Inheritance of blast resistance and its allelic relationship with five major $R$ genes in a rice landrace “Vanasurya”


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ABSTRACT: A rice landrace “Vanasurya” was identified to be blast resistant after multilocation evaluation under uniform blast nursery (UBN). The $F_2$ population derived from the cross between Vanasurya x susceptible rice cultivar, CO 39 showed a segregation of 3R:1S, which revealed that the resistance to blast disease is governed by a single dominant gene. Test of allelic relationship of blast resistance in the landrace, Vanasurya with known major blast resistance genes, viz. $Pi_1$, $Pi_9$, $Pita$, $Piz5$ and $Pi54$ by crossing it with the monogenic blast differentials showed that blast resistance gene in Vanasurya was allelic to $Pita$ whereas it was non-allelic to other genes tested. This was further confirmed through molecular marker analysis with a gene based marker YL155/87 for $Pita$.

Key words: Host resistance, Magnaporthe oryzae, PCR, rice blast

Rice as a staple food crop is grown all over the world and Asian subcontinent contributes for around 90% production and consumption. Further there is more pressure to increase production due to the rapid population growth in this part of the world (Khush, 2005). Rice blast caused by the fungal pathogen Magnaporthe oryzae is a devastating disease occurring in more than 85 countries resulting in major crop losses. The dynamic change in the race composition of pathogen has often resulted in breakdown of resistance in most of the improved varieties. Occurrence of new races of the pathogen in Japan have resulted in frequent breakdown of resistance causing 20-100% of crop losses despite utilization of effective blast resistance genes in local varieties (Khush and Jena, 2009). Till date about 101 blast resistance genes have been mapped from different rice lines. Out of these 22 genes have been cloned and functionally validated (Sharma et al., 2012), providing opportunities for breeding durable blast resistance with multiple genes. Sharma et al. (2012) have stressed the importance of landraces in exploring valuable genes for resistance to blast.

Genetic studies on blast resistance have been conducted extensively in Japan. Sasaki (1922) carried out genetic analysis by inoculation with fungus strains, and showed that the Japanese cultivar, Tsurugi possesses a single dominant gene for blast resistance. Majority of the studies have shown that resistance to blast disease is dominant. The inheritance of resistance to blast disease is easily traced in $F_2$ mapping population specifically developed to identify genes/QTLs associated with it (Mackill and Bonman, 1992). The virulence in $M. oryzae$ is subjected to the selection pressure created by resistant genotypes. The high rate of natural mutations due to selection pressure created by resistance genes has led to accumulation of more virulent genes. Thus, cultivars carrying a single resistance gene are more prone to breakdown of resistance leading to short life-span of resistant genotypes in the field due to instability of $M. oryzae$ avirulence genes (Bonman et al., 1989; Valent and Chumley, 1994; Zhou et al., 2004). A dynamic interaction exists between host plant resistance gene and pathogen virulence gene. Disease resistance is largely controlled by one or two pairs of genes (Chen et al., 2005; Notteghem et al., 1994; Padmavathi et al., 2005; Pan et al., 1999; Sharma et al., 2007; Yu et al., 1996). The most popular work on blast resistance was carried out by Kiyosowa and his colleagues in Japan, wherein they were able to identify as many as 13 genes (Kiyosowa, 1972). Therefore, a need to identify new gene sources is imperative for gene pyramiding using various donors having divergent genes (Padmavathi et al., 2005).

Allelism tests would be much easier in NILs than in donor cultivars where resistance is often conferred by two or more major genes. The genetic basis of broad-spectrum resistance to blast pathogen in rice land races was reported using the standard blast differential system consisting of the standard isolates and differential varieties for analysing segregation ratio governing either dominant or recessive genes for blast resistance (Koide et al., 2013).

Keeping this in view, the present study was conducted to study the nature of inheritance for the blast resistance in rice landrace Vanasurya, and confirm its
allelic status with reference to already reported major R-genes for blast resistance in rice.

