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Molecular analysis and phenotypic validation of blast resistance genes *Pita* and *Pita²* in landraces of rice (*Oryza sativa* L.)

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Abstract

Rice (*Oryza sativa* L.) crop confronts several major and minor diseases of which blast disease caused by *Magnaporthe oryzae* is a serious constraint causing moderate to huge economic losses world over. Exploitation of genetic resistance in cultivars is a preferred management strategy. Though the number of genes for resistance to blast have been cloned and mapped, however, as because of their variable efficacy and donor traits there remains a scope for discovery of alleles and hence fresh donors among local collections and landraces. Present investigation was undertaken with the objective towards screening of 100 germplasm accessions for *Pita-Pita²*, the two tightly linked dominant genes governing major gene blast resistance. An attempt was made to identify the genotypes carrying individually or in combination the genes *Pita* and *Pita²* with the help of molecular markers and differential screening using diagnostic isolates. Based on PCR assay using two gene based markers, 27 accessions were found carrying resistance specific alleles. Differential screening results coupled with marker information helped to classify the genotypes in five categories. Out of the marker positive accessions, 11 were supposed to possess *Pita* and in 9 other genotypes the presence of *Pita* and (or) *Pita²* was validated. Besides this, the validation of markers linked to *Pita-Pita²* locus was performed across varieties and landraces grown in India to assess their use and relevance in MAS. The identified gene sources may serve as new donors and a subject for further genetic and genomic investigations.

Key words: Rice blast, resistance, *Pita, Pita²*, markers, landraces

Introduction

Rice (*Oryza sativa*) is grown in varied agro-climatic regions ranging from tropical, sub-tropical to temperate. Its wide cultivation pre-disposes rice crop to range of biotic stresses of which blast disease caused by *Magnaporthe oryzae* remains scourge to farmers and has been reported from around 85 countries. Natural Resources Institute, London declared blast disease most deadly of all biotic stresses on world basis [1]. Annual yield losses attributable to blast range from 10-30%, even 10% being sufficient to cater 60 million people for one year. Protection strategies safeguard ~38% of attainable rice production [2]. Chemical control in blast is effective but has serious ecological ramifications and also adds to cost of cultivation. Exploiting genetic resistance is safe and reliable option for sustenance of yield potential of otherwise successful cultivars.

India is a niche to enormous genetic diversity and about 50000 landraces of rice are believed to be existing [3]. More than 95% rice germplasm collections worldwide have never been utilized in breeding programs [4], that is consequently reflected in low genetic diversity and high vulnerability to biotic stress in commercially grown cultivars [5]. Sharma *et al.* [6] have underlined the importance of landraces in exploring valuable genes for resistance to blast.

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Collection and evaluation of landraces thus becomes an integral part of pre-breeding process. So far, more than 100 blast resistance genes from japonica (45%), indica (51%) and other (4%) genotypes have been identified [6]. While, many of these genes are located in a close physical proximity to one another in rice genome, yet they exhibit differential reaction specificities at host pathogen interface. For example, tightly linked genes *Pi9-Piz5* and *Pik*^{m} - *Pik* vary in their resistance spectrum [7, 8]. *Pita* locus in rice has been effectively used to manage rice blast worldwide [9] and also in India [10]. The genes *Pita* and *Pita*^{2} occupy near-centromeric region on chromosome 12 and are linked closely at 0.4 cM [11] with *Pita*^{2} reportedly having broader resistance [12]. Even though, these genes have been utilized in many varieties bred through conventional breeding approaches by virtue of their origin/ pedigree, the information regarding actual status on the presence of these genes in the released varieties and the landraces is lacking. The present study was carried out with the objective of screening the landraces and varieties for *Pita*^{2} and *Pita* and validating the presence of gene(s) by using differential isolates between these tightly linked genes. Also, molecular markers were validated for their efficacy for identification of these genes.

Materials and methods

Germplasm

The germplasm screened comprised of 46 released varieties from different centres and 54 landraces collected from various parts of India. The monogenic lines IRBLta-K1 (*Pita*) and IRBLta^2-PI (*Pita*^{2}) in the genetic background of LTH (Lijiangxintuanheigu) were used as resistant checks while LTH was included as susceptible control.

