Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci. (Jan–Mar 2017) 87(1):67–72
DOI 10.1007/s40011-015-0548-3

RESEARCH ARTICLE

Molecular Screening of Blast Resistance Gene *Pi2* in Indian Rice Landraces (*Oryza sativa L.*) and its Verification by Virulence Analysis

Shamshad Alam[1,2] · Jahangir Imam[1] · Mukesh Nitin[1] · Chandeshwar Prasad[2] · Mukund Variar[1]

Received: 9 August 2014 / Revised: 23 February 2015 / Accepted: 4 May 2015 / Published online: 30 June 2015

Abstract The *Pi2* gene is a member of a multigene family which confers resistance to strains of blast pathogen *Magnaporthe oryzae*. The gene encodes a typical NBS-LRR type protein which confers broad spectrum resistance to a wide range of races of blast pathogen prevalent in eastern India. In the present study, the presence of *Pi2* gene in 61 landraces was determined using a pair of primers NBS2P3 and NBS2R followed by restriction digestion and the results were further confirmed by pathotyping using *M. oryzae* isolates avirulent for *Pi2* gene. The *Pi2* gene was found in four rice landraces from Sikkim and one landrace from Jharkhand though the latter was susceptible to the isolate bearing corresponding avirulence gene. Presence of *Pi2* gene in independent *indica* landraces from the eastern Indian region suggested that the gene might have originated and evolved in *indica* rice and exist in different allelic forms in blast endemic zones of eastern India. The present study not only identifies the presence of *Pi2* gene in landraces but also demonstrates the usefulness of molecular markers and virulence analyses for rapid identification of resistant genes in rice landraces. These characterized landraces can be used for genetic studies and marker assisted breeding for improving blast resistance in rice.

Keywords Blast resistance · *Magnaporthe oryzae* · Marker assisted selection · *Pi2* gene · Rice landraces

Introduction

Rice blast caused by filamentous ascomycete *Magnaporthe oryzae* [1], is a devastating disease affecting rice production every year worldwide [2]. The blast fungus infects all aerial parts of the rice plant at every stage of its growth starting from seedling to grain filling stage, often resulting in total loss of rice grain [3]. Many rice varieties with resistance to blast have been developed but their resistance is rapidly reduced by the emergence of strong virulent races which are ascribed to the high genetic instability in the avirulence/virulence genes of the pathogen [3, 4].

Magnaporthe oryzae, a filamentous ascomycete, is a model organism for the study of host pathogen interactions as it has a small genome size ($\sim$40 Mb). It can be cultured in defined media, facilitating biochemical and molecular analyses [5]. Resistance to *M. oryzae* follow the classical gene-for-gene relationship in which a major resistance gene prevents infection by races of pathogen containing the corresponding avirulence (*Avr*) gene [6]. Differential races of *Magnaporthe oryzae* were used earlier to identify resistance gene(s) in landraces, cultivars and wild relatives [7]. Detection of resistance genes in rice germplasm by traditional pathogenicity test using differential races is time consuming, less efficient and requires strict environmental control. Availability of gene specific or tightly linked DNA markers has helped overcome these constraints and

Electronic supplementary material The online version of this article (doi:10.1007/s40011-015-0548-3) contains supplementary material, which is available to authorized users.

✉ Mukund Variar
mukund.variar@gmail.com

[1] Biotechnology Laboratory, Central Rainfed Upland Rice Research Station (CRRI), Post box 48, Hazaribagh 825301, Jharkhand, India