**MATERIALS AND METHODS**

**Plant material**

Rice seed material for present study was obtained from NRCPB, New Delhi, (component 4 NAIP-4150). *M. oryzae* cultures were available in the Division of Plant Pathology, IARI, New Delhi. Vanasurya, blast resistant genotype Tetep and susceptible cultivar, CO 39 were screened under Uniform Blast Nursery (UBN) in five blast hot spot locations, Malan (HP), Hazaribagh (Jharkhand), Gerua (Assam) and Gangavati Mugad (Karnataka). Among them, the rice landrace, Vanasurya identified to be resistant, was further evaluated singly with monogenic rice lines, IRBL1-CL, IRBL9-W, IRBLta-K1, IRBLz5-CA carrying *Pi1, Pi9, Pita, Piz5* and Pusa 1603 an isogenic line carrying *Pi54* in PRR 78 background (Singh et al., 2012) respectively, along with resistant check Tetep and susceptible genotype CO 39 under artificial inoculation conditions with four pathotypes of *M. oryzae*. To study the nature of inheritance, Vanasurya was crossed with susceptible rice cultivar, CO 39 and F1 plants were raised from the crossed seed to obtain F2 seeds. Test for allelism of the blast resistance in Vanasurya was conducted through crossing with above monogenic lines carrying major R-gene/(s), *Pi1, Pi9, Pita, Piz5* in Lijiangxintuanheigu (LTH) background and *Pi54* in PRR78, respectively.

**Genomic DNA extraction and PCR amplification**

Genomic DNA of all the F1 plants derived from respective crosses was extracted from fresh, healthy and young leaf tissue by CTAB (Cetyl- Tri Methyl Ammonium Bromide) method (Murray and Thompson, 1980). The DNA was purified by adding Rnase (10mg/ml) to the sample at the rate of 1µl/100 µl of crude DNA. DNA quantification was done using 0.8% agarose gel. The rate of 1µl/100 µl of crude DNA. DNA quantification was done using 0.8% agarose gel. The final concentration was considered resistant and those rated 4-5 as susceptible.

To study the nature of inheritance, Vanasurya was crossed with susceptible rice cultivar, CO 39 under artificial inoculation conditions with four pathotypes of *M. oryzae*. In addition to the corresponding avirulence gene (Silue et al., 1992). In addition to the conventional genetic approach, Sharma et al. (2012) emphasized the importance of molecular marker technology in combination with classical genetic approach for understanding the nature of blast resistance in new source of resistance to *M. Oryzae*. The mode of

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**Table 1. Details of Magnaporthe oryzae isolates used for phenotyping under artificial inoculation.**

<table>
<thead>
<tr>
<th>Isolates used</th>
<th>Year of collection</th>
<th>Place of collection</th>
<th>Infected plant part</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mo-nwi–kas1</td>
<td>2011</td>
<td>Anatnag, Kashmir</td>
<td>Panicle</td>
</tr>
<tr>
<td>Mo-ei-mbi1</td>
<td>2012</td>
<td>Madhubani, Bihar</td>
<td>Leaf</td>
</tr>
<tr>
<td>Mo-ni-0049</td>
<td>2011</td>
<td>Dehradoon, Uttarakhand</td>
<td>Panicle</td>
</tr>
<tr>
<td>Mo-ni-0033</td>
<td>2010</td>
<td>Kaul, Kaithal, Haryana</td>
<td>Panicle</td>
</tr>
</tbody>
</table>
inheritance of blast resistance in rice has been extensively studied and many dominant R-genes conferring high level of resistance to M. oryzae have been identified (Khumbar et al., 2013). Till-date, 101 blast resistance genes and 350 QTLs covering almost all the chromosomes of rice lines have been identified (Ballini et al., 2008; Ghaley et al., 2012, Sharma et al., 2012). Thakur et al. (2013) analysed allelic variants of Pi-ta from 48 rice lines selected after phenotyping of 529 rice landraces across three eco-geographical blast hot spot regions of India based on nucleotide polymorphism.