Pathogenicity assay

The two blast isolates Mo-ni-0066 and Mo-ni-0052 maintained at Division of Plant Pathology, IARI, New Delhi were used for pathogenicity on IRBLta-K1 (*Pita*) and IRBLta^2-PI (*Pita*^{2}). Of these Mo-ni-0052 is avirulent to both *Pita* and *Pita*^{2} and Mo-ni-0066 is a differential isolate between IRBL monogenic lines with compatible and incompatible reaction response respectively. Mo-nwi-31 (maintained at CSKHPKV, Palampur) is a virulent isolate for both *Pita and Pita*^{2} and was used as cross check to confirm the disease reaction. Artificial inoculation on a set of germplasm was performed using blast isolates Mo-ni-0052, Mo-ni-0066 and Mo-nwi-31. Seeds were grown in 5 x 4 pro-trays with 6 seedlings per well, replicated twice. The clean soil was fertilized with well decomposed organic matter, N_2 and P_2O_5 as per recommendation. The inoculum was prepared following protocol given by Bonman *et al.* [13]. The seedlings were sprayed using hand atomizer (100 kPa) at 3-4 leaf stage with *M. oryzae* spore suspension adjusted to 5 x 10^4 spores per ml. Two drops of 0.02% Tween20 was added to the suspension prior to spray. The trays were kept in dark for 24 hrs. inside disinfected dew chambers and subsequently maintained at 25±1^{o}C and 85% RH under proper light regime. After 6 DAI scoring was done following Mackill and Bonmann [14]. This was followed by second scoring after 3 days interval. The whole experiment was repeated twice.

Molecular analysis

Isolation of genomic DNA from leaf tissue was carried out following CTAB method as described by Murray and Thompson [15]. DNA quantification was done using 0.8% Agarose gel. The λ uncut DNA was used as a comparison and the final concentration was adjusted to ~25 ng/µl. The samples were subjected to Polymerase Chain Reaction using oligo-nucleotide gene based primers YL153/154, YL155/87 and linked primers RM101, OSM89 and RM7102 (Table 1). PCR reaction mixture contained 25 ng of DNA, 10 x PCR buffer (10 mM Tris, pH 8.4, 50 mM KCl, 1.8 mM $MgCl_2$), 0.05 mM dNTPs (MBI, Fermentas, Lithuania, USA), 5 pmol each of forward and reverse primer and 0.5 U of Taq DNA polymerase (Bangalore Genei Pvt. Ltd., India) in a reaction volume of 10 µl. Polymerase chain reaction (PCR) was performed in a thermal cycler (G-Storm, Somerset, UK). Initial denaturation was performed at 95^{o}C for 5 min. followed by 35 PCR cycles maintaining following thermal conditions: denaturation at 94^{o}C, annealing at 55^{o}C and extension at 72^{o}C; also a final extension for 7 min at 72^{o}C was provided. The amplified products from reactions involving YL-primers were resolved on 1.5% agarose gel, visualized in UV trans-illuminator and documented in gel documentation system (Bio-Rad Laboratories Inc., USA). PCR products amplified with the help of linked primers were resolved and analyzed with MultiNA microchip electrophoresis (MCE-202, Shimadzu Corporation). PIC was calculated as per the formula 1-Σx^2, where x is the frequency of ith allele.

Results and discussion

The possibility of identifying novel alleles of already

Table 1. Molecular markers used for screening of blast resistance genes *Pita-Pita*^{2} in wide germplasm

Gene/chromo-soaml location	Primer name	Oligo-nucleotide sequence	Centi-morgan distance from gene	Reference
Pita-Pita^2 / *Chromosome-12*	YL155/ YL87	YL155: AGCAGGTTATAAGCTAGGCC YL87 : CTACCAACAAGTTCATCAAA	Gene based	[19]
	YL153/ YL154	YL153: CAACAATTTAATCATACACG YL154: ATGACACCCTGCGATGCAA	Gene based	[19]
	RM101	F: GTGAATGGTCAAGTGACTTAGGTGGC R: ACACAACATGTTCCCTCCCATGC	2.7 cM	[27]
	RM7102	F: TAGGAGTGTTTAGAGTGCCA R: TCGGTTTGCTTATACATCAG	1.2 cM	[27]
	OSM89	F: TTGGTCAAAGTTAGCATGGGAGGG R: TTTGAACCGGGTGGCCCACATG	2.7cM	[33]

known genes with even broad resistance spectrum can be met by screening wild species and germplasm accessions as is evident from the recent works carried out by Das *et al.* [16]. The use of molecular markers for screening diverse germplasm for tapping valuable genes for blast resistance has been demonstrated by Cho *et al.* [17]. Gene derived markers directly sample variation in transcribed regions of the genome, which may enhance their value in MAS, comparative genetic studies and for exploiting rich genetic resources by providing a more direct estimate of functional diversity [18]. In the present study, the dominant markers, YL153/154 and YL155/87 based on genic regions within the *Pita* locus showed amplification in 27 of the 100 accessions screened. The germplasm set comprised of 46 released varieties and 54 landraces, out of which 12 (26%) and 15 (28%) accessions respectively were found positive for two markers. YL153/154 amplified a fragment of 440 bp and YL155/87 a fragment of 1042 bp on 1.5% agarose gel. The marker YL155/87 is based on internal nucleotide polymorphisms between resistant *Pita* and susceptible *pita* alleles whereas the marker YL153/154 is based on sequence polymorphism at translation start site of the gene [19]. The accessions testing positive for the gene based markers under present study included the popular varieties IR64, IR50 and ADT37 (Table 2, Fig 1a&b). In earlier studies, IR64 has been reported to possess *Pita* as one of the blast resistance genes [20]. In RFLP analysis of *japonica* NILs, Rybka *et al.,* [21] found Tadukan type segments around *Pita*^{2} locus while *Pita* NILs had non-Tadukan type regions flanking the gene (Tadukan is a Philippino *indica* rice variety which has been used as donor of *Pita*). Such distinct introgressions in the evolution of *Pita*^{2} and *Pita* were attributed as a cause to the difference in race specificity of these two nearly linked genes. While, a common set of DNA markers has been referred for both *Pita-Pita*^{2} [12, 22-24], they exhibit different reaction pattern. Therefore, this opens up the possibility that these genes could be identified individually by pathotyping using a differential isolate between these two genes.

