Molecular Screening of Blast Resistance Genes in Rice using SSR Markers

A. K. Singh1*, P. K. Singh1, Madhuri Arya1, N. K. Singh1 and U. S. Singh3
1Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221005, India
2National Research Centre on Plant Biotechnology, Indian Agricultural Research Institute, New Delhi 110 012, India
3STRASA-IRRI-India Office, Rajendra Place, 9th Floor, Aggrawal Corporate Tower, New Delhi 110 008, India

(Received on June 12, 2014; Revised on December 9, 2014; Accepted on November 17, 2014)

Rice blast is the most devastating disease causing major yield losses in every year worldwide. It had been proved that using resistant rice varieties would be the most effective way to control this disease. Molecular screening and genetic diversities of major rice blast resistance genes were determined in 192 rice germplasm accessions using simple sequence repeat (SSR) markers. The genetic frequencies of the 10 major rice blast resistance genes varied from 19.79% to 54.69%. Seven accessions IC337593, IC346002, IC346004, IC346813, IC356117, IC356422 and IC383441 had maximum eight blast resistance gene, while FR13B, Hourakani, Kala Rata 1-24, Lemont, Brown Gora, IR87756-20-2-2-3, IC282418, IC356419, PKSLGR-1 and PKSLGR-39 had seven blast resistance genes. Twenty accessions possessed six genes, 36 accessions had five genes, 41 accessions had four genes, 38 accessions had three genes, 26 accessions had two genes, 13 accessions had single \( R \) gene and only one accession IC438644 does not possess any one blast resistant gene. Out of 192 accessions only 17 accessions harboured 7 to 8 blast resistance genes.

Keywords: blast, marker assisted selection, resistance genes, rice, SSR markers

Rice blast is one of the most destructive diseases affecting rice production worldwide, which caused an economic loss up to 65% yield in susceptible cultivars (Li et al., 2007). Losses are dependent on the growth stage of the plant at which infection occurs, level of resistance and prevailing environmental conditions. It occurs more frequently in rainfed areas in wet season due to favourable environmental conditions for disease development. The identification and isolation of additional host blast resistance (\( R \)) genes and pathogen avirulence gene are now required to deepen understanding of molecular mechanisms involved in the host-pathogen interaction (Valent, 1990). Generally \( R \) genes are identified in land races, cultivars and wild rice collections using differential physiological races of \textit{Magnaporthe oryzae} (Tanksley et al., 1997). With fine mapping and cloning of many blast resistance genes, many PCR-based markers have been developed to screen and identify different blast resistance genes. DNA markers closely linked to a blast \( R \) gene that confers resistance to a particular race of the pathogen can be effectively employed for marker assisted selection (MAS), which is much faster than traditional pathogenicity assays. Accurate identification of a particular \( R \) gene in diverse elite germplasm using DNA markers and differential blast races is an essential step for ensuring the accuracy of \( R \) gene utilization in using MAS for different rice breeding programs (Roy-Chowdhury et al., 2012a).

Recently, more than 100 major blast resistance genes from \textit{japonica} (45%), \textit{indica} (51%) and other (4%) genotypes have been identified and documented (Ballini et al., 2008; Berruyer et al., 2003; Chauhan et al., 2002; Chen et al., 2002; Huang et al., 2010; Liu et al., 2004; Liu et al., 2005; Sharma et al., 2012; Xiao et al., 2010; Zhu et al., 2004); and many rice varieties with complete resistance to \textit{M. grisea} have been developed, but in many cases this resistance has been breakdown within a few years of the initial cultivation owing to the emergence of stronger virulent isolates of rice blast fungus (Han et al., 2001). Partial and field resistance of rice blast has received much attention as a means of effective control of a parasite under natural field condition and conferring durable blast resistance when exposed to new races of that parasite (Hittalmani et al., 2000; Liu et al., 2005). These \( R \) genes are distributed throughout the 12 rice chromosomes except chromosome 3 (Liu et al., 2010; Yang et al., 2008). Out of them, 22 have been

*Corresponding author.
Phone: +91-9451173551, FAX: +91-5422369971
E-mail: anilbhu987@gmail.com
Table 1. List of blast resistance genes, markers and germplasm used as check variety

| S.No. | Gene | Chromosome locus | Check variety | Linked marker | Linkage distance (cM) | Expected PCR product size (bp) | SSR motif | Annealing temp. (°C) | References |
|-------|------|------------------|---------------|---------------|----------------------|-------------------------------|-----------|---------------------|------------|
| 1     | Pi-9 | 6                | PB-1460       | RM 541        | 0.6                  | 158                          | (TC)16    | 55                  | Cho et al., 2008 |
| 2     | Pi-1 | 11               | PB-1460       | RM 224        | 0.0                  | 157                          | (AAG)8(AG)13 | 56.4                | Fuentes et al., 2007 |
| 3     | Pi5-t| 11               | PB-1460       | RM 21         | 0.0                  | 157                          | (GA)18    | 60.4                | Cuong et al., 2006 |
| 4     | Piz-5| 6                | PB-1460       | RM 527        | 0.3                  | 233                          | (GA)17    | 55                  | Fjellstrom et al., 2006 |
| 5     | Pi-b | 2                | PB-1460       | RM 208        | 1.2                  | 173                          | (CT)17    | 57.85               | Hayashi et al., 2006 |
| 6     | Pi-ta| 12               | PB-1460       | RM 247        | 5.0                  | 131                          | (CT)16    | 55.25               | Eizenga et al., 2006 |
| 7     | Pi33 | 8                | IR-64         | RM 72         | 11.5                 | 166                          | (TAT)5C(ATT)15 | 56.95 | Berruyer et al., 2003 |
| 8     | Pi-27(t)| 1           | Tepet         | RM 259        | 9.7                  | 162                          | (CT)17    | 56.65               | Zhu et al., 2004 |
| 9     | Pitp(t)| 1           | Tepet         | RM 246        | 0.0                  | 116                          | (CT)20    | 58.50               | Barman et al., 2004 |
| 10    | Pi-k | 11               | Tepet         | RM 206        | 0.6                  | 147                          | (CT)21    | 56.30               | Sharma et al., 2005 |

Materials and Methods

Plant materials. The experimental materials comprised of 192 rice germplasm accessions received from Networking Project National Research Centre on Plant Biotechnology, New Delhi, Birsa Agricultural University, Ranchi and Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu Varanasi, India, and their seeds were multiplied in wet season 2013. The details of the source of 192 rice accessions are presented in Table 2.

DNA extraction. Young leaves were collected from two week old plantlets of each germplasm grown in the plant growth chamber. The 40 mg leaves were taken from each accession placed in 1.2 ml collection microtubes (Qiagen Tissue Lyser II, Qiagen, U.S.A.) and in each microtube 3 mm tungsten beads were dispensed by bead dispenser and kept at –80°C for 4 hrs. Tissues were disrupted and homogenized by qiangen tissue lyser to a fine powder at frequency of 30 vibrations/seconds for 30 seconds. Fine powdered leaf samples were used for isolation of genomic DNA using CTAB (hexadecyl trimethyl ammonium bromide) method (Doyle and Doyle, 1987). The DNA was quantified spectrophotometrically (Perkin Elmer, Singapore) by measuring A260/A280, and DNA quality was checked by electrophoresis in 0.8% agarose gel.