[2] Department of Botany, Vinoba Bhave University, Hazaribagh 825301, Jharkhand, India

represent a significant advantage for identification and incorporation of resistance gene in a breeding program [8–10]. During the past decade, genetics of blast resistance has been extensively studied. So far, 100 race specific resistance genes and about 350 QTLs have been identified [11, 12]. Most of the blast resistance genes and their analogues are located on chromosomes 4, 6, 11 and 12. Out of 100, 22 resistance genes have been cloned, among which 20 are major (*Pib, Pita, Pi9, Pi2, Piz-t, Pid2, Pi36, Pi37, Pik-m, Pit, Pi5, Pid3, pi21, Pb1, Pish, Pik, Pik-p, Pi54, Pia, NLS1)* and 2 minor (*Pi25 and Pi54rh)* [13]. Most of the blast resistance genes encode NBS-LRR type of resistance protein except *Pid2*, which encodes a receptor-like kinase protein with a predicted extracellular domain of a bulb-type mannose specific binding lectin (B-lectin), and *pi21,* which encodes a proline-rich protein that includes a putative heavy metal-binding domain and protein–protein interaction motifs. Though these genes are of the same gene families, their efficiencies against different pathotypes are different, some exhibiting more broad spectrum resistance than others.

Pi-2 gene is a member of a multigene family, located on the short arm and near the centromere of chromosome six. At least eight blast resistance genes were identified from the *Pi2/9* locus [*Pi9, Pi2, Piz-t, Pi26(t), Pigm(t), Piz(t), Pi40(t)* and *Pi50(t)*] [14–20]. Among them *Pi9, Pi2, Piz-t* were successfully cloned. The *Pi-2* gene was introgressed from the highly resistant *O. sativa* spp. *indica* cultivar 5173 into the susceptible cultivar C039 and the derived isogenic line was named C101 A51 [21]. Greenhouse inoculation revealed that C101 A51 was resistant to 455 isolates collected from Philippines and most of the 792 isolates from China [22]. Extensive inoculation tests in several other countries also indicated that *Pi-2* is one of the broad-spectrum blast resistance genes [23]. *Pi2* gene was also found resistant to 66 out of 72 isolates from different regions of eastern and north eastern India, by virulence analysis [24], indicating that *Pi2* gene is resistant to most of the pathotypes prevalent in the region. India is having rich biodiversity in rice, but no serious effort has been made to exploit this extensive bioresource. Local landraces and wild germplasm of rice play an important role in maintaining this diversity as they are thought to be at an intermediate stage in the domestication process from wild ancestors to cultivated rice [25]. They represent a unique and critical source of genetically valuable traits for tolerance to biotic and abiotic stresses, particularly disease resistance. Molecular screening of *Pi2* gene among local landraces might provide rare/superior alleles of *Pi2* with broad resistance spectra. The objectives of this study were to identify the existence of *Pi2* gene in landraces using a gene based marker and its validation by virulence analysis.

Material and Methods

Plant Material

For the identification of *Pi2* gene, 61 accessions of rice originating from different geographical locations of India, mainly from eastern and north-eastern region, were selected from the germplasm collection maintained at Central Rainfed Upland Rice Research Station, Hazaribag (25°58′N, 85°26′E; 604 m above sea level) (Table 1). *A* monogenic line IRBLz5-CA, harbouring *Pi2* gene was used as positive control as it is resistant to *M. oryzae* isolates containing *Avr-Pi2* gene. CO39 was used as a negative control as it is susceptible to *M. oryzae* isolates containing *Avr-Pi2* gene. Five to seven seeds of each landrace and checks were germinated on moistened filter paper for 3 days in a plant growth chamber, planted in plastic pots, transferred and maintained in a green house at 25 °C with 16 h light for 3–4 weeks until the emergence of fourth foliar leaf for pathogenicity tests and genomic DNA isolation.