All the 100 germplasm lines and cultivars along with checks *viz.,* IRBLta-K1 (*Pita*), IRBLta^2-PI (*Pita*^{2}) and LTH (Susceptible control) were screened using isolates Mo-ni-0066 (*Avr-Pita*^{2}, *avir-Pita*), Mo-ni-0052 (*Avr-Pita*^{2}*-Pita*) and Mo-nwi-31 (*avr- Pita*^{2}*-Pita*). The entries with a score 0 were recorded as immune, 1-2 as Resistant and 3-5 as Susceptible (Table 2). The entire set of germplasm was classified in 4 reaction types: Susceptible against Mo-ni-0066 and resistant against Mo-ni-0052 (SR); resistant to Mo-ni-0066 and susceptible to Mo-ni-0052 (RS); susceptible (SS) and resistant (RR) against both the isolates. Out of 27 gene positive accessions identified under marker analysis, 11 had profile SR, 9 followed RR, 5 belonged to SS and 2 were RS (Table 3). The reaction SR indicates the presence of *Pita* and absence of *Pita*^{2}. The accessions in this group included Kalinga I, Suphala, IR64, Pant Dhan 12, Pratikshya, ND118, Baubhog, Birui, Agnibaou, Paoba and Sadhajhumur. If *Pita*^{2} was present in these lines they should have expressed incompatible reaction with Mo-ni-0066, however, they were susceptible to it and therefore, the presence of *Pita* in these lines was confirmed by resistant reaction to Mo-ni-0052. The second class

Table 2. Molecular analysis, validation of molecular markers and disease reaction to blast isolates under artificial conditions (S.No 1-42 and 97-100 are released varieties; 43-96 are landraces)

S.No.	Germplasm	Cross combination/origin	YL155/87	YL153/154	RM 101	RM 7102	Mo-ni-0066 (*Avr-Pita*^{2} and *avr-Pita*	Mo-ni-0052 (*Avr-Pita*^{2} *Pita)*	Mo-nw-31 (*avr-Pita*^{2}*-Pita)*
	1	2	3	4	5	6	7	8	9
1	Jaldi Dhan 6	India			263	170	4	4	4
2	CSR 10	M40-431-24-114/Jaya			263	186	4	4	4
3	IR 50	IR-2153-14-1-6-2 x IR-28 x IR-36	+	+	263	170	0	2	4
4	Sona Mahsuri	Sona/Mahsuri			263	170	1	3	1
5	Swarna	Vasistha/Mahsuri			263	170	0	0	1
6	MTU 2067 (Chaitanya)	Sowbhagya x ARC-5984			263	170	3	4	1
7	Rasi	TN 1/CO.29			263	170	0	2	4
8	Kohsaar (K-429)	Shenei/ Ginmasari			310	170	3	4	5
9	Pusa 33	Sabarmati/Ratna			263	186	4	4	5
10	Tai Pei 309	Japan			263	170	4	3	3
11	Heibao	China			263	160	1	2	1
12	Kalinga-I	India	+	+	286	170	5	1	5
13	MTU 1001 (Vijetha)	MTU5249/MTU7014			263	170	0	2	1
14	K 332	Shenei/Norin-11			235	160	4	4	4
15	Chandrahasini	Abhaya/Phalguna	+	+	235	160	0	1	5
16	Varun Dhan	Pure line selection from JUN JEN-4			318	160	5	4	4
17	Manhar	IR20/Cauvery			263	170	2	0	5
18	Anjali	RR19-2/RR149-1129			263	186	1	4	1
19	ADT 37	BG280-12/BTP33	+	+	235	170	1	0	4
20	Suphala	India	+	+	263	186	4	2	4
21	Subhadra	TN1/SR26B			263	186	4	4	2
22	Keshari	SR26B/Jagannath			263	170	3	2	1
23	Rudra	HR19/TN1			263	170	3	3	2
24	Shankar	Parijat/IET3225			251	160	4	4	5
25	Pathara	Hema/CO18			263	186	1	2	5
26	Badami	Suphala/Annapurna			263	150	4	4	5
27	Nialgiri	Suphsla/DZ-12			263	160	4	0	5
28	IR-8	Dee-geo-woo-gen x Peta			263	170	4	4	5
29	IR-64	IR5657-33-2-1/IR2061-465-1-5-5	+	+	263	186	4	2	5
30	Keshav	WGL28712/IR36-1996	+	+	263	170	2	0	1
31	Indira Sugandh Dhan-1	Madhuri/Surekha			263	186	4	4	4
32	Pant Dhan 12	Govind/UPR201-1-1	+	+	251	170	5	2	4

Table 2 Contd....