SSR analysis. Ten previously reported SSR markers synthesized by Eurofins Genomics (Bangalore, India), were used to analyze the status of the blast resistance genes (Table 1). The amplification was carried out in 15 μl of reaction...
Table 2. List of one hundred ninety two rice germplasm accessions, their source and genotypic screening for blast resistance gene with SSR markers

| S.No. | Accession Number | Source                  | Blast resistance genes |
|-------|------------------|-------------------------|------------------------|
|       |                  |                         | Pi-9 (RM 541)          | Pi-ta (RM 247) |
|       |                  |                         | Pi-t (RM 224)          | Total number of genes |
|       |                  |                         | Pi-1 (RM 21)           |                 |
|       |                  |                         | Pi-5 (RM 208)         |                 |
|       |                  |                         | Pi-33 (RM 259)        |                 |
|       |                  |                         | Pi-6 (RM 206)         |                 |
|       |                  |                         | Total                 |                 |
| 1     | Dular            | IRRI, Philippines       | 0 1 0 0 1 0 0 0 1 0 3 |                 |
| 2     | FR13A            | IRRI, Philippines       | 1 1 0 0 1 0 0 0 1 0 5 |                 |
| 3     | FR13B            | IRRI, Philippines       | 1 1 0 1 1 1 0 1 0 7 4 |                 |
| 4     | Karkati 87       | IRRI, Philippines       | 0 0 1 1 1 0 1 0 0 4 3 |                 |
| 5     | Birpala          | IRRI, Philippines       | 1 0 1 1 1 0 0 0 1 0 5 |                 |
| 6     | Goda Heenati     | IRRI, Philippines       | 1 0 1 0 0 0 0 1 0 0 3 |                 |
| 7     | IR-64 Sub-1      | NRCPB, New Delhi, India | 1 0 0 0 0 1 1 0 0 0 3 |                 |
| 8     | Heensalai        | IRRI, Philippines       | 1 0 1 1 0 0 0 1 0 0 4 |                 |
| 9     | Madal            | IRRI, Philippines       | 1 0 1 1 0 1 0 0 0 0 5 |                 |
| 10    | Mahadikwée       | IRRI, Philippines       | 1 0 0 1 1 1 0 0 0 0 4 |                 |
| 11    | Ausboro          | IRRI, Philippines       | 1 0 0 0 0 1 1 1 0 5 5 |                 |
| 12    | Jaldungi         | IRRI, Philippines       | 1 0 0 0 0 1 0 1 1 0 5 |                 |
| 13    | Nirboi           | IRRI, Philippines       | 1 1 0 0 0 0 0 1 0 0 3 |                 |
| 14    | CR-1009          | IRRI, Philippines       | 1 1 0 0 0 0 0 1 1 0 5 |                 |
| 15    | Houranaki        | IRRI, Philippines       | 1 0 0 1 1 1 1 1 0 7 4 |                 |
| 16    | Nadal            | IRRI, Philippines       | 1 1 0 0 1 0 1 1 0 0 5 |                 |
| 17    | Lunishree        | CRRI, Cuttack, India    | 1 1 0 1 0 0 0 1 1 0 6 |                 |
| 18    | Swarna Sub-1     | NRCPB, New Delhi, India | 1 1 0 0 0 0 1 1 1 0 6 |                 |
| 19    | Sambha Sub-1     | NRCPB, New Delhi, India | 1 0 1 1 0 1 1 1 0 0 6 |                 |
| 20    | Kariywa          | NRCPB, New Delhi, India | 1 1 0 1 0 1 1 0 1 0 6 |                 |
| 21    | S-155            | CRRI, Cuttack, India    | 0 1 0 0 0 0 1 0 0 0 3 |                 |
| 22    | Tsao Wanching    | IRRI, Philippines       | 1 1 1 1 0 1 0 1 0 0 6 |                 |
| 23    | JC-1             | IRRI, Philippines       | 0 1 1 0 0 1 0 1 0 0 4 |                 |
| 24    | Lumbini          | IRRI, Philippines       | 0 0 0 0 0 1 0 1 1 1 1 5 |                 |
| 25    | Madhukar         | IRRI, Philippines       | 1 1 1 0 0 1 0 0 0 0 4 |                 |
| 26    | N-22             | IRRI, Philippines       | 0 0 0 0 0 1 1 1 1 0 5 |                 |
| 27    | Binulavan        | IRRI, Philippines       | 0 1 0 0 1 0 1 0 0 0 3 |                 |
| 28    | Kala Rata-2-14   | IRRI, Philippines       | 1 1 0 1 1 1 1 1 0 0 7 |                 |
| 29    | Tatan            | IRRI, Philippines       | 1 0 1 0 0 0 0 0 1 1 4 |                 |
| 30    | Bapakribuna      | IRRI, Philippines       | 1 0 1 0 0 1 0 0 0 0 3 |                 |
| 31    | Gota             | IRRI, Philippines       | 1 0 0 1 1 1 0 1 0 0 6 |                 |
| 32    | Khaiyen          | IRRI, Philippines       | 0 0 0 1 0 1 1 1 1 0 5 |                 |
| 33    | Kamnan           | IRRI, Philippines       | 1 1 1 0 0 0 0 0 0 0 3 |                 |
| 34    | Lernont          | IRRI, Philippines       | 1 1 1 1 1 1 0 0 1 0 7 |                 |
| 35    | Latska           | IRRI, Philippines       | 0 0 0 0 0 0 1 0 0 0 4 |                 |
| 36    | Akita Komachi    | IRRI, Philippines       | 1 1 0 0 0 0 0 0 0 0 2 |                 |
| 37    | M-202            | IRRI, Philippines       | 0 1 1 0 0 1 1 0 0 0 5 |                 |
| 38    | Amaroo           | IRRI, Philippines       | 0 1 1 0 1 0 1 0 0 0 4 |                 |
| 39    | Nipponbare       | IRRI, Philippines       | 0 1 1 0 1 0 0 0 0 0 4 |                 |
| 40    | Asami Dhan       | IRRI, Philippines       | 0 0 1 0 1 0 0 0 0 0 3 |                 |
| 41    | Chanygat         | IRRI, Philippines       | 0 0 1 1 1 1 0 1 0 0 5 |                 |
| 42    | Budkodi          | IRRI, Philippines       | 1 0 1 1 1 1 0 1 0 0 6 |                 |
| 43    | Dub Gelong       | IRRI, Philippines       | 0 0 1 0 1 1 1 0 1 0 5 |                 |
| 44    | Sawitri          | IRRI, Philippines       | 0 0 0 0 0 1 1 1 0 0 3 |                 |
## Table 2. Continued