**Reaction of Vanasurya to blast disease in the UBN**

The rice landrace, Vanasurya was evaluated under uniform blast nursery (UBN) in blast hot spot locations at Malan (HP), Hazaribag (Jharkhand), Mugad and Gangavati (Karnataka) and Gerua (Assam) on 0-9 SES scale. Vanasurya recorded blast scores of 3, 2, 1 and 2 at four locations namely Malan (HP), Mugad (Karnataka), Gangavati (Karnataka) and Gerua (Assam), respectively, while it recorded a score of 4 at Hazaribag (Jharkhand) and was considered as moderately resistant. Tetep used as resistant check was free from blast at all these hot spot locations whereas the susceptible check CO 39 had severe incidence of blast.

**Inheritance of blast resistance in the cross between rice landrace Vanasurya x CO 39**

Crosses between the blast resistant landrace, Vanasurya and susceptible cultivar CO 39 produced 5 F1 seeds. The hybridity was confirmed through molecular analysis (Singh et al., 2012) with a polymorphic microsatellite marker, RM 224 (Fig. 1).

The PCR validated heterozygous F1 hybrids were further raised to harvest F2 seeds for studying inheritance of resistance. The seeds harvested from a single F1 plant were used for phenotyping under artificial inoculation with 4 different M. oryzae isolates.

The isolates used for phenotyping of F2 population were first tested on monogenic differential lines harbouring single blast resistance gene in LTH background. The isolate Mo-ni-0049 and Mo-ni-0033 were from Basmati rice growing region, and Mo-nwi-kas1 and Mo-ei-mbi1 belonged to non-Basmati rice growing region. The phenotypic reaction of isolate Mo-ni-0049 was avirulence to Pi9, Pi54 and virulence to Pi1, Pita and Piz5 (Table 2). Isolate Mo-nwi-kas1 was incompatible with Pi1 and Pi9 and compatible with Pita, Piz5 and Pi54 blast resistance genes. Mo-ni-0033 isolate was virulent to Pi1 but avirulent to other R-genes. Mo-ei-mbi1 isolate displayed varied reaction as it was avirulent to Pi1, Pi9, Pita, Piz5 and Pi54 and virulent to CO 39.

The phenotypic data on the blast disease incidence showed that the F2 population derived from F1 hybrid between Vanasurya and CO 39 segregated in 3R:1S ratio. This indicated that the blast resistance in landrace, Vanasurya is governed by single dominant gene (Table 3). In studies involving blast resistant rice lines, Kumbhar et al. (2013), Ashkani et al. (2011), Sharma et al. (2007) and Pan et al. (1999) showed similar kind of segregation ratios to indicate genetics of resistance gene.

**Allelic relationship with major R-genes for blast resistance**

To characterise the specificity of rice blast resistance gene/s in Vanasurya, the test of allelism was carried out by crossing it with five monogenic lines in the background of LTH carrying major blast resistance genes namely Pi1, Pi9, Pita, Piz5 and Pi54. The F2 seeds were collected from the crosses and tested with different pathotypes having varying virulence pattern on monogenic blast differential lines. The segregation in the F2 progenies showed two distinct classes. In a similar study involving rice accession IR49830-7-1-2-2, harbouring five blast resistance genes Pia, Pib, Pik-s, Pita and Pi11(t), Koide et al. (2011) validated the allelic nature of the corresponding genes using a differential system, allelism.

**Table 2. Phenotypic reaction of genotypes to four M. oryzae isolates**

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>R-gene/s</th>
<th>Mo-ni-0049</th>
<th>Mo-ni-kas1</th>
<th>Mo-ni-0033</th>
<th>Mo-ei-mbi1</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRBL1-CL</td>
<td>Pi1</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>IRBL9-W</td>
<td>Pi9</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>IRBLta-K1</td>
<td>Pita</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>IRBLz5-CA</td>
<td>Piz5</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Pusa 1603</td>
<td>Pi54</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Vanasurya</td>
<td>-</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Tetep</td>
<td>Pikh, Pi1, Pita, Pita(t) (Barman et al., 2004)</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>CO 39</td>
<td>Pi-CO39(t) (Chauhan et al., 2002)</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>
The presence of Pita in Vanasurya was further confirmed and Pi1 blast differential monogenic line showed 15R:1S monogenic blast differential line containing Piz5 gene, dominant gene interaction) present on different loci. Depicting two dominant resistance genes (duplicate containing Pi9 gene was inoculated with Mo-nwi-kas1 contributing to its blast resistance along with Pita cannot unknown/undetected genes present in Vanasurya blast resistance. However, the possibility of other alleles in the breeding programmes aimed at improving this information would be of use for selecting better

