markers | Forward Primer | Reverse Primer |
|------|------------------|--------------------|----------------|---------------|
| 1.   | Pi9              | NBS4               | ACTTTGTTGTGCTTGATAAC | ATGGTGAACGCTATCTGTAT |
| 2.   | Pi54             | Pi54MAS            | CAATCTCAGAAGTTTCCAGG | GCCTCAATCAGTCATAGACC |

**MATERIALS AND METHODS**

**Genetic materials used:**
CO51: The rice cultivar CO51 has high yield potential and fine rice grain type. It is a Short duration (110-115 days) variety, suitable for cultivation during all rice seasons in Tamil Nadu

562-4: A NIL of CO43, It is a semi dwarf variety, it has lodging-resistant and high yield potential with fine grain type.

PCR was carried out in 15 μl reactions containing 25 to 50 ng of DNA template, 8.0 μl of sterile water, 1.5 μl of Assay buffer (10 X), 0.50 μl of 2.5 mM dNTPs, 0.20 μl (3 ng) of Taq DNA polymerase and 1.00 μl each of 10 μM forward primer and reverse primer. PCR amplification was done with a profile of 35 cycles at 94°C for 1 min initial denaturation, 94°C for 1 min denaturation, 55°C for 1 min annealing, 72°C for 1 min extension, 72°C for 10 min final extension and 4°C for hold. The PCR products were resolved by agarose gel (3.0%) electrophoresis in 1X TBE buffer and bands were observed after Ethidium Bromide staining and documented using a gel documentation system (BIO-RAD, USA) and banding pattern were scored.

Genotypes were evaluated by following Uniform Blast Nursery (UBN) as described earlier (Selvaraj et al., 2011). Parental lines CO 51, 562-4 (NIL of CO43), Improved White Ponni and susceptible check CO39 were sown in single rows with a length of 100 cm and 10 cm gap between the successive rows. Entire uniform blast nursery bed was surrounded by a known susceptible check CO39, which are sown in a single row for the spread of blast fungal pathogen. Nitrogen fertilizers were also applied for increasing the blast infection rate in the nursery bed. Blast scoring was done following the IRRI standard evaluation system (SES) scale. Two to three readings were made based on the blast disease incidence from 30 days old seedlings at 7-10 days interval (Immanuel et al., 2011; Roumen et al., 1997 and IRRI, 2002 (Table 1).

**Table 1. IRRI Standard evaluation system for Blast resistance**

| Scale | Damage | Resistance level     |
|-------|--------|----------------------|
| 0     | No lesions | Highly Resistant |
| 1     | Small brown specks of pinhead size without sporulating centre | Resistant |
| 3     | Small roundish to slightly elongated necrotic grey spots, about 1-2 mM in diameter with distinct margin, lesions are mostly found on the lower leaves. But significant number lesions are on the upper leaves | Moderately Resistant |
| 5     | Typical blast lesions infecting 2-10% of the leaf area | Moderately susceptible |
| 7     | Blast lesions infecting 26-50% of the leaf area | Susceptible |
| 9     | More than 75% leaf area affected | Highly Susceptible |

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Table 2. List of blast resistance genes and their donors

| Gene name   | Chromosome | Donor          | Reference              |
|-------------|------------|----------------|------------------------|
| Pi37        | 1          | St. No. 1      | Lin et al., 2007       |
| Pi7         | 1          | K59            | Hayashi and Yoshida, 2009 |
| Pi35        | 1          | Shin-2         | Takahashi et al., 2010 |
| Pi35        | 1          | Hokkai 188     | Fukuoka et al., 2014   |
| Pi64        | 1          | Yangmaogu      | Ma et al., 2015        |
| Pi2         | 2          | Tohoku IL9     | Wang et al., 1999      |
| Pi21        | 4          | Owarihatamochi | Fukuoka et al., 2009   |
| Pi63/Pikahei-1(t) | 4 | Kahei         | Xuet al., 2014        |
| Pi-d2       | 6          | Digu           | Chen et al., 2006      |
| Pi9         | 6          | 75-1-127       | Quet al., 2006         |
| Pi2         | 6          | C101A51        | Zhou et al., 2006      |
| Pi-z        | 6          | Toride 1       | Zhou et al., 2006      |
| Pi-d3       | 6          | Digu           | Shang et al., 2009     |
| Pi25        | 6          | Gumei 2        | Chen et al., 2011      |
| Pi50        | 6          | Er-Ba-zhan (EBZ)| Su et al., 2015        |
| Pigm        | 6          | Gumei 4        | Deng et al., 2017      |
| Pid3-11     | 6          | MC276          | Inukai et al., 2019    |
| Pi36        | 8          | Q61            | Liu et al., 2007       |
| Pi5         | 9          | Moroberekan    | Lee et al., 2009       |
| Pi2         | 9          | Hitomebore     | Takagi et al., 2013    |
| Pi2k        | 11         | Tsuyuake       | Ashikawa et al., 2008  |
| Pi-b        | 11         | Modan          | Hayashi et al., 2010   |
| Pi54        | 11         | Tetep          | Sharma et al., 2010    |
| Pi-a        | 11         | Aichi Asahi    | Okuyama et al., 2011   |
| Pi-k-p      | 11         | K60            | Yuan et al., 2011      |
| Pi2k        | 11         | Kusabue        | Zhai et al., 2011      |
| Pi1         | 11         | C101LAC        | Hua et al., 2012       |
| Pi3k        | 11         | Xiangzao 143   | Chen et al., 2015      |
| Pi-ta       | 12         | Yashiro-mochi  | Bryan et al., 2000     |
| Pi2k        | 12         | Katy           | Zhao et al., 2018      |