| S.No. | Accession Number | Source                      | Blast resistance genes | Total number of genes |
|-------|------------------|-----------------------------|------------------------|-----------------------|
|       |                  |                             | Pz-5 (RM 527)          |                       |
|       |                  |                             | Pz-9 (RM 541)          |                       |
|       |                  |                             | Pitp(t) (RM 224)       |                       |
|       |                  |                             | Pi-1 (RM 246)          |                       |
|       |                  |                             | Pi-5(t) (RM 529)       |                       |
|       |                  |                             | Pi-33 (RM 208)         |                       |
|       |                  |                             | Pi-b (RM 72)           |                       |
|       |                  |                             | Pik-h (RM 206)         |                       |
|       |                  |                             | Pi-ka (RM 247)         |                       |
| 45    | Asthu Bhejna     | IRRI, Philippines           | 0 0 0 0 0 0 1 0 0 0 1 0|                       |
| 46    | Tundianiya       | IRRI, Philippines           | 1 0 1 0 0 0 1 0 1 0 4 0|                       |
| 47    | MTU-7029         | BHU, Varanasi, India        | 1 0 1 0 1 1 0 1 1 0 6 0|                       |
| 48    | Sarjoo-52        | BHU, Varanasi, India        | 1 0 1 0 1 1 1 0 1 0 4 0|                       |
| 49    | HUR-105          | BHU, Varanasi, India        | 1 0 1 0 1 1 0 0 0 0 4 0|                       |
| 50    | HUR-3022         | BHU, Varanasi, India        | 1 0 0 0 0 0 1 0 0 0 2 0|                       |
| 51    | HUR-38B          | BHU, Varanasi, India        | 1 0 0 0 1 1 1 0 0 0 4 0|                       |
| 52    | HUBR-40          | BHU, Varanasi, India        | 1 1 0 0 0 1 1 0 0 1 5 0|                       |
| 53    | HUBR-21          | BHU, Varanasi, India        | 1 1 1 0 0 1 0 0 0 4 0 0|                       |
| 54    | CB05-753-3       | BAU, Ranchi, India          | 1 1 1 0 0 0 1 0 1 0 5 0|                       |
| 55    | IR73000-981-2-1  | BAU, Ranchi, India          | 1 1 0 0 0 1 0 1 1 0 5 0|                       |
| 56    | Brown Gora       | BAU, Ranchi, India          | 0 1 1 1 1 1 1 1 0 0 7 0|                       |
| 57    | IR82912-B-B-10   | BAU, Ranchi, India          | 0 1 1 0 0 0 0 0 0 0 2 0|                       |
| 58    | IR82910-B-B-67-2 | BAU, Ranchi, India          | 1 0 1 1 0 0 0 0 0 0 3 0|                       |
| 59    | UPI2102-16       | BAU, Ranchi, India          | 0 1 0 1 0 0 0 0 0 0 2 0|                       |
| 60    | CR3488-1-2-1-2   | BAU, Ranchi, India          | 1 1 1 0 1 0 0 0 0 0 4 0|                       |
| 61    | IR82912-B-B-13   | BAU, Ranchi, India          | 0 1 0 0 0 0 0 0 0 0 1 0|                       |
| 62    | IR83926-B-B-71-4 | BAU, Ranchi, India          | 1 1 1 0 0 1 0 0 0 4 0 0|                       |
| 63    | CT15678-2-3-3-1  | BAU, Ranchi, India          | 1 0 1 0 0 0 1 0 0 3 0 0|                       |
| 64    | B10580-E-KN-28   | BAU, Ranchi, India          | 1 1 1 1 1 0 0 0 0 1 0 5|                       |
| 65    | IR82635-B-B-93-2 | BAU, Ranchi, India          | 1 1 1 0 0 0 0 0 0 0 3 0|                       |
| 66    | BV7109           | BAU, Ranchi, India          | 1 1 1 1 0 0 0 0 1 0 5 0|                       |
| 67    | IR71146-97-1-2-1 | BAU, Ranchi, India          | 1 1 1 1 0 0 0 0 0 0 3 0|                       |
| 68    | IR82635-B-B-143-1| BAU, Ranchi, India          | 1 1 1 1 0 0 0 0 0 0 4 0|                       |
| 69    | BAU408-05        | BAU, Ranchi, India          | 0 1 0 0 0 0 0 0 0 0 1 0|                       |
| 70    | IR82912-B-B-2    | BAU, Ranchi, India          | 0 1 0 0 0 0 0 0 0 0 1 0|                       |
| 71    | IR82912-B-B-7    | BAU, Ranchi, India          | 1 1 1 0 0 0 0 0 0 0 3 0|                       |
| 72    | IR83928-B-B-132-3| BAU, Ranchi, India          | 0 0 0 0 0 0 0 0 0 1 1 0|                       |
| 73    | IRAT-112         | BAU, Ranchi, India          | 1 1 1 0 0 0 0 0 1 0 4 0|                       |
| 74    | IR87756-20-2-2   | BAU, Ranchi, India          | 1 1 1 0 0 1 0 1 1 1 7 0|                       |
| 75    | IR67017-124-2-4  | BAU, Ranchi, India          | 0 0 0 1 0 0 0 0 1 1 0 3|                       |
| 76    | B3688-TB-25-MR-2 | BAU, Ranchi, India          | 1 0 0 0 1 0 0 0 1 1 0 4|                       |
| 77    | BP1R768-2-3-7-81-1| BAU, Ranchi, India          | 1 0 0 1 1 0 0 1 1 0 5 0|                       |
| 78    | IR78933-B-B-24-B-4 | BAU, Ranchi, India        | 1 0 0 1 1 0 0 1 1 0 5 0|                       |
| 79    | BP13510-1-2-PK-3-1| BAU, Ranchi, India       | 1 0 1 0 0 0 0 1 1 1 0 5|                       |
| 80    | RR617-B-B-3      | BAU, Ranchi, India          | 1 0 1 0 1 0 0 1 0 0 4 0|                       |
| 81    | CR3423-1         | BAU, Ranchi, India          | 0 0 1 0 0 0 0 1 0 0 2 0|                       |
| 82    | CR1946-2-1       | BAU, Ranchi, India          | 0 0 1 0 0 0 0 1 0 0 2 0|                       |
| 83    | Kala Bunde       | NRCPB, New Delhi, India     | 0 0 0 0 0 0 1 0 1 0 2 0|                       |
| 84    | IC260937         | DBT, New Delhi, India       | 1 0 0 0 1 0 0 0 0 0 2 0|                       |
| 85    | IC260961         | DBT, New Delhi, India       | 1 0 0 0 1 0 0 0 0 0 2 0|                       |
| 86    | IC260964         | DBT, New Delhi, India       | 1 0 0 1 1 0 0 0 0 0 3 0|                       |
| 87    | IC267416         | DBT, New Delhi, India       | 0 0 0 1 0 0 0 0 0 0 2 0|                       |
| 88    | IC278777         | DBT, New Delhi, India       | 1 0 0 0 0 0 0 0 0 0 1 0|                       |
| 89    | IC280466         | DBT, New Delhi, India       | 1 0 1 0 0 0 0 0 0 0 2 0|                       |
Table 2. Continued