	1	2	3	4	5	6	7	8	9
33	Prasad	IR7474B-26-3/IR57948			263	170	2	0	1
34	Swarna mukhi	CICA4/IR625-23-3-1//Tetep	+	+	263	186	0	0	1
35	Sarathi	T90/IT8//W1263			263	186	2	0	4
36	PR118	Pusa44/PR110			263	230	3	0	4
37	CSR23	(IR64//IR4630-22-2-5-1-3/IR9764-45-2-2)			263	186	4	0	4
38	Pratikshya	Swarna/IR64	+		263	170	4	0	1
39	Samleshwari	R310-37/R308-6	+	+	263	170	2	2	4
40	Narendra Dhan-118	IR-36 x Hansraj-A	+	+	235	170	4	0	5
41	Haryana Basmati1	Sona/ Basmati 370			263	186	0	4	4
42	Super Basmati				263	186	1	2	2
43	Basmati (Orissa)				263	186	3	2	3
44	Latasal		+	+	263	186	5	5	4
45	Paani dooba				327	230	1	2	4
46	Banstana				263	170	0	0	4
47	Baubhog		+	+	263	186/230	5	2	4
48	Kalo mota				263	170	1	1	3
49	Mehandi				263	210	5	4	4
50	Sonashree				263	170	5	4	1
51	Jata Dhan		+	+	263	186	2	2	4
52	Lal dusari		+	+	263	170	5	4	4
53	Tulsi mukul				209	210	3	5	1
54	Kishori				-	170	4	2	1
55	Khaja		+	+	263	170	2	4	3
56	Bangla Patni				263	186	4	3	4
57	Lal patri				330	220	2	5	4
58	Dehraduni Gaudeshwari				263	170	5	5	3
59	Kumargarh				263	186	4	0	4
60	Tulsa				263	186	1	3	3
61	Kalo bhutia		+	+	330	200	2	0	1
62	Birui		+	+	263	170	5	0	1
63	Mayur pankhi				318	186	4	4	4
64	Boarti		+	+	263	186	5	4	4
65	Sada kaijam				263	170	1	4	2
66	Paran kalas				235	130	1	4	4
67	Mourisal				263	170	4	2	1
68	Sabita		+	+	263	186	1	4	4
69	Lakki kajal				263	170	1	0	2
70	Kala munia				209	200	4	4	1
71	Lad sal				310	170	3	4	4

Table 2 Contd....

	1	2	3	4	5	6	7	8	9
72	Agni baou		+	+	263	186	4	2	4
73	Durga sudami		+	+	291	170/186	5	5	2
74	Kakhow		+	+	235	200/230	4	5	2
75	Chima kamin				263	186	4	2	4
76	Kala jeera		+	+	263	170	1	2	1
77	Lakhi Chura				263	170	2	0	3
78	Pakhri				330	200	0	2	2
79	Geetanjali				263	186	1	3	4
80	Badshah bhog				263	186	2	4	4
81	Paoba		+	+	263	170	4	2	4
82	Leela bati				330	220	4	5	4
83	Mala				263	186	4	0	4
84	Sadhajhumur		+		263	186	4	0	4
85	Super sugandhamati				330	170	4	2	4
86	Katori bhog				263	130	4	3	1
87	Tangra				263	170	4	4	5
88	Jugal				263	170	3	4	2
89	Pak Basmati				310	170	3	4	4
90	Bankra				251	170	5	2	4
91	Poonti kaami				263	186	2	3	1
92	Lalmeeta				263	170	2	3	2
93	Khayersal				263	170	1	4	1
94	Janghi jata				263	160	2	3	1
95	Tuniaslet				263	170	2	3	4
96	Randhuni pagal				263	170	1	4	4
97	Shalimar Rice 1	China 1007/ Rasi			263	186	1	2	4
98	Jehlum	China 1007/ Jikkoku			263	170	4	3	5
99	Sneha	Annanda x CR-143-2-2			263	170	4	4	4
100	Pusa Basmati 1	Pusa-150 x Karnal Local			263	170	4	4	5
	IRBLtaK1 (Pita)				263	186	4	0	4
	IRBLta2-PI (Pita^2)				263	186	0	0	4
	LTH				310	170	4	4	4