Fig. 1. PCR analysis of parental lines for screening of Pi9 and Pi54 blast resistance genes. (A) Amplification with NBS4 marker linked to Pi9 resistance gene. An amplicon size of 1kb found in 562-4 alone. (B) Amplification with Pi54MAS marker linked to Pi54 resistance gene. An amplicon size of 216bp found in CO51.360 bp amplicon size found in 562-4 (a NIL of CO43) and Improved White Ponni (IWP).

Phenotypic evaluation of parental lines against blast pathogen.
RESULTS AND DISCUSSION
*M. oryzae* population has higher variability and new virulent races are emerged frequently, often results in losing resistance in tolerant variety within 3-4 years and becomes susceptible. Based on previous research findings, the list of broad spectrum resistance and effective blast genes mentioned in Table 2. (Wu et al., 2007).

The presence of *Pi9* and *Pi54* blast resistance genes was confirmed by using functional markers NBS4 and Pi54MAS respectively (Fig. 1).

Results of blast screening revealed complete susceptibility of CO39 against Tamil Nadu isolate of blast pathogen (Fig. 2). CO39 started developing symptom within 18 days and severe symptom was observed in 45 days. Similarly, Improved White Ponni (IWP) showed severe symptom in 45 days. CO51 (containing *Pi54* gene) and 562-4 (containing *Pi9* gene) were found to exhibit moderate resistance against blast pathogen. At 45 days, they had small round spots with a score of 3 (Fig. 2; Table 3). Blast is a major biotic stress, which causes severe yield losses in rice globally. In recent times majority of high yielding rice varieties have shown highly susceptible reaction to blast disease. However, the use of resistant varieties or cultivars has been shown effective and economical method to control rice blast disease. Studying the effects of different genes as individual in parental lines is highly important, and the results of the experimental study will give useful information for identification of suitable genes for rice breeding programs in future. Identification of resistance spectrum for blast disease in parental lines has created opportunity to develop resistance for devastating blast disease in elite rice cultivar/genotypes. Breakdown of resistance is known against blast disease due to the evolution of new fungal races of pathogen, so pyramiding of more than one gene (available and effective genes which gives resistance against blast) for blast disease is highly important and considered as practical solution to avoid the breakdown of resistance to the pathogen (Hittalmani et al., 2000).

The purpose of this study was to evaluate the efficacy of two major blast resistance genes namely *Pi9* and *Pi54* against Tamil Nadu isolate of blast pathogen. The *Pi9* gene was identified from the rice cultivar *O.minuta* in China and it is mapped on chromosome 6 (Bordeos et al., 1992; Qu et al., 2006). Whereas the *Pi54* gene was identified from rice variety Tetep and it is mapped on the long arm of chromosome 11. In India *Pi54* was shown to provide resistance to many isolates of blast pathogen (Sharma et al., 2005, 2010). *Pi54* gene is widely used by many researchers in breeding program of rice for blast resistance (Singh et al., 2011 and Ellur et al, 2016). The different variants in *Pi54* alleles from wild rice species and different rice cultivars exhibit numerous sequence variation is responsible for its broad spectrum resistance (Kumari et al, 2013, Thakur et al, 2015 and Vasudevan et al., 2015).

**Table 3. Responses of parental lines against blast fungal pathogen**

| Parental line          | Disease Score | Reaction |
|------------------------|---------------|----------|
| CO39 (Susceptible check) | 9             | HS       |
| CO51                   | 3             | MR       |
| 562-4                  | 3             | MR       |
| Improved White Ponni   | 7             | S        |

S- Susceptible, MR- Moderately Resistant;
In recent research studies a resistance mechanisms comparison in Pusa basmati 1 (PB1) NIL population shown cumulatively that, Pi9 gene revealed 52% resistance against M. oryzae, Pi54 gene revealed 43% resistance level against M. oryzae. So evaluation of Pi9 and Pi54 resistance genes against Tamil Nadu isolate will help in formulating breeding programs for developing broad spectrum resistant varieties (Jain et al., 2019). In this study, we have demonstrated that Pi9 and Pi54 resistance genes will be more effective by providing resistance (R) reaction to the blast disease. However, by pyramiding both Pi9 and Pi54 resistance genes in elite/popular rice cultivar can confer enhanced/increased resistance reaction to the blast disease. While improved Blast screening experiment demonstrated that single gene.

Blast screening experiment demonstrated that the parental lines CO51 and S62-4 were moderately resistant to the blast disease (seedling stage). While improved white ponni (IWP) parent line does not have either Pi9 or Pi54 resistance gene and it had shown susceptible reaction to blast disease. Pyramiding of Pi9 and Pi54 genes will provide durable blast resistance in rice cultivar. Parental lines can serve as a useful genetic resource for future breeding program to develop blast resistance in other popular/elite rice variety.

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