| S.No. | Accession Number | Source | Blast resistance genes |
|-------|------------------|--------|------------------------|
|       |                  |        | $P_{i-5}$ (RM 527) | $P_{i-9}$ (RM 541) | $P_{ipt(t)}$ (RM 246) | $P_{i-1}$ (RM 224) | $P_{i-5(t)}$ (RM 21) | $P_{i-33}$ (RM 72) | $P_{i-h}$ (RM 208) | $P_{i-27(t)}$ (RM 259) | $P_{i-k-h}$ (RM 206) | $P_{i-ta}$ (RM 247) | Total number of genes |
| 90    | IC281785         | DBT, New Delhi, India | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 3 |
| 91    | IC281786         | DBT, New Delhi, India | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 6 |
| 92    | IC282418         | DBT, New Delhi, India | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 7 |
| 93    | IC282443         | DBT, New Delhi, India | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 4 |
| 94    | IC282463         | DBT, New Delhi, India | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 6 |
| 95    | IC282471         | DBT, New Delhi, India | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 6 |
| 96    | IC282514         | DBT, New Delhi, India | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 5 |
| 97    | IC282808         | DBT, New Delhi, India | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 4 |
| 98    | IC282815         | DBT, New Delhi, India | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 5 |
| 99    | IC282816         | DBT, New Delhi, India | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 4 |
| 100   | IC282822         | DBT, New Delhi, India | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| 101   | IC282824         | DBT, New Delhi, India | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| 102   | IC331196         | DBT, New Delhi, India | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 103   | IC334180         | DBT, New Delhi, India | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| 104   | IC337051         | DBT, New Delhi, India | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 3 |
| 105   | IC337367         | DBT, New Delhi, India | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| 106   | IC337558         | DBT, New Delhi, India | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 |
| 107   | IC337578         | DBT, New Delhi, India | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 4 |
| 108   | IC337582         | DBT, New Delhi, India | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 2 |
| 109   | IC337588         | DBT, New Delhi, India | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 6 |
| 110   | IC337593         | DBT, New Delhi, India | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 8 |
| 111   | IC341351         | DBT, New Delhi, India | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 6 |
| 112   | IC346602         | DBT, New Delhi, India | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 8 |
| 113   | IC346604         | DBT, New Delhi, India | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 8 |
| 114   | IC346813         | DBT, New Delhi, India | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 8 |
| 115   | IC346880         | DBT, New Delhi, India | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 5 |
| 116   | IC356117         | DBT, New Delhi, India | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 8 |
| 117   | IC356419         | DBT, New Delhi, India | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 7 |
| 118   | IC356422         | DBT, New Delhi, India | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 8 |
| 119   | IC356429         | DBT, New Delhi, India | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 5 |
| 120   | IC356431         | DBT, New Delhi, India | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 5 |
| 121   | IC356432         | DBT, New Delhi, India | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 5 |
| 122   | IC356448         | DBT, New Delhi, India | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 4 |
| 123   | IC362206         | DBT, New Delhi, India | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 4 |
| 124   | IC382604         | DBT, New Delhi, India | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 6 |
| 125   | IC383396         | DBT, New Delhi, India | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 4 |
| 126   | IC383404         | DBT, New Delhi, India | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 4 |
| 127   | IC383441         | DBT, New Delhi, India | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 8 |
| 128   | IC383559         | DBT, New Delhi, India | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 3 |
| 129   | IC384176         | DBT, New Delhi, India | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 3 |
| 130   | IC384190         | DBT, New Delhi, India | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 5 |
| 131   | IC384260         | DBT, New Delhi, India | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 3 |
| 132   | IC391524         | DBT, New Delhi, India | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 4 |
| 133   | IC418382         | DBT, New Delhi, India | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 2 |
| 134   | IC426012         | DBT, New Delhi, India | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 5 |
| S.No. | Accession Number | Source                  | Blast resistance genes                  |
|-------|----------------|-------------------------|-----------------------------------------|
|       |                |                         | $P_{iz-5}$ (RM 527) | $P_{z-9}$ (RM 541) | $P_{itp(t)}$ (RM 246) | $P_{i-1}$ (RM 224) | $P_{ir-5(t)}$ (RM 21) | $P_{i-33}$ (RM 72) | $P_{i-b}$ (RM 208) | $P_{i27(t)}$ (RM 259) | $P_{i-k-h}$ (RM 206) | $P_{i-ta}$ (RM 247) | Total number of genes |
| 135   | IC426013       | DBT, New Delhi, India   | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 5                        |
| 136   | IC426017       | DBT, New Delhi, India   | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 6                        |
| 137   | IC426058       | DBT, New Delhi, India   | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 5                        |
| 138   | IC426060       | DBT, New Delhi, India   | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 3                        |
| 139   | IC426061       | DBT, New Delhi, India   | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 3                        |
| 140   | IC426137       | DBT, New Delhi, India   | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1                        |
| 141   | IC438644       | DBT, New Delhi, India   | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0                        |
| 142   | IC446975       | DBT, New Delhi, India   | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2                        |
| 143   | EC545051       | DBT, New Delhi, India   | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 3                        |
| 144   | EC545061       | BHU, Varanasi, India    | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 3                        |
| 145   | PKSGLR-1       | BHU, Varanasi, India    | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 7                        |
| 146   | PKSGLR-2       | BHU, Varanasi, India    | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 4                        |
| 147   | PKSGLR-3       | BHU, Varanasi, India    | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 5                        |
| 148   | PKSGLR-4       | BHU, Varanasi, India    | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 3                        |
| 149   | PKSGLR-5       | BHU, Varanasi, India    | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 4                        |
| 150   | PKSGLR-6       | BHU, Varanasi, India    | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 3                        |
| 151   | PKSGLR-7       | BHU, Varanasi, India    | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 5                        |
| 152   | PKSGLR-8       | BHU, Varanasi, India    | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 6                        |
| 153   | PKSGLR-9       | BHU, Varanasi, India    | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 2                        |
| 154   | PKSGLR-10      | BHU, Varanasi, India    | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 2                        |
| 155   | PKSGLR-11      | BHU, Varanasi, India    | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 2                        |
| 156   | PKSGLR-12      | BHU, Varanasi, India    | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 3                        |
| 157   | PKSGLR-13      | BHU, Varanasi, India    | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1                        |
| 158   | PKSGLR-14      | BHU, Varanasi, India    | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1                        |
| 159   | PKSGLR-15      | BHU, Varanasi, India    | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 2                        |
| 160   | PKSGLR-16      | BHU, Varanasi, India    | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 2                        |
| 161   | PKSGLR-17      | BHU, Varanasi, India    | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 3                        |
| 162   | PKSGLR-18      | BHU, Varanasi, India    | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 2                        |
| 163   | PKSGLR-19      | BHU, Varanasi, India    | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2                        |
| 164   | PKSGLR-20      | BHU, Varanasi, India    | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 4                        |
| 165   | PKSGLR-21      | BHU, Varanasi, India    | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 4                        |
| 166   | PKSGLR-22      | BHU, Varanasi, India    | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2                        |
| 167   | PKSGLR-23      | BHU, Varanasi, India    | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2                        |
| 168   | PKSGLR-24      | BHU, Varanasi, India    | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 4                        |
| 169   | PKSGLR-25      | BHU, Varanasi, India    | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 6                        |
| 170   | PKSGLR-26      | BHU, Varanasi, India    | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 4                        |
| 171   | PKSGLR-27      | BHU, Varanasi, India    | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 5                        |
| 172   | PKSGLR-28      | BHU, Varanasi, India    | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 4                        |
| 173   | PKSGLR-29      | BHU, Varanasi, India    | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 3                        |
| 174   | PKSGLR-30      | BHU, Varanasi, India    | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 6                        |
| 175   | PKSGLR-31      | BHU, Varanasi, India    | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1                        |
| 176   | PKSGLR-32      | BHU, Varanasi, India    | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1                        |
| 177   | PKSGLR-33      | BHU, Varanasi, India    | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 3                        |
| 178   | PKSGLR-34      | BHU, Varanasi, India    | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 3                        |
| 179   | PKSGLR-35      | BHU, Varanasi, India    | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 5                        |
The rice blast resistance gene scored as the presence (1) and absence (0) of amplicon linked to ten allele specific SSR markers.

| S.No. | Accession Number | Source | Blast resistance genes |
|-------|------------------|--------|------------------------|
|       |                  |        | $Piz-5$ | $Piz-9$ | $Pitp(t)$ | $Pitp(t)$ | $Pi-1$ | $Pi-5(t)$ | $Pi-33$ | $Pi-b$ | $Pi-27(t)$ | $Pi-h$ | $Pi-t$ | Total number of genes |
| 180   | PKSLGR-36        | BHU, Varanasi, India | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 4 |
| 181   | PKSLGR-37        | BHU, Varanasi, India | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 4 |
| 182   | PKSLGR-38        | BHU, Varanasi, India | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 6 |
| 183   | PKSLGR-39        | BHU, Varanasi, India | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 7 |
| 184   | PKSLGR-40        | BHU, Varanasi, India | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 6 |
| 185   | PKSLGR-41        | BHU, Varanasi, India | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 4 |
| 186   | PKSLGR-42        | BHU, Varanasi, India | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 4 |
| 187   | PKSLGR-43        | BHU, Varanasi, India | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 3 |
| 188   | PKSLGR-44        | BHU, Varanasi, India | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| 189   | PKSLGR-45        | BHU, Varanasi, India | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 3 |
| 190   | PKSLGR-46        | BHU, Varanasi, India | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 2 |
| 191   | PKSLGR-47        | BHU, Varanasi, India | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 |
| 192   | PKSLGR-48        | BHU, Varanasi, India | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 4 |

Frequency (%) | 54.69 | 52.60 | 47.92 | 44.27 | 40.63 | 40.10 | 39.06 | 33.85 | 27.08 | 19.79
Approx. size (bp) | 216 | 170 | 120 | 140 | 147 | 160 | 170 | 150 | 130 | 126

The rice blast resistance gene scored as the presence (1) and absence (0) of amplicon linked to ten allele specific SSR markers.

