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Research Article

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 *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 *Magnaporthegrisea* Barr. (Miah *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 *et al*., 2011;Bevitori and Ghini, 2014;Motlagh *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 *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 *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*, *Pi33*, *Pii*, *Pi36*, *Pi37*,

Pikm, *Pit*, *Pi5*, *Pid3*, *Pia*, *PiCO39*, *Pi25*, *Pi1*, *pi21*, *Pi50* and *Pi65(t)* have been cloned and functionally validated at molecular level (Su *et al*., 2015; Zheng *et al*., 2016; zhu *et al*., 2016). Identifying effective combinations of genes conferring broad spectrum resistance against prevailing pathogen races is very important in blast resistance breeding (Thippeswamy *et al*, 2016). The present study was aimed at validating resistance reaction of two major genes *Pi9* and *Pi54* against local isolates of blast pathogen in Tamil Nadu located at southern part of India. Results of this study enabled the selection of suitable parental lines for blast resistance breeding rice.

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.

Improved White Ponni (IWP): It is a medium duration type, popularly cultivated in Tamil Nadu. It has high yield potential and superior grain and cooking qualities.

CO39: It is used as susceptible check for blast screening

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, where 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	ATGGTGAACGGTATCTGTAT
2.	*Pi54*	Pi54MAS	CAATCTCCAAAGTTTTCAGG	GCTTCAATCACTGCTAGACC

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 mMdNTPs, 0.20 µl (3 U/µl) 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 5 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 CO 39 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 sproulating 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

Table 2.List of blast resistance genes and their donors

Gene name	Chromosome	Donor	Reference
Pi37	1	St. No. 1	Lin *et al*, 2007
Pit	1	K59	Hayashi and Yoshida, 2009
Pish	1	Shin-2	Takahashi *et al*, 2010
Pi35	1	Hokkai 188	Fukuoka *et al*, 2014
Pi64	1	Yangmaogu	Ma *et al*, 2015
Pib	2	Tohoku IL9	Wang *et al*, 1999
Pi21	4	Owarihatamochi	Fukuoka *et al*, 2009
Pi63/Pikahei-1(t)	4	Kahei	Xu*et al*, 2014
Pi-d2	6	Digu	Chen *et al*, 2006
Pi9	6	75-1-127	Qu*et al*, 2006
Pi2	6	C101A51	Zhou *et al*, 2006
Piz-t	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
Pii	9	Hitomebore	Takagi *et al*, 2013
Pikm	11	Tsuyuake	Ashikawa*et al*, 2008
Pb1	11	Modan	Hayashi *et al*, 2010
Pi54	11	Tetep	Sharma *et al*, 2010
Pia	11	Aichi Asahi	Okuyama*et al*, 2011
Pik-p	11	K60	Yuan *et al*, 2011
Pik	11	Kusabue	Zhai*et al*, 2011
Pi1	11	C101LAC	Hua*et al*, 2012
Pike	11	Xiangzao 143	Chen *et al*, 2015
Pi-ta	12	Yashiro-mochi	Bryan *et al*, 2000
Ptr	12	Katy	Zhao *et al*, 2018

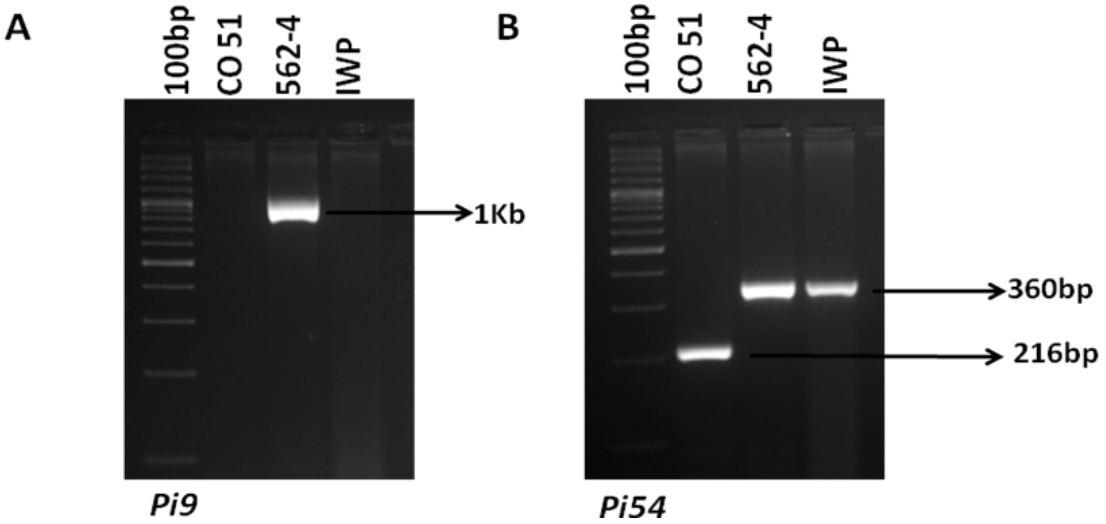

Fig. 1.PCR analysis of parental lines for screening of *Pi9* and *Pi54* blast resistance genes. (A) Amplification with NBS4 marker linked to *Pi9* resistance gene. An amplicon size of 1kb found in 562-4 alone. (B) Amplification with Pi54MAS marker linked to *Pi54* resistance gene. An amplicon size of 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

Fig. 2. Blast screening evaluation of 562-4 harboring Pi9, CO51 harboring Pi54 and Improved White Ponni without Pi9 and Pi54 resistance genes, along with CO39 susceptible check.

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- Moderatery Resistant;

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).

In recent research studies a resistance mechanisms comparison in Pusa basmati 1 (PB1) NIL population shown cumulatively that, *Pi9* generevealed 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 gene in elite/popular rice cultivar can confer enhanced/increased resistance reaction to the blast disease. Based on previous studies it is evident that the genes in combination were providing high level of resistance level and would be more effective than a single gene.

Blast screening experiment demonstrated that the parental lines CO51 and 562-4 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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