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Marker assisted pyramiding of major blast resistance genes *Pi9* and *Pita* in the genetic background of an elite Basmati rice variety, Pusa Basmati 1

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Abstract

Basmati is a premium quality rice of India which is highly priced in the international market. Pusa Basmati 1, an elite Basmati rice variety is highly susceptible to rice blast caused by *Magnaporthe oryzae*. Therefore, pyramiding blast resistance genes is essential to effectively combat the blast disease and increase the durability of resistance genes. The blast resistance genes *Pi9* and *Pita* have been earlier demonstrated to be effective in Basmati growing regions of the country. Therefore, in the present study, monogenic-near isogenic lines Pusa 1637-18-7-6-20 and Pusa 1633-3-8-8-16-1 carrying *Pi9* and *Pita*, respectively, were intercrossed to generate pyramided lines through marker assisted foreground, background and phenotypic selection for recurrent parent phenotype. The pyramided lines carrying *Pi9+Pita* were found to be either at par or superior to the recurrent parent Pusa Basmati 1 for agro-morphological, grain and cooking quality traits. Further, these pyramided lines were also found to show resistance against three virulent pathotypes of *M. oryzae* namely, Mo-nwi-kash 1, Mo-nwi-lon2 and Mo-ei-ran1, when evaluated under artificial inoculation conditions as well as in the natural epiphytotic conditions of uniform blast nursery at two locations. The developed pyramided lines are the potential sources of blast resistance genes in the Basmati improvement program and can also be released for commercial cultivation after required testing.

Key words: Basmati rice, blast resistance, gene pyramiding, marker assisted selection

Introduction

Basmati rice is the pride possession of Indian sub-continent which is cultivated in the Indo-Gangetic region of the country comprising of seven states namely, J&K, Himachal Pradesh, Punjab, Haryana, Delhi, western UP and Uttarakhand. Basmati rice being characterized by unique grain and cooking qualities with attractive grains and pleasant aroma, it is widely accepted worldwide. Therefore, it is highly prized in the international market. The annual forex earning of the country due to export of Basmati rice was 29,300 crores during 2014-15 (APEDA, 2015).

The rice blast disease is caused by an ascomycete fungus *Magnaporthe oryzae*. The fungal spore germinates on the leaf surface forming a germ tube which differentiates into a peculiar dome shaped structure called appressoria, which engenders mechanical force to rupture the host cuticle and navigate the underlying epidermal tissues. Further, the

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cell cycle-regulated autophagic programmed cell death occurs to initiate infection in the host (Howard et al. 1991, De Jong et al. 1997, Veneault-Fourrey et al. 2006). The pathogen is prominent in mutilating 10-100% of the rice harvest globally and upto 46% in the north-eastern and eastern hot spot blast locations of the country (Kihoro et al. 2013; Ngachan et al. 2011). Fungicides such as Carbendazim 50 WP, Chlorothalonil 75% WP, Ediphenphos 50% EC, Eprobenfos 48% EC, etc. worth Rs. ~222 crores were used during 2010-11 on rice crop to combat blast disease (Kumar et al. 2013). However, use of chemical pesticides have caused concerns regarding rejection of consignments by the importing nations due to presence of pesticide residues in the produce. Also, use of chemical pesticides is not considered as an eco-friendly approach and therefore, developing genetic resistance is most feasible. More than 100 blast resistance (R) genes and 350 QTLs governing blast resistance have been identified. Among them, 26 blast resistance genes *viz., Pib*, *Pita*, *Pi54*, *Pid2*, *Pi9*, *Pi2*, *Pizt*, *Pi36*, *Pi37*, *Pikm*, *Pi5*, *Pit*, *Pid3*, *pi21*, *Pish*, *Pb1*, *Pik*, *Pikp*, *Pia*, *Pi25*, *Pid3A4, Pi35, NLS1, Pikh, Pi54of* and *Pi54rh* have been cloned and functionally validated (Sharma et al. 2012; Lv et al. 2013; Fukuoka et al. 2014).

The blast resistance gene *Pita* was introduced from the Vietnamese cultivar 'Tetep' into the background of rice cultivar Katy (Jia et al. 2004; Moldenhauer et al. 1990). It has been mapped adjacent to the centromere of chromosome 12 and found to encode a cytoplasmic protein with 928 amino acids possessing a nucleotide binding site (NBS) and leucine rich repeat (LRR) domain (Bryan et al. 2000; Chen et al. 2002; Jia et al. 2003). The resistant and susceptible alleles of the gene have been demarcated by a single amino acid difference at 918 residues with alanine present in the former and serine in the latter (Bryan et al. 2000).

Another dominant gene, *Pi9* conferring resistance to blast derived from the wild species *O. minuta* was introgressed into an *indica* rice variety 75-1-127 (Liu et al. 2002). *Pi9* was mapped to the *Piz* locus present on chromosome 6. This region constitutes six NBS-LRR domains namely, Nbs 1-*Pi9* to Nbs 6-*Pi9*, however, map based cloning and functional validation studies revealed that the Nbs2-*Pi9* is the functional blast resistance gene *Pi9* (Qu et al. 2006). Further, expression analysis revealed that it is a constitutively expressed gene. From the previous studies, it was concluded that dominant blast resistance genes *Pi9* and *Pita* were the most promising among the six blast resistance genes *Pi9*, *Pita*, *Pib*, *Pi5*, *Pi1* and *Pi54* (Khanna et al. 2015). Pyramiding major blast resistance genes has been