### Results

#### Allelic diversity of rice blast resistance genes

The results of genotypic screening of 192 accessions for the presence or absence of 10 major rice blast resistance genes using SSR markers are presented in Table 2 and electrophoresis pattern of each SSR marker linked to blast resistant gene with few accessions are shown in Fig. 1A&B. The germplasms PB-1460 for $P_i-9$, $P_i-1$, $P_i-5(t)$, $Piz-5$, $Piz-b$, $Pi-t$; IR-64 for $P_i-33$, $Pitp(t)$ and Tetep for $Pitp(t)$, $Piz-k$ were used as gene differential lines. Estimation of PCR results for 10 blast resistance genes viz. $Piz-5$, $Piz-9$, $Pitp(t)$, $Pitb$, $Pitp(t)$, $Piz-9$, $Piz-5(t)$, $Piz-33$, $Piz-b$, $Pitp(t)$, $Piz-k$ and $Pi-t$ were determined by visualization of amplicons on near 216 bp, 170 bp, 120 bp, 140 bp, 147 bp, 160 bp, 170 bp, 150 bp, 130 bp and 126 bp of positive fragments, respectively. The genetic frequencies of the 10 major rice blast resistance genes were ranged from 19.79% to 54.69%. Seventy three accessions containing at least five positive bands of the 10 rice blast resistance markers. The blast resistance gene $Piz-5$ was widely distributed in 54.69% accessions followed by $Piz-9$ in 52.60%, $Pitp(t)$ in 47.92%, $Piz-b$ in 44.27%, $Piz-5(t)$ in 40.63%, $Piz-33$ in 40.10%, $Pib$ in 39.06%, $Pitp(t)$ in 33.85%, $Pik-h$ in 27.08% and $Pi-t$ in only 19.79% accessions. Seven accessions IC337593, IC346002, IC346004, IC346813, IC356117, IC356422 and IC383441 had maximum eight blast resistance gene, while FR13B, Hourakani, Kala Rata 1-24, Lemont, Brown Gora, IR87756-20-2-2-3, IC282418, IC356419, PKSLGR-1 and PKSLGR-39 possessed seven blast resistance genes, and 20 accessions had six genes, 36 accessions had five genes, 41 accessions had four genes, 38 accessions had three genes, 26 accessions had two genes, 13 accessions had single $R$ gene and only one accession IC438644 does not possess any one blast resistant gene.
Genetic diversity of Pi-1 and Pi-9 gene. Estimation of PCR results for the Pi-1 and Pi-9 rice blast resistance genes were determined by visualization of amplicons on 140 bp and 170 bp of positive fragments using SSR primer RM 224 and RM 541 on the chromosome number 11 and 6, respectively. Pi-1 gene was scored on 85 accessions and Pi-9 gene in 101 accessions. Pi-9 gene fragment was the second most prevalent among the germplasm accessions studied. Fifty one accessions amplify both SSR markers corresponding to the resistance check (PB 1460) while 57 accessions did not amplify either of the two markers and hence negative for these two genes.
Genetic diversity of *Pi-5(t)* and *Piz-5* gene. The SSR marker RM 21 is linked to blast resistance gene *Pi5-(t)* on chromosome no. 11, revealed the presence of a 147 bp fragment specific for *Pi5-(t)* mediated blast resistance in the differential line PB 1460. Presence of rice blast resistance gene *Piz-5* on chromosome 6 was determined by visualization of positive fragments using SSR primer RM 527 on 216 bp of positive fragment corresponding to the resistance differential line PB 1460. *Piz-5* gene fragment was the most prevalent among the accessions studied. Forty accessions possessing both genes corresponding to the resistance check (PB 1460) while 51 accessions did not amplify either of the two markers and hence negative for the two genes.
Molecular Screening of Blast Resistance Genes in Rice using SSR Markers

Genetic diversity of *Pi-ta* and *Pi-b* gene: PCR based screening of *Pi-ta* and *Pi-b* genes on chromosome 12 and 2 showed that only 38 and 75 accessions under study produced positive bands on 126 bp and 170 bp with SSR marker RM 247 and RM 208, respectively. Eighty-nine accessions possessed at least one of *Pi-ta/Pi-b* genes. Twelve accessions amplify both SSR markers corresponding the resistance check (PB 1460) while 91 accessions did not amplify either of the two markers and hence negative for the two genes.

Genetic diversity of *Pi-27(t)* and *Pi-33* gene. The *Pi-27(t)* and *Pi-33* specific PCR primer RM 259 and RM 72 were produced 150 bp and 160 bp amplicon based on its sequence on chromosome 6 and 8, respectively. Screening of *Pi-27(t)* and *Pi-33* blast resistance gene were determined by visualization of positive fragments from 65 and 77 accessions with SSR marker RM 259 and RM 72, respectively. The 37 accessions amplify both SSR markers corresponding to the resistance check (IR 64) while 87 accessions did not amplify either of the two SSR markers which showed negative for the two genes.

Genetic diversity of *Pi-k* and *Pitp(t)* gene. Fifty two accessions show the positive fragment of *Pi-k* gene located on chromosome 11 with tightly linked SSR markers RM 206, while 92 accessions show the positive fragment of *Pitp(t)* located on chromosome 11 with tightly linked SSR markers RM 246. Result indicates the presence of an approx 130 bp and 120 bp fragment specific for blast resistance genes *Pi-k* and *Pitp(t)* in the differential Tetep, respectively. Eighteen accessions showed positive bands for both genes while 68 accessions did not amplify any of the two genes.

Discussion

The marker-assisted selection of rice blast resistance genes will help in the breeding program in multi-diseases resistant rice varieties in genetic resources of rice. Some of these germplasm accessions may have special properties that are important to breeding program. In the present study, the genetic frequencies of the 10 major rice blast resistance genes *Piz-5, Pi-9, Pitp(t), Pi-1, Pi-5(t), Pi-33, Pi-b, Pi27(t), Pi-k* and *Pi-ta* were ranged from 19.79% to 54.69%. Similar results were reported by Kim et al. (2010) in 84 accessions of rice germplasms possessed more than three positive bands of the eight rice blast resistance genes, and Imam et al. (2014) reported the genetic frequency of the nine major rice blast resistance genes *Piz, Piz-t, Pik, Piz-5, Pik-h, Pita-2, pita, Pt9* and *Pih*, ranged from 6 to 97% in the select set of rice germplasms. Our result also showed that the analysis of the distribution of resistance genes in ancient populations of landraces can direct the rice blast resistance breeding program and rice blast control by genetic diversity. Many rice varieties have been developed as completely resistant to *M. oryzae* strains, but soon breakdown of rice blast resistant genes occurred because of the emergence of stronger virulent isolates of rice blast fungus (Mackill and Bonman, 1992). Genotyping of the accessions with allele specific markers helped to identify 10 major blast resistance genes in 192 rice germplasm accessions from different ecological regions.