Genomic DNA Isolation and PCR Amplification

Leaf samples of all the landraces with controls were collected, frozen in liquid nitrogen and stored at −80 °C for DNA extraction. Genomic DNA was isolated using Plant Mini Kit (Qiagen, Valencia, CA, USA) according to manufacturer's instruction. The extracted genomic DNA was estimated on 0.8 % agarose gel in 1× TBE buffer, stained with ethidium bromide and visualized under Gel Documentation System (Alpha Innotec). The quantity of extracted DNA was estimated by NANO Drop spectrophotometer (Thermo, USA) and diluted to 100 ng/µl with 1× TE buffer and stored at −20 °C. A pair of primers NBS2P3 (5′-GATTTAGTTCAGGAAAACACTC-3′) and NBS2R (5′-TGGAAGCCTCATTGATCATC-3′) specific for *Pi2* was used to amplify the gene [15]. PCR reaction mixture contained: 100 ng of genomic DNA, 1× Taq buffer, 0.2 mM of dNTPs mix, 1.5 mM of $MgCl_2$, 0.5 µM of each primer, 1 unit of Taq DNA polymerase (Fermentas Life Sciences, Burlington, Canada) and MilliQ water to make final volume of 20 µl. The PCR amplification program for this marker consisted of (i) 5 min. initial denaturation at 94 °C (ii) 35 cycles of 45 s denaturation at 94 °C, 45 s annealing at 55 °C, 2 min. extension at 72 °C, and a final extension for 5 min at 72 °C. After amplification, the PCR products were resolved in 1.5 % (W/V) agarose gel in 1× TBE buffer, stained with ethidium bromide and visualized under gel documentation system. All PCR reactions for each sample were repeated at least twice to confirm the results.

Table 1 Geographical origin of landraces

Sl no.	Geographical origin	Landraces
1	Andhra Pradesh	MTU 17
2	Assam	ARC 7102, ARC 10372, ARC 11775, Dumai, Dumai, Chakula, ARC 7098, ARC 7059, ARC 6248, ARC 6248
3	Gujarat	Sathi 34-36, Junagarh 68
4	Himachal Pradesh	Lalnakanda
5	Jharkhand	Brown gora(14), Brown gora(113), Charka gora, Black gora, Pathari, Gora, Dahia(1), Dahia(2), Raria, Bhojani, Gora(1), Tilasar, Gora(2), Gora(3), Chipiti, Lalka, Bherakabar, Jonga, Nardha, Alsanga Gora, Charka Gora, Safed Gora, Dani Gora, Bangla Gora
6	Maharashtra	Tuljapur 1, Teteco Ratnagiri24
7	Odisha	Tikradhan, Kalakeri
8	Sikkim	Fudangi, Thule Atte (Thima), Sanu Atte (Thima), Atte, Chirakey B, Champae, Jhappaka (Scented)
9	Uttar Pradesh	N 22, Lalmati 14, Lalmati, Narendra, Teenpakhia, Bhadia,
10	West Bengal	Dular, Panke, AUS 257, AUS 196, AUS 454

Restriction Digestion of PCR Products

PCR amplification of susceptible Co39 and monogenic resistant line *IRBLz5-CA* and 61 land races with NBS2P3 and NBS2R primers [15] generated monomorphic bands. However, restriction digestion of the PCR product with fast-digest EcoR1 enzyme (Fermentas Life Sciences, Burlington, Canada) generated polymorphism between susceptible Co39 and monogenic resistant line IRBLz5-CA (*Pi2*). Restriction enzyme digestion was carried out directly in the PCR tube. The enzyme mix contained 2 µl 10X FD buffer, 2 units of Fast digest EcoR1 enzyme, 10 µl of PCR product and 17 µl MilliQ water to make final volume 30 µl. After a brief spin (microfuge) the mixture was incubated at 37 °C at 20 min to digest the PCR product. The digested PCR product was resolved on 1.5 % agarose gel. Restriction digestion of PCR product produced a polymorphic band of 850 bp corresponding to the resistant allele of *Pi2* gene but not in susceptible Co39. PCR amplification and restriction digestion were repeated twice to confirm the results.