with reaction response RR had genotypes IR50, Chandrahasini, ADT37, Keshav, Swarna mukhi, Jata Dhan, Kalo bhutia, Kala jeera and Samleshwari. Neverthless, as discussed earlier, *Pita*^{2} functioning would require *Pita* and *Ptr,* therefore, it can be concluded that *Pita* and *Pita2* are invariably present in all lines showing resistance to Mo-ni-0066. The susceptible reaction of these genotypes against virulent isolate Mo-nwi-31 validates the presence of *Pita* and (or) *Pita*^{2}. The another group SS that covers five genotypes is a case of susceptibility in marker positive genotypes towards both the isolates under consideration. The perfect markers YL155/87 and YL153/154 are based on the nucleotide differences at 3' primer binding sites between *Pita* resistant *indica* and *pita* susceptible *japonica*. It is possible that the fragment amplifies because the YL-marker specific polymorphism is conserved in these genotypes but

Table 3. Grouping of germplasm on the basis of marker genotype and reaction response against differential isolates

Reaction type		Marker profile using YL155/87 and YL153/154	Probable gene at *Pita-Pita*^{2} locus	Genotypes
Mo-ni-0066 (*Avr-Pita*^{2} and *avir-Pita*)	Mo-ni-0052 (*Avr-Pita*^{2}*-Pita*)			
Susceptible	Resistant	+	*Pita*	Kalinga I, Suphala, IR64, Pant Dhan 12, Pratikshya, ND118, Baubhog, Birui, Agnibaou, Paoba and Sadhajhumur
Resistant	Resistant	+	*Pita*^{2} + *Pita ?**	IR50, Chandrahasini, ADT37, Keshav, Swarna mukhi, Jata Dhan, Kalo bhutia, Kala jeera and Samleshwari
Susceptible	Susceptible	+	_	Latasal, Lal dusari, Baorti, Durga sudami, Kakhow
Resistant	Susceptible	+	R_ **	Khaja and Sabita
Resistant	Resistant	_	*??...****	Rasi, Manhar, Pathara, Sarathi, Paani dooba, Banstana, Kalo mota, Shalimar Rice 1 and Lakhi chura

*Resistant response from *Pita* if present would be masked by presence of *Pita*^{2}; **Pita*^{2} may be present and the isolates show difference in pathogenicity; ***Resistance may be attributed to presence of some other unknown genes