In another cross derived from Vanasurya and monogenic blast differential line containing Piz5 gene, the phenotypic response of F2 population challenged with blast isolate Mo-ni-0033, virulent on P1t gene showed a segregation ratio of 15R:1S ratio for dominant duplicate gene action and the results indicate that the two genes are non-allelic to each other. The segregation pattern of F2 population obtained from cross between Vanasurya and P1t blast differential monogenic line showed 15R:1S ratio for Mo-ri-mbi1 isolate, which is avirulent to P1t, P19, Pita, Piz5 and P1s4 blast resistance genes. This finding shows that the genes present in Vanasurya and P1t genes are non-allelic to each other. The allelism test involving the F2 population developed by crossing Vanasurya and Pusa 1603 was done by challenging the F2 plants with Mo-ri-0049 isolate, which was avirulent to P1s4 as well as P19 and virulent to other blast resistance genes P1t, Pita and Piz5. F2 population segregated in a typical duplicate dominant gene interaction ratio of 15R:1S suggesting non allelic nature for P1s4 and P19 with the genes present in Vanasurya.

When 430 F2 seedlings from a cross between Vanasurya and monogenic line IRBLta-K1 carrying Pita were challenged with Mo-ri-0033 isolate, which was incompatible for both Pita and resistant parent Vanasurya, did not show any segregation, indicating the confirmation for the presence of Pita gene in Vanasurya. The presence of Pita in Vanasurya was further confirmed with molecular marker analysis with gene based marker, YL155/87 (Fig. 2). The allelic relationship study confirmed that the candidate resistance gene present in Vanasurya was allelic to the major blast resistance gene Pita and non-allelic to other R-genes, i.e. P1t, P19, Piz5 and P1s4. This information would be of use for selecting better alleles in the breeding programmes aimed at improving blast resistance. However, the possibility of other unknown/undetected genes present in Vanasurya contributing to its blast resistance along with Pita cannot be ruled out at this stage.

Table 3. Segregation of F2 population obtained from cross between Vanasurya with susceptible line CO 39 and other monogenic lines

<table>
<thead>
<tr>
<th>Crosses</th>
<th>Resistant</th>
<th>Susceptible</th>
<th>Total</th>
<th>Expected ratio (R:S)</th>
<th>Chi-square value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vanasurya x CO 39</td>
<td>134</td>
<td>41</td>
<td>175</td>
<td>3:1</td>
<td>0.230</td>
<td>0.63</td>
</tr>
<tr>
<td>Vanasurya x IRBLta-K1 (Pita)</td>
<td>430</td>
<td>0</td>
<td>430</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vanasurya x IRBL9-L (P19)</td>
<td>172</td>
<td>10</td>
<td>182</td>
<td>15:1</td>
<td>0.177</td>
<td>0.67</td>
</tr>
<tr>
<td>Vanasurya x IRBL25-CA (Piz5)</td>
<td>210</td>
<td>20</td>
<td>230</td>
<td>15:1</td>
<td>2.347</td>
<td>0.12</td>
</tr>
<tr>
<td>Vanasurya x IRBL1-CL (P1t)</td>
<td>175</td>
<td>15</td>
<td>190</td>
<td>15:1</td>
<td>0.877</td>
<td>0.34</td>
</tr>
<tr>
<td>Vanasurya x Pusa 1603 (P1s4)</td>
<td>187</td>
<td>13</td>
<td>200</td>
<td>15:1</td>
<td>0.021</td>
<td>0.88</td>
</tr>
</tbody>
</table>

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Fig. 2. PCR amplification of Pita gene from Vanasurya with gene based marker (L- 100bp ladder, S- CO 39, R- IRBLta-K1, V- Vanasurya)


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