Disease Evaluation in Uniform Blast Nursery (UBN)

An initial evaluation of all the landraces and controls (IRBLz5-CA, R Check and Co 39, S. check) were done for their reaction to blast in uniform blast nursery at Central Rainfed Upland Rice Research Station, Hazaribag, Jharkhand, during wet season of 2013 in two replications. Thirty to 35 seeds of each line were planted with a row spacing of 10 cm surrounded by two rows of highly susceptible spreaders Co39 and B40. Abundant nitrogen 100–120 kg/ha in the form of urea was applied, half at seeding and the other half 15 days after seeding. Disease reaction of each line was recorded 30 days after sowing and continued at 5 days intervals until the susceptible check had more than 80 % infection. Disease reaction was scored visually on 0–9 SES scale (Standard Evaluation System, IRRI, 1996). Scores 0–3 were considered resistant (R), 4 as moderately resistant (MR) and 5–9 as susceptible (S).

Virulence Analysis

To validate the presence of *Pi2* gene in landraces virulence analysis was done with a mixture of two avirulent isolates, Mo-ei-118 and Mo-ei-241. Monogenic line IRBLz5-CA having *Pi2* gene was used as positive control as it is resistant to Mo-ei-118 and Mo-ei-241. Co39 was used as negative control as it is susceptible to both the isolates. Inoculum of isolates was prepared with a concentration of 1.5×10^5 spores/ml and sprayed on to 21 days old seedlings of landraces and control. The inoculated trays were kept in darkness at ±25 °C with more than 90 % relative humidity in a specialized blast phenotyping facility. Disease reaction of all the seedlings after seven days of inoculation was measured on a 0–5 disease assessment scale [26]. Reaction types 0–3 were considered resistant, while 4–5 were considered as susceptible.

Results and Discussion

Identification of *Pi2* Gene in Landraces

Molecular screening of *Pi2* gene, conducted on 61 landraces of rice using gene based marker NBS2P3 and NBS2R derived from *Nbs4-Pi2* candidate gene [15] generated a monomorphic band of 1.8 kb. Restriction digestion of PCR product with *EcoR1* enzyme, however, revealed polymorphism between susceptible and resistant

lines (Fig. 1, Fig. S1). Three different types of banding patterns of alleles of *Pi2* gene were observed among the landraces by NBS2P3 and NBS2R markers as shown in Fig. 2. IRBLz5-CA and the landraces having *Pi2* gene produced a unique band of 850 bp after restriction digestion but negative control of Co39 did not have this band. Out of 61 landraces, five landraces had *Pi2* gene type banding pattern. The five landraces positive for *Pi2* gene originated from Sikkim (four) and Jharkhand (one). Thirty four landraces had Co39 susceptible type banding pattern and the remaining 22 landraces had null allele. Failure to generate amplicons may be attributed to sequence variation, insertion, deletion or substitution in one or both the primer binding sites [27, 28]. The landraces which were not positive for *Pi2* gene did not generate desired band of 850 bp (Fig. 2). Screening of *Pi2* gene in 162 local landraces by Hittalmani et al. [27] using RG64 revealed 31 landraces having the null allele.

Disease Reaction of Landraces in UBN

Disease reaction of landraces (61) and checks (2) to blast in uniform blast nursery during wet season 2013 is summarized in Table 2. Disease reaction ranged from score 0 to 7 in SES scale when susceptible spreaders (Co39 and B40) had more than 80 % infection. Out of 61 landraces, 9 landraces were resistant (Score 0–3), 25 landraces were moderately resistant (Score 4), 23 were susceptible (Score 5–6) while four landraces failed to germinate (Table 2). The resistant check IRBLz5CA having *Pi2* gene had a score of '3' and the susceptible check scored '7'. Among the five land races positive for *Pi 2* gene, four recorded resistant reaction while the fifth, Brown gora (HRC No 113), was susceptible with a score of '5'.