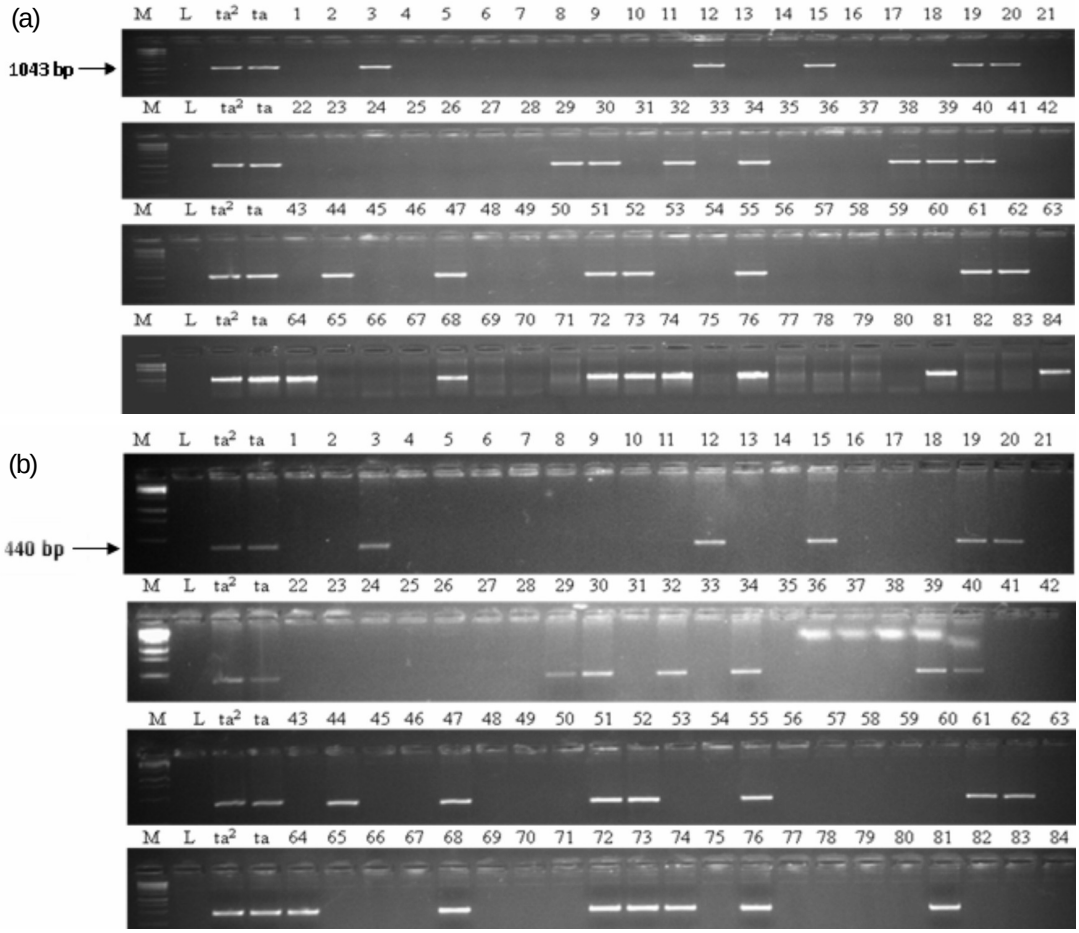

Fig. 1. Screening of germplasm lines for the blast resistance genes *Pita*^{2} and *Pita* with the dominant gene based markers: (a) YL155/87; (b) YL153/154 (a) Marker: YL155/87; M: 1Kb Ladder; ta^2: IRBLta^{2}PI (*Pita*^{2}); ta: IRBLtaK1 (*Pita*); 1-84: Germplasm accessions and (b) Marker: YL153/154 M: 1Kb Ladder; ta^2: IRBLta^{2}PI (*Pita*^{2}); ta: IRBLtaK1 (*Pita*); 1-84: Germplasm accessions; L : LTH

they may differ with respect to some other genic regions influencing resistance. According to Bryan *et al.* [12], a single amino acid difference exists between *Pita* and *pita*. Resistant *Pita* has alanine in place of serine in susceptible *pita* at position 918. Finally, the reaction type RS was rare with only two accessions, Khaja and Sabita. This reaction pattern is opposite to the hypothesis reported earlier. Kiyosawa *et al.* [25], reported that reaction spectrum of *Pita*^{2} against blast races includes that of *Pita*. This view is also held by Bryan *et al.* [12], according to whom no isolate knocking down *Pita*^{2} was found avirulent to *Pita*. In these types *Pita*^{2} is suspected on the basis of marker profile and incompatibility with *Avr-Pita*^{2}.

The germplasm entries Swarna, Heibao, MTU1001, Prasad, Super Basmati, Lakki Kajal and Pakhri recorded resistant reaction to all the three isolates Mo-ni-0066, Mo-ni-0052 and Mo-nwi-32 but were negative for pair of YL markers. Also, the genotypes Rasi, Manhar, Pathara, Sarathi, Paani dooba, Banstana, Kalo mota, Shalimar Rice 1 and Lakhi chura showed resistant reaction to the two isolates Mo-ni-0066 and Mo-ni-0052. This upholds the possibility that these genotypes harbour some other unknown genes those need to be analyzed in future studies. The preliminary pathotyping data on LTH background monogenic differentials revealed that the isolate Mo-ni-0066 was avirulent to *Pi11(t), Pi19, Pi9, Pib, Pizt*; Mo-ni-0052 showed avirulence to *Pi12*, *Pik*^{h}, *Pit* and *Pizt* and Mo-nwi-31 was avirulent on *Pish, Pik*^{h}*, Pik*^{m}*, Pi20, Pi3, Pii, Pi5*). Therefore, it is likely that germplasm accessions with a score of 0-2 might possess one or more of the aforesaid genes or even some new gene(s).

The gene *Pita*^{2} shows broad spectrum resistance than *Pita* and it also confers resistance to panicle blast [22]. The resistance specificity of *Pita*^{2} is conditioned by a combination of *Pita* and yet another gene *Ptr(t)*