The 54.69% accessions possessing resistance gene *Piz-5* on chromosome 6. These accessions were distributed in different ecosystem across the globe. Similarly, Yan et al. (2007) evaluated a core subset of the USDA 1790 rice germplasms and they found some accessions contained *Piz-5* gene with additional R genes. In addition we verified the accuracy of our results, 105 accessions possessed identical alleles for *Piz-5* gene at approximate 216 bp fragments corresponding to the check (PB 1460). The presence of the same marker alleles in these accessions suggests that they contain a *Piz-5* gene. This finding is important because these 105 accessions were collected from different geographic regions. The most likely reason for this similarity is that the original donor parent for the *Piz-5* gene may contain the same genomic fragment for all these cultivars. In contrast, 87 accessions do not showed similar marker allele suggesting that these accessions presumably either inherited from different donors or the result of recombination during the breeding process. In conclusion, we not only verified the *Piz-5* gene in 105 accessions using previously identified DNA markers but also demonstrated the usefulness of DNA markers with differential blast resistance gene for germplasm characterization. Similar report also made earlier by Roy-Chowdhury et al. (2012b).

The resistance pattern of the accessions is examined for the presence of amplicon products of the major genes. It was noted that though resistance is generally proportional to the frequency of the resistant gene(s). The present study was taken up with a selected set of rice accessions covering a wider geographical region where certain genes (*Piz-5, Pi9, Pitp (t) and Pi-1*) were more diverse than others and these were identified in 105, 101, 92 and 85 accessions on chromosome 6, 6, 1 and 11, respectively. Similarly, Imam et al. (2014) reported in his study that the genes (*Pi9, Pita-2, Piz-t*) were more effective than others in thwarting infection. Thirty eight accessions produced positive bands near 126 bp fragments corresponding to the check (PB 1460) for
blast resistance gene $Pi$-$ta$, which has been located near the centromere of chromosome 12 (Wang et al., 2002). Identification and validation of $Pi$-$ta$ genes reveals that the Indian rice germplasm are diverse and potential source of blast resistant lines which can be exploited in rice blast breeding programs (Shikari et al., 2013). The $Pi$-$ta$ genes commonly used in rice breeding programs worldwide have originated from several traditional indica cultivars, including Tetep from Vietnam and Tadukan from the Philippines (Cho et al., 2008). Transferring blast resistance genes to different genetic backgrounds is very cumbersome and tedious. Since, it would be difficult to identify under field conditions using conventional approaches in order for marker-assisted selection to facilitate at early selection phase with greater accuracy (Gu et al., 2005).

Molecular screening of $Pik$-$h$ and $Pi$-$b$ blast resistance gene were determined by visualization of positive fragments from 52 and 75 accessions, respectively. Members of the $Pik$-$h$ multi-gene family and $Pi$-$b$ were moderately distributed genes in the present study but neither the germplasm possessing them nor the isogenic lines in the previous evaluations had exhibited resistance (Variar et al., 2009). Presence of major rice blast resistance gene $Pitp(t)$ and $Pi$-$27(t)$ on chromosome 1 and $Pi$-$33$ on chromosome 8 was determined by visualization of 120 bp, 150 bp and 160 bp positive fragments, respectively. The gene-specific marker RM 246 for resistance gene $Pitp(t)$ amplified positive bands in 85 accessions, while $Pi$-$27(t)$ and $Pi$-$33$ genes were identified in 65 and 77 accessions, respectively. This study illustrated the utility of SSR markers to identify rice varieties likely carried the same $R$ genes with potentially novel resistance. Rice varieties with a number of alleles in common with any specific resistance might have a similar blast $R$ gene, and understanding the natural diversity at the specific gene is important for incorporation of specific $R$ gene using DNA marker into rice breeding program (Jia et al., 2003).

Genetic diversity among the rice accessions and within the pathogen often leads to inconsistent marker and phenotype analysis. MAS have the advantage in identifying $R$ genes, but its power lies in the robustness of the markers used. The identification and analysis of rice blast resistance genes suggests that DNA primers derived from the gene is a valuable tool for blast gene identification and screening among the rice germplasm (Roy-Chowdhury et al., 2012a, b). In this study, the PCR based markers employed for screening of different blast resistance genes are well established and effective. The consistent results showed with the selected SSR markers for respective genes was highly reliable and make them the marker of choice for molecular screening of rice blast resistance genes among the rice accessions. Plant breeders often use cultivars developed in other countries to broaden the genetic background of the improved cultivars being developed such as the major fungal diseases of blast, but most breeding programs of rice have a narrow genetic diversity of breeding resources. Our results showed that 17 accessions harboured 7 to 8 blast resistance genes, which can be suggested that these accessions could be used as sources of resistance genes in designing future breeding programmes, and there is good possibility of obtaining enhanced resistance through gene pyramiding.

Acknowledgement

Authors are thankful to Department of Biotechnology, New Delhi, Ministry of Agriculture, Government of India for their financial support through Grant No. 07/473, BHU Varanasi India.

References

Ashikawa, I., Hayashi, N., Yamane, H., Kanamori, H., Wu, J., Matsumoto, T., Ono, K. and Yano, M. 2008. Two adjacent nucleotide-binding site-leucine-rich repeat class genes are required to confer Pkm-specific rice blast resistance. *Genetics* 180:2267–2276.

Ballini, E., Morel, J. B., Droz, G., Price, A., Courtois, B., Notteghem, J. L. and Tharreau, D. 2008. A genome-wide meta-analysis of rice blast resistance genes and quantitative trait loci provides new insights into partial and complete resistance. *Mol. Plant-Microbe Interact.* 21:859–868.

Barman, S. R., Gowda, M., Venu, R. C. and Chattoo, B. B. 2004. Identification of a major blast resistance gene in the rice cultivar Tetep. *Plant Breed.* 123:300–302.

Berruyer, R., Adreit, H., Milazzo, J., Gaillard, S., Berger, A., Dioh, W., Lebrun, M. H. and Tharreau, D. 2003. Identification and fine mapping of $Pi$-$33$, the rice resistance gene corresponding to the *Magnaporthe grisea* avirulence gene *ACE1*. *Theor. Appl. Genet.* 107:1139–1147.

Bryan, G. T., Wu, K. S., Farrall, L., Jia, Y. L., Hershey, H. P., McAdams, S. A., Faulk, K. N., Donaldson, G. K., Tarchini, R. and Valent, B. 2000. A single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene $Pt$-$ta$. *Plant Cell* 12:2033–2045.

Chauhan, S., Farman, M. L., Zhang H. B. and Leong, N. 2002. Genetic and physical mapping of a rice blast resistance locus, *PICO39(t)*, that corresponds to the avirulence gene *AVR1*: *CO39* of *Magnaporthe grisea*. *Mol. Genet. Genomics* 267:603–612.

Chen, J., Shi, Y., Liu, W., Chai, R., Fu, Y., Zhuang, J. and Wu, J. 2011. A $Pd$-$3$ allele from rice cultivar Gumei-2 confers resistance to *Magnaporthe oryzae*. *J. Genet. Genomics* 38:209–
Molecular Screening of Blast Resistance Genes in Rice using SSR Markers

216.