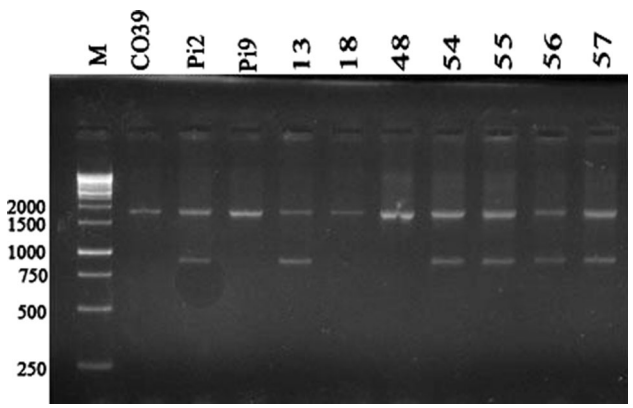

Fig. 1 Gel analysis of PCR products after digestion with restriction enzyme *EcoR1*. *M* 1 kb ladder, *Lanes 2* and *3* represent the susceptible control CO39 and resistant control *IRBLz5CA*, respectively. *Lane 4* IRBL9-w *(Pi9)*, *13* represent Brown gora, *18* Dahia, *48* Safed Gora, *54* Sanu Atte, *55* Atte, *56* Chirakey B, *57* Champae

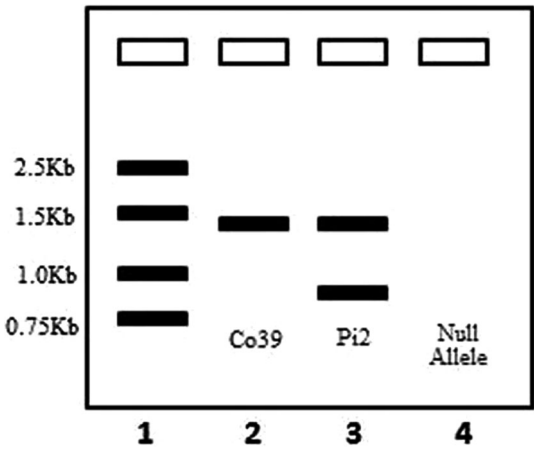

Fig. 2 Banding pattern of alleles of *Pi2* gene by NBS2P3 and NBS2R marker, *lane 1* 1 Kb ladder, *lane 2* Co39 type susceptible allele, *lane 3 Pi2* type resistant allele, *lane 4* null allele

Verification of *Pi2* Gene in Landraces by Virulence Analysis

The five landraces identified to possess *Pi2*, based on amplification of the gene specific markers and the checks (IRBLz5CA and Co39*)* were inoculated with a mixture of *M. oryzae* isolates Mo-ei-118 and Mo-ei-241. The controls exhibited expected resistant and susceptible reaction for the mixture of isolates, with the Near Isogenic Line C101 A51 (*Pi2*) exhibiting resistant reaction and Co 39 showing susceptible reaction (Table 3). Among the five landraces harbouring *Pi2* gene, four were resistant [Sanu Atte (Thima), Atte, Chirakey B, Champae] to the mixture of avirulent isolates but one landrace (Brown gora, HRC 113) exhibited susceptible reaction even though the amplicon product was positively identified indicating the presence of *Pi2* gene (Table 3). Mutation in the coding region of the gene may lead to loss of resistant function [10]. Virulence analysis with avirulent isolates for *Pi2* gene however, confirmed that four landraces of Sikkim [Sanu Atte (Thima), Atte, Chirakey B, Champae] have the functional *Pi2* gene (Table 3).

Wild species and landraces are unique source of valuable alleles for various biotic and abiotic stresses. Local landraces can serve as donors for future breeding programme. These untapped genetic resources exist in wild species and local landraces of crop which can be utilized successfully to identify novel or superior allele(s)/gene(s). Several blast resistance genes have already been identified and cloned from wild species like *Pi9* from *Oryzae minuta*, *Pi40* from *Oryza australiensis*, *Pi54rh* from *O. rhizomatis*, bacterial blight resistant gene *Xa21* from *O. longistaminata* etc. which provide resistance against a wide range of isolates. Present study confirmed the existence of *Pi2* gene in four landraces of Sikkim. Most deployed R genes have