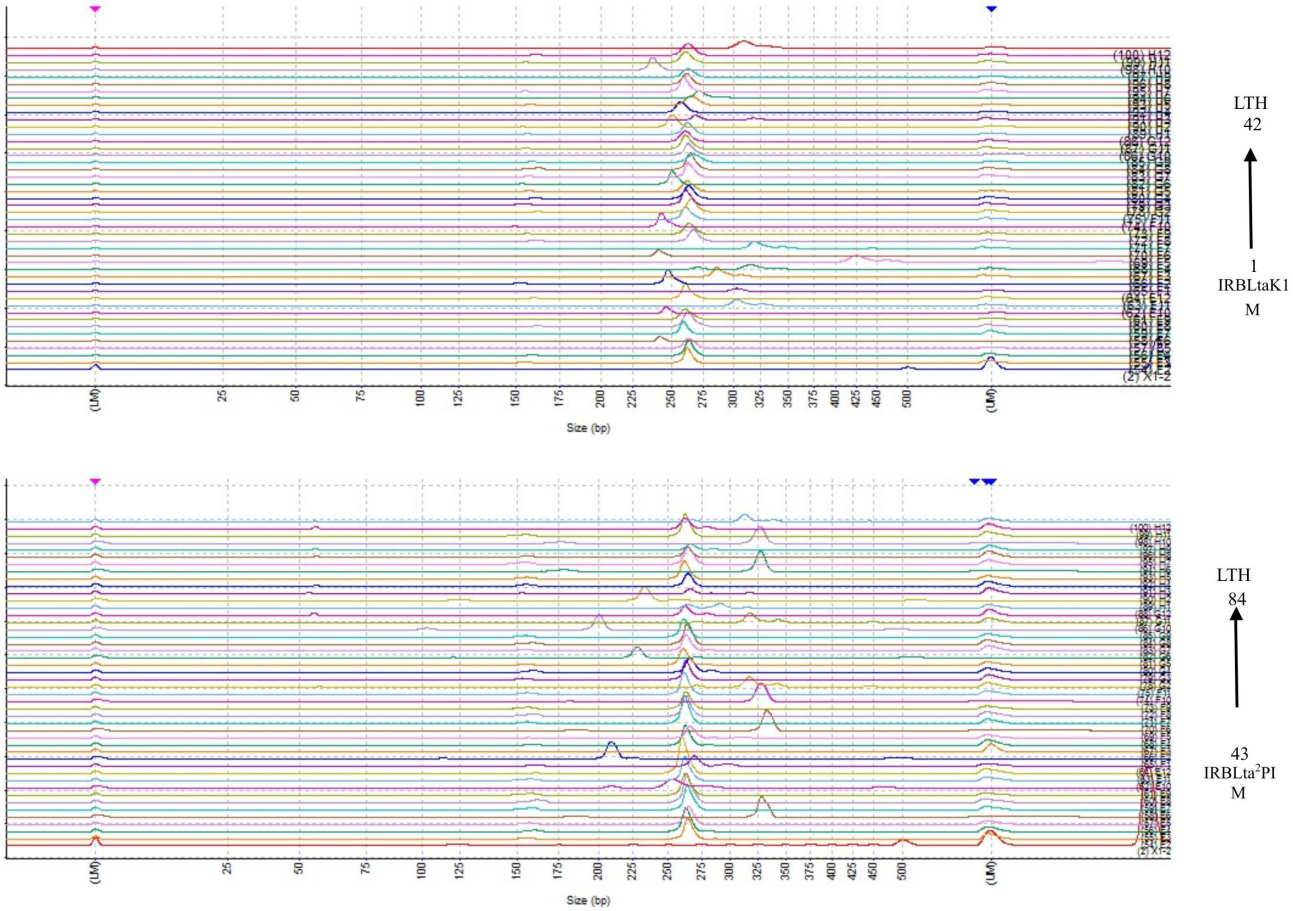

Fig. 2. Electropherogram of molecular marker analysis of germplasm lines using *Pita* linked marker RM101: M: Marker; Monogenic differential lines, IRBLtaK1 (*Pita*); IRBLta^{2}PI (*Pita*^{2}); 1-84: Germplasm; LTH (Susceptible check)

[*Pita* required (temporary)]. Identified from Katy, this gene was mapped within 9Mb region spanning *Pita* [21, 26]. This indicates the role of chromosomal segments outside the *Pita* locus to be important for *Pita* mediated resistance and explains the susceptibility of our marker positive genotypes. Conaway *et al.,* [27]) placed markers RM101 and RM7102 near *Pita*^{2}. Three *indica* landrace cultivars *viz*., Tadukan from Phillippines, Tetep from Vietnam and TeQing from China are known as the sources of *Pita* resistance worldwide [28]. Tetep was used to derive *Pita* cultivars in USA and Tadukan was used as source of *Pita* for cultivars like K1. Depending upon the lineage, the LD blocks near *Pita* are conserved in wide accessions because of recombination suppression due to centromeric proximity, where 1Mb of physical distance corresponds to 1cM [29]. A study on validation of markers linked to *Pita* locus was carried out to find out the distribution of *Pita* across the set of diverse rice germplasm. SSR marker OSM89 reported to be at a distance of 2Mb from *Pita* showed lack of polymorphism between LTH and *Pita*/ *Pita*^{2} monogenic lines with respect to resistant allele size of 300bp. This marker is derived from the same cDNA sequence as RM101, however, the later was polymorphic and recorded PIC of 0.41 with 7 different alleles. The fragment size at RM101 ranged from 209 bp to 330 bp with 76% accessions amplifying 263 bp allele (Fig. 2). Another marker RM7102 at a distance of 1.2cM from *Pita* had a significant PIC of 0.66 with 7 alleles per locus which indicates its usefulness in polymorphism assays across bi-parental populations. This marker showed a fragment size range of 150-230bp with 48% of the accessions at 170bp (Fig. 3). *Pita* near centromeric region on chromosome 12 is clustered by some other genes like *Pi20* [30], *Pi39* [31], *Pi42* [32]. The distinction between clustered R-genes in terms of degree and distribution of resistance is difficult to work out and remains technically a challenging task in the absence of specific differential isolates with non-