Chen, M., Presting, G. and Barbazuk, W. B. 2002. An integrated physical and genetic map of the rice genome. *Plant Cell* 14:537–545.

Chen, X., Shang, J., Chen, D., Lei, C., Zhou, Y., Zhai, W., Liu, G., Xu, J., Ling, Z., Cao, G., Ma, B., Wang, Y., Zhao, X., Li, S. and Zhu, L. 2006. A Blectin receptor kinase gene conferring rice blast resistance. *Plant J.* 46:794–804.

Cho, Y. C., Jeung, J. U., Park, H. J., Yang, C. I., Choi, Y. H., Choi, I. B., Won, Y. J., Yang, S. J. and Kim, Y. G. 2008. Haplotype diversity and durability of resistance genes to blast in Korean Japonica rice varieties. *J. Crop Sci. Biotech.* 11:205–214.

Cuong, N. M., Lang, T. and Buu, B. C. 2006. Applications of microsatellite and sequence tagged site marker to detect blast resistance in local rice (*Oryza sativa* L.). Hanoi: Nong Nghiệp. 52–68 pp.

Das, A., Soubham, D., Singh, P. K., Thakur, S., Singh, N. K. and Sharma, T. R. 2012. A novel blast resistance gene, *Pi54rb* cloned from wild species of rice, *Oryza rhizomatiss* confers broad spectrum resistance to *Magnaporthe oryzae*. *Func. Integr. Genomics* 12:215–228.

Doyle, J. J. and Doyle, J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19:11–15.

Eizenga, G. C., Agrama, H. A., Lee, F. N., Yan, W. G. and Jia, Y. 2006. Identification of novel resistance genes in newly introduced blast resistant rice germplasm. *Crop Sci.* 46:1870–1878.

Fjellstrom, R., Mc-Clung, A. M. and Shank, A. R. 2006. SSR markers closely linked to the *Pi-2* locus are useful for selection of blast resistance in a broad array of rice germplasm. *Mol. Breed.* 17:149–157.

Fuentes, J. L., Correa-Victoria, F. J., Escobar, F., Prado, G., Aricapa, G., Duque, M. C. and Tohme, J. 2007. Identification of microsatellite markers linked to the blast resistance gene *Pi-1(t)* in rice. *Euphytica* 160:295–304.

Fukuo, S., Saka, N., Koga, H., Ono, K., Shimizu, T., Ebana, K., Hayashi, N., Takahashi, A., Hirochika, H., Okuno, K. and Yano, M. 2009. Loss of function of a proline-containing protein confers durable disease resistance in rice. *Science* 325:998–1001.

Gu, K., Yang, B., Tian, D., Wu, L., Wang, D., Sreekala, C., Yang, F., Chu, Z., Wang, G. L., White, F. F. and Yin, Z. 2005. R gene expression induced by type-III effector triggers disease resistance in rice. *Nature* 435:1122–1125.

Han, S. S., Ryu, J. D., Shim, H. S., Lee, S. W., Hong, Y. K. and Cha, K. H. 2001. Breakdown of resistant cultivars by new race *Kl-III* and race distribution of rice blast fungus during 1999–2000 in Korea. *Res. Plant Dis.* 7:86–92.

Hayashi, K. and Yoshida, H. 2009. Refunctionalization of the ancient rice blast disease resistance gene *Pit* by the recruitment of a retrotransposon as a promoter. *Plant J.* 57:413–425.

Hayashi, K., Yoshida, H. and Ashikawa, I. 2006. Development of PCR-based allele specific and InDel marker sets for nine rice blast resistance genes. *Theor. Appl. Genet.* 113:251–260.

Hayashi, N., Inoue, H., Kato, T., Funao, T., Shirota, M., Shimizu, T., Kanamori, H., Yamane, H., Hayano-Saito, Y., Matsumoto, T., Yano, M. and Takatsuji, H. 2010. Durable panicle blast-resistance gene *Phb* encodes an atypical CC-NBS-LRR protein and was generated by acquiring a promoter through local genome duplication. *Plant J.* 64:498–510.

Hittalmani, S., Parco, T. A., Mew, T. V. and Zeigler, R. S. 2000. Fine mapping and DNA marker-assisted pyramiding of the three major genes for blast resistance in rice. *Theor. Appl. Genet.* 100:1121–1128.

Huang, H., Huang, L., Feng, G., Wang, S., Wang, Y., Liu, J., Jiang, N., Yan, W., Xu, L., Sun, P., Liu, Z., Pan, S., Liu, X., Xiao, Y., Liu, E., Dai, L. and Wang, G. 2010. Molecular mapping of the new blast resistance genes *Pi47* and *Pi48* in the durably resistant local rice cultivar *Xiangzi 3150*. *Phytopathology* 101:620–626.

Imam, J., Alam, S., Mandal, N. P., Variar, M. and Shukla, P. 2014. Molecular screening for identification of blast resistance genes in North East and Eastern Indian rice germplasm (*Oryza sativa* L.) with PCR based makers. *Euphytica* 196:199–211.

Jia, Y., Bryan, G. T., Farrall, L. and Valent, B. 2003. Natural variation at the *Pi-7a* rice blast resistance locus. *Phytopathology* 93:1452–1459.

Kim, J. S., Ahn, S. N., Kim, C. K. and Shim, C. K. 2010. Screening of rice blast resistance genes from aromatic rice germplasms with SNP markers. *Plant Pathol. J.* 26:70–79.

Lee, S. K., Song, M. Y., Seo, Y. S., Kim, H. K., Ko, S., Cao, P. J., Suh, J. P., Yi, G., Roh, J. H., Lee, S., An, G., Hahn, T. R., Wang, G. L., Ronald, P. and Jeon, J. S. 2009. Rice *Pi3*-mediated resistance to *Magnaporthe oryzae* requires the presence of two coiled-coilnucleotide-binding-leucine-rich repeat genes. *Genetics* 181:1627–1638.

Li, Y. B., Wu, C. J., Jiang, G. H., Wang, L. Q. and He, Y. Q. 2007. Dynamic analyses of rice blast resistance for the assessment of genetic and environmental effects. *Plant Breed.* 126:541–547.

Lin, F., Chen, S., Que, Z., Wang, L., Liu, X. and Pan, Q. 2007. The blast resistance gene *Pi37* encodes a nucleotide binding siteleucine-rich repeat protein and is a member of a resistance gene cluster on rice chromosome 1. *Genetics* 177:1871–1880.

Liu, B., Zhang, S. H., Zhu, X. Y., Yang, Q. Y., Wu, S. Z., Mei, M. T., Mauleon, R., Leach, J., Mew, T. and Leung, H. 2004. Cadidate defense genes as predictors of quantitative blast resistance in rice. *Mol. Plant-Microbe Interact.* 17:1146–1152.

Liu, J., Wang, X., Mitchell, T., Hu, Y., Liu, X., Dai, L. and Wang, G. L. 2010. Recent progress and understanding of the molecular mechanisms of the rice *Magnaporthe oryzae* interaction. *Mol. Plant Pathol.* 11:419–427.

Liu, X. Q., Wang, L., Chen, S., Lin, F. and Pan, Q. H. 2005. Genetic and physical mapping of *Pi36(t)*, a novel rice blast resistance gene located on rice chromosome 8. *Mol. Genet. Genomics* 274:394–401.

Liu, X., Lin, F., Wang, L. and Pan, Q. 2007. The in silico map-
based cloning of Pti36, a rice coiled-coil nucleotide-binding site leucine-rich repeat gene that confers race-specific resistance to the blast fungus. *Genetics* 176:2541–2549.