Table 2 List of landraces and their reaction to blast under UBN

Score	Landraces
Resistant (score 0–3) (9)[a]	Atte, Champae, Kalakeri, Tetco, Dahia, Sanu Atte (Thima), Chirakey B, ARC 7059, Lalka
Moderately resistant (score 4) (25)[a]	N 22, Lalnakanda, Brown gora, Panke, Sathi 34-36, Junagarh 68, Lalmati 14, Lalmati, Raria, Tilasar, Gora (2), ARC 7102, ARC 11775, Chakula, ARC 7098, Teenpakhia, AUS 257, AUS 196, AUS 454, Bherakabar, Chain gora, Fudangi, Thule Atte (Thima), Jhappaka (Scented), Ratnagiri 24
Susceptible (score 5–7) (23)[a]	Dular, Narendra, Brown gora, Charka gora, Black gora, Pathari, Gora, Dahia, Bhojani, Gora (1), Gora (3), Chipti, ARC 10372, Dumai, Jonga, Nardha, Tuljapur 1, ARC 6248, Safed gora, Bangla gora, Tapari, Tikradhan, Charka gora

[a] Number of landraces

Table 3 Summary of disease reactions of landrace, virulence analysis against mixture of avirulent isolates for *Pi2* gene and PCR result of *Pi2* gene

LR (Landraces)	HRC (Hazaribag rice collection) number	Landrace	Origin	Marker for *Pi2* gene[a]	Disease reaction[b]	*Pi2**
13	113	Brown gora	JHARKHAND	+	S	0
54	1276	Sanu Atte (Thima)	SIKKIM	+	R	1
55	1291	Atte	SIKKIM	+	R	1
56	1295b	Chirakey B	SIKKIM	+	R	1
57	1334	Champae	SIKKIM	+	R	1
		Co 39 (Control)		−	S	0
		IRBLz5CA (Control)		+	R	1

* 1 indicate presence of *Pi2* gene, 0 indicate *Pi2* gene not present

[a] Marker profile for analysis of *Pi2* gene, + indicate it contain specific band and − indicate it do not contain a specific band for *Pi2* gene

[b] Reaction against mixture of avirulent isolates for *Pi2* gene

been identified from indica rice, including *Pi2* which was originally identified from the indica cultivar 5173 from which the isogenic line C101A51was derived by Mackill and Bonman [21]. Presence of *Pi2* gene in independent *indica* landraces from the eastern Indian region confirms that the gene originated and evolved in *indica* rice existing in different allelic forms in blast endemic zones of western and eastern India. Greenhouse inoculation test in India and in several other countries also indicated that *Pi2* is a broad-spectrum blast resistance gene [22–24, 29]. These findings not only reflect that *Pi2* gene has been durable in India but also suggests that it has potential for broader application worldwide. Survey for identification of alleles of *Pi2* gene among local landraces, therefore, might provide a valuable source for *Pi2* gene with broad resistance spectrum and agronomic fitness. Landraces having *Pi2* gene identified in this study can be immediately used as donor in resistance breeding program. Since the landraces possessing the gene have evolved in the eastern Indian region, it may also provide additional genes for environmental fitness. Additionally, the gene based marker used in this study can be reliably used in screening of *Pi2* gene from rice landraces and subsequent selection of the *Pi2* gene in a marker assisted backcross breeding program. Recent work published by RoyChowdhury et al. [10, 30] on the identification of rice blast resistance gene *Pib* and analysis of rice blast resistance gene *Piz* also suggested that DNA markers derived from the gene and pathogenicity assays can be valuable tools for detection of novel alleles and their deployment.

Conclusion

Detection of *Pi2* gene in four land races of rice from blast endemic zones of eastern India using gene specific markers and evaluation of their resistance spectra with diagnostic isolates opened up possibilities of their use in varietal improvement programme for blast resistance. *Pi2* is highly effective in eastern India because it confers resistance to most pathotypes prevalent in this region [24]. Usefulness of the gene specific DNA marker for germplasm characterization further demonstrated that the process of gene tracking in resistance breeding programmes can be made precise with such molecular tools. Sequence analyses of the segments identified positive for the marker would further clarify allelic variation and the evolutionary significance of this gene in different landraces.