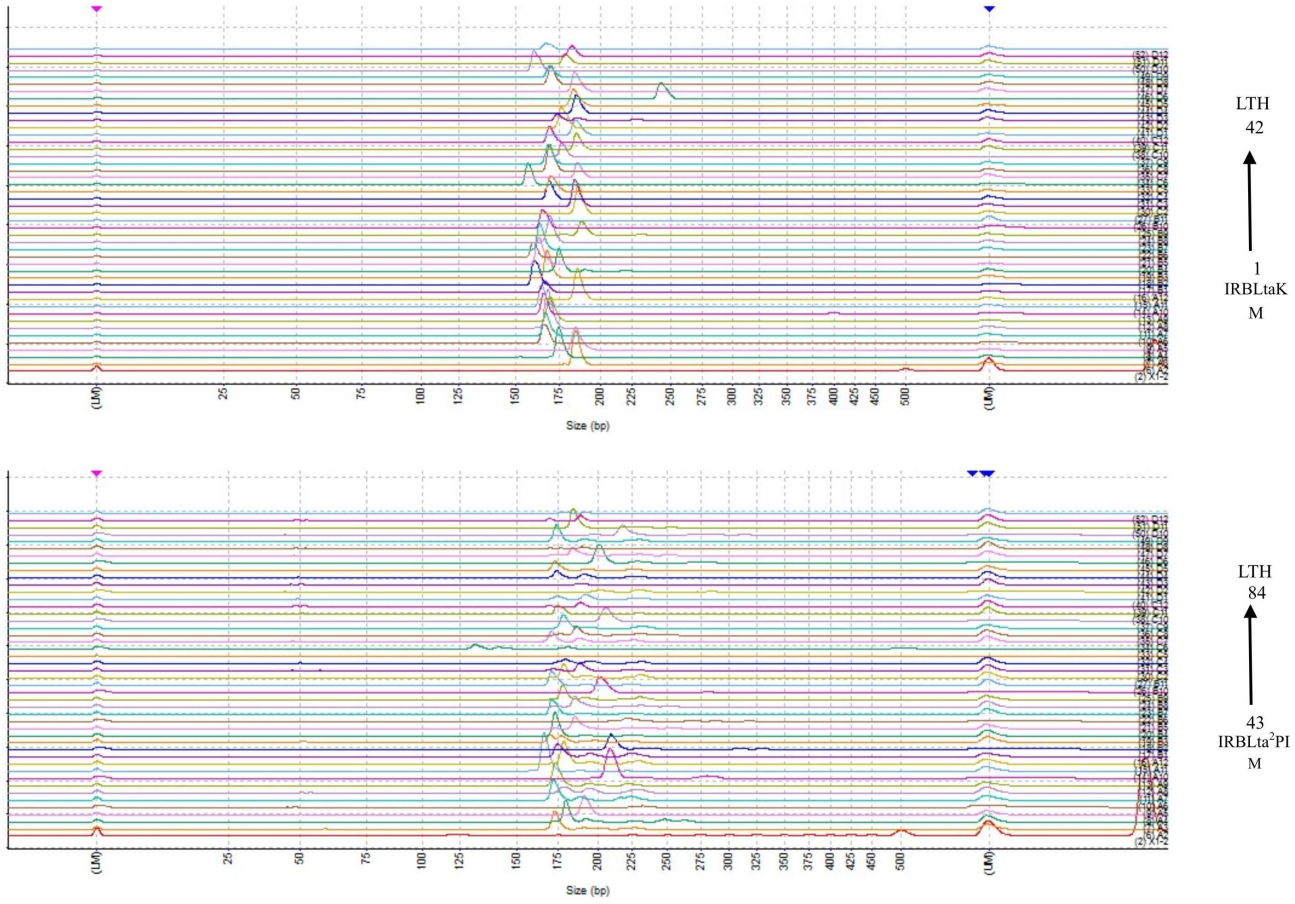

Fig. 3. Electropherogram of molecular marker analysis of germplasm lines using Pita linked marker RM7102: M: Marker; Monogenic differential lines, IRBLtaK1 (*Pita*); IRBLta^{2}PI (*Pita*^{2}); 1-84: Germplasm; LTH (Susceptible check)

overlapping reaction. The task is complicated further by the lack of knowledge of the number of genes present in a given cultivar. Thus, the identified sources of resistant *Pita* allele in the present study need to be screened through wide races and analysed for other resistance genes. Nevertheless, the information generated about the positive resistant accessions may help in utilization of these genetic resources in future breeding programs and also the germplasm may be used as subject for allele mining of other possible R-genes.

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