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

Okuyama, Y., Kanzaki, H., Abe, A., Yoshida, K., Tamiru, M., Saitoh, H., Fujibe, T., Matsumura, H., Shenton, M., Galam, D. C., Undan, J., Ito, A., Sone, T. and Terayuchi, R. 2011. A multi-faceted genomics approach allows the isolation of rice Pita blast resistance gene consisting of two adjacent NBS-LRR protein genes. *Plant J.* 66:467–479.

Qu, S., Liu, G., Zhou, B., Bellizzi, M., Zeng, L., Dai, L., Han, B. and Wang, G. L. 2006. The broad spectrum blast resistance gene Pib9 encodes a nucleotide-binding site leucine-rich repeat protein and is a member of a multigene family in rice. *Genetics* 172:1901–1914.

Roy-Chowdhury, M., Jia, Y., Jackson, A., Jia, M. H., Fjellstrom, R. and Cartwright, R. 2012a. Analysis of rice blast resistance gene Pi-z using pathogenicity assays and DNA markers. *Euphytica* 184:35–47.

Roy-Chowdhury, M., Jia, Y., Jia, M. H., Fjellstrom, B. and Cartwright, R. 2012b. Identification of the rice blast resistance gene Pib in the national small grains collection. *Phytopathology* 102:700–706.

Shang, J., Tao, Y., Chen, X., Zou, Y., Lei, C., Wang, J., Li, X., Zhao, X., Zhang, M., Lu, Z., Xu, J., Cheng, Z., Wan, J. and Zhu, L. 2009. Identification of a new rice blast resistance gene Pid3 by genome-wide comparison of paired nucleotide-binding site-leucine-rich repeat genes and their pseudogene alleles between the two sequenced rice genomes. *Genetics* 182:1303–1311.

Sharma, T. R., Madhav, M. S., Singh, B. K., Shanker, P., Jana, T. K., Dalal, V., Pandit, A., Singh, A., Gaikwad, K., Upeti H. C. and Singh, N. K. 2005. High-resolution mapping, cloning and molecular characterization of the Pa-kh gene of rice, which confers resistance to *Magnaporthe grisea*. *Mol. Genet. Genomics* 274:569–578.

Sharma, T. R., Rai, A. K., Gupta, S. K., Vijayan, J., Devanna, B. N. and Ray, S. 2012. Rice blast management through host-plant resistance: retrospect and prospects. *Agrim. Res.* 1:37–52.

Shikari, A. B., Khanna, A., Krishnan, S. G., Singh, U. D., Ratnou, 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 *Pita2* in landraces of rice (*Oryza sativa* L.). *Indian J. Genet.* 73:131–141.

Takahashi, A., Hayashi, N., Miyao, A. and Hirochika, H. 2010. Unique features of the rice blast resistance *Pish* locus revealed by large scale retrotransposon-tagging. *BMC Plant Biol.* 10:175.

Tang, J., Zhu, X., Wang, Y., Liu, L., Xu, B., Li, F., Fang, J. and Chu, C. 2011. Semi-dominant mutations in the CC-NB-LRR-type R gene, NLS1, lead to constitutive activation of defense responses in rice. *Plant J.* 66:996–1007.

Tanksley, S. D. and McCouch, S. R. 1997. Seeds banks and molecular maps: unlocking genetic potential from the wild. *Science* 277:1063–1066.

Valent, B. 1990. Rice blast as a model system for plant pathology. *Phytopathology* 80:33–36.

Variar, M., Vera, C. C. M., Carrillo, M. G., Bhatt, J. C. and Sanger, R. B. S. 2009. Rice blast in India and strategies to develop durably resistant cultivars. In: Xiaofan, W. and Valent, B. (eds). Advances in genetics, genomics and control of rice blast disease. Springer, New York, 359–374 pp.

Wang, C., Hirano, K. and Kawasaki, S. 2002. Cloning of Pita-2 in the centromeric region of chr-12 with HEGS: high efficiency genome scanning. Third International Rice Blast Conference. 25 p.

Wang, X., Fjellstrom, R. G., Jia, Y., Yan, W., Jia, M. H., Scheffler, B. E., Wu, D., Shu, Q. and Mc-Clung, A. M. 2010. Characterization of Pita blast resistance gene in an international rice core collection. *Plant Breed.* 129:491–501.

Wang, X., Z., Yano, M., Yamanouchi, U., Iwamoto, M., Monna, L., Hayasaka, H., Katayose, Y. and Sasaki, T. 1999. The *Pib* gene for rice blast resistance belongs to the nucleotide-binding and leucine-rich repeat class of plant disease resistance genes. *Plant J.* 19:55–64.

Xiao, W. M., Yang, Q. Y., Wang, H., Guo, T., Liu, Y. Z., Zhu, X. Y. and Chen, Z. Q. 2010. Identification and fine mapping of a resistance gene to *Magnaporthe oryzae* in a space-induced rice mutant. *Mol. Breed.* 28:303–312.

Yan, W., Rutger, J. N., Bockelman, H. E., Fjellstrom, R. G., Chen, M. H., Tai, T. and Mc-Clung, A. M. 2007. Development and evaluation of a core subset of the USDA rice (*Oryza sativa* L.) germplasm collection. *Crop Sci.* 47:869–878.

Yang, J. Y., Chen, S., Zeng, L. X., Li, Y. L., Chen, Z., Li, C. Y. and Zhu, X. Y. 2008. Race specificity of major rice blast resistance genes to *Magnaporthe grisea* isolates collected from indica rice in Guangdong. *Rice Sci.* 15:311–318.

Yuan, B., Zhai, C., Wang, W., Zeng, X., Xu, X., Hu, H., Lin, F., Wang, L. and Pan, Q. 2011. The *Pik-p* resistance to *Magnaporthe oryzae* in rice is mediated by a pair of closely linked CC-NBSLRR genes. *Theor. Appl. Genet.* 122:1017–1028.

Zhai, C., Lin, F., Dong, Z., He, X., Yuan, B., Zeng, X., Wang, L. and Pan, Q. 2011. The isolation and characterization of *Pik*, a rice blast resistance gene which emerged after rice domestica-

Zhao, X., Zhang, M., Lu, Z., Xu, J., Cheng, Z., Wan, J. and Zhu, L. 2009. Identification of a new rice blast resistance gene *Pib3* by genome-wide comparison of paired nucleotide-binding site-leucine-rich repeat genes and their pseudogene alleles between the two sequenced rice genomes. *Genetics* 182:1303–1311.

Zhao, X., Zhang, M., Lu, Z., Xu, J., Cheng, Z., Wan, J. and Zhu, L. 2009. Identification of a new rice blast resistance gene *Pib3* by genome-wide comparison of paired nucleotide-binding site-leucine-rich repeat genes and their pseudogene alleles between the two sequenced rice genomes. *Genetics* 182:1303–1311.

Zhao, X., Zhang, M., Lu, Z., Xu, J., Cheng, Z., Wan, J. and Zhu, L. 2009. Identification of a new rice blast resistance gene *Pib3* by genome-wide comparison of paired nucleotide-binding site-leucine-rich repeat genes and their pseudogene alleles between the two sequenced rice genomes. *Genetics* 182:1303–1311.

Zhao, X., Zhang, M., Lu, Z., Xu, J., Cheng, Z., Wan, J. and Zhu, L. 2009. Identification of a new rice blast resistance gene *Pib3* by genome-wide comparison of paired nucleotide-binding site-leucine-rich repeat genes and their pseudogene alleles between the two sequenced rice genomes. *Genetics* 182:1303–1311.