Acknowledgments The authors thank the National Agriculture Innovative Project—Component 4, on Allele mining blast resistance genes, for financial support for this research. This work was performed at the Central Rainfed Upland Rice Research Station, Hazaribag, Jharkahnd.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Couch BC, Kohn LM (2002) A multilocus gene genealogy concordant with host preference indicates segregation of a new species, *Magnaporthe oryzae*, from *M. grisea*. Mycologia 94:683–693
2. Liu Y, Liu B, Zhu X, Yang J, Bordeos A, Wang G, Leach JE, Leung H (2013) Fine-mapping and molecular marker development for *Pi56(t),* a NBS-LRR gene conferring broad-spectrum resistance to *Magnaporthe oryzae* in rice. Theor Appl Genet 126:985–998
3. Dean RA, Talbot NJ, Ebbole DJ, Farman ML, Mitchell TK, Orbach MJ, Thon M, Kulkarni R, Xu JR, Pan H, Read ND, Lee YH, Carbone I, Brown D, Oh YY, Donofrio N, Jeong JS, Soanes DM, Djonovic S, Kolomiets E, Rehmeyer C, Li W, Harding M, Kim S, Lebrun MH, Bohnert H, Coughlan S, Butler J, Calvo S, Ma LJ, Nicol R, Purcell S, Nusbaum C, Galagan JE, Birren BW (2005) The genome sequence of the rice blast fungus *Magnaporthe grisea*. Nature 434:980–986
4. Bonman JM, Khush GS, Nelson RJ (1992) Breeding rice for resistance to pest. Ann Rev Phytopathol 30:507–528
5. Saleh D, Milazzo J, Adreit H, Fournier E, Tharreau D (2014) South-east Asia is the center of origin, diversity and dispersion of the rice blast fungus, *Magnaporthe oryzae*. New Phytol 201:1440–1456
6. Flor HH (1971) Current status of the gene for gene concept. Annu Rev Phytopathol 9:275–296
7. Tanksley SD, McCouch SR (1997) Seeds banks and molecular maps: unlocking genetic potential from the wild. Science 277:1063–1066
8. Hayashi et al (2006) Development of PCR-based allele-specific and InDel marker sets for nine rice blast resistance gene. Theor Appl Genet 113:251–260
9. Wang Z, Jia Y, Rutger JN, Xia Y (2007) Rapid survey for presence of a blast resistance gene *Pi-ta* in rice cultivars using the dominant DNA markers derived from portions of the *Pi-ta* gene. Plant Breed 126:36–42
10. RoyChowdhury M, Jia Y, Jackson A, Jia MH, Fjellstrom R, Cartwright RD (2012) Analysis of rice blast resistance gene Pi-z in rice germplasm using pathogenictiy assays and DNA markers. Euphytica 184:35–46
11. Thakur S, Gupta YK, Singh PK, Rathour R, Variar M, Prashanthi SK, Singh AK, Singh UD, Chand D, Rana JC, Singh NK, Sharma TR (2013) Molecular diversity in rice blast resistance gene *Pi-ta* makes it highly effective against dynamic population of *Magnaporthe oryzae*. Funct Integr Genomics 13:309–322
12. Koide Y, Kobayashi N, Xu D, Fukuta Y (2009) Resistance genes and selection DNA markers for blast disease in rice (*Oryza sativa* L.). JARQ 43:255–280. http://www.jircas.affrc.go.jp
13. Imam J, Alam S, Variar M, Shukla P (2013) Identification of rice blast resistance gene *Pi9* from Indian rice land races with STS marker and its verification by virulence analysis. Proc Natl Acad Sci India Sect B Biol Sci 83:499–504
14. Qu S, Liu G, Zhou B, Bellizzi M, Zeng L, Dai L, Han B, Wang GL (2006) The broad spectrum blast resistance gene *Pi9* encodes a nucleotide-binding site leucine-rich repeat protein and is a member of a multigene family in rice. Genetics 172:1901–1914
15. Zhou B, Qu S, Liu G, Dolan M, Sakai H, Lu G, Bellizzi M, Wang GL (2006) The eight amino-acid differences within three leucine-rich repeats between *Pi2* and *Piz-t* resistance proteins determine the resistance specificity to *Magnaporthe grisea*. Mol Plant Microbe Interact 19:1216–1228
16. Wu JL et al (2005) Genetic control of rice blast resistance in the durably resistant cultivar Gumei 2 against multiple isolates. Theor Appl Genet 111:50–56
17. Deng Y, Zhu X, Shen Y, He Z (2006) Genetic characterization and fine mapping of the blast resistance locus *Pigm(t)* tightly linked to *Pi2* and *Pi9* in a broad-spectrum resistant Chinese variety. Theor Appl Genet 113:705–713
18. Fjellstrom R, McClung AM, Shank AR (2006) SSR markers closely linked to the *Pi-z* locus are useful for selection of blast resistance in a broad array of rice germplasm. Mol Breed 17:149–157
19. Jeung JU, Kim BR, Cho YC, Han SS, Moon HP, Lee YT, Jena KK (2007) A novel gene, *Pi40(t),* linked to the DNA markers derived from NBS-LRR motifs confers broad spectrum of blast resistance in rice. Theor Appl Genet 115:1163–1177
20. Zhu X, Chen S, Yang J, Zhou S, Zeng L, Han J, Su J, Wang L, Pan Q (2012) The identification of *Pi50(t),* a new member of the rice blast resistance *Pi2/Pi9* multigene family. Theor Appl Genet 124:1295–1304
21. Mackill DJ, Bonman JM (1992) Inheritance of blast resistance in near-isogenic lines of rice. Phytopathology 82:746–749
22. Chen D, Zeigler RS, Ahn SW, Nelson RJ (1996) Phenotypic characterization of the rice blast resistance gene *Pi2(t)*. Plant Dis 80:52–56
23. Leung H, Borromeom ES, Bernardoa MA, Notteghem JL (1988) Genetic analysis of virulence in the rice blast fungus *Magnaporthe grisea*. Phytopathology 78:1227–1233
24. Alam S, Imam J, Maiti D, Prasad C, Sharma TR, Mandal NP, Variar M (2013) Diversity of *Magnaporthe Oryzae* and resistance spectrum of major genes to rice blast in eastern India (abstr.) In: Proceedings of ARRW Golden Jubilee International Symposium, pp 287–288, CRRI, Cuttack
25. Pusadee T, Jamjod S, Chiang Y, Rerkasem B, Schaal BA (2009) Genetic structure and isolation by distance in a landrace of Thai rice. PNAS. doi: 10.1073/pnas.0906720106
26. Bonman JM, Vergel De Dios TI, Khin MM (1986) Physiologic specialization of *Pyricularia oryzae* in the Philippines. Plant Dis 70:767–769
27. Hittalmani S, Kahani F, Dhangond SM, Rao AM (2013) DNA marker characterization for allele mining of blast and bacterial blight resistance genes and evaluation for grain yield. Afr J Biotechnol 12:2331–2340
28. Saito M, Konda M, Vrinten P, Nakamura K, Nakamura T (2004) Molecular comparision of Waxy null alleles in common wheat and identification of unique null allele. Theor Appl Genet 108:1205–1211
29. Rathour R, Singh BM, Plaha P (2006) Virulence Structure of the *Magnaporthe grisea* rice population from the northwestern Himalayas. Phytoparasitica 34:281–291
30. RoyChowdhury M, Jia Y, Jia MH, Fjellstrom R, Cartwright RD (2012) Identification of rice blast resistance gene *Pib* in national small grains collection. Phytopathology 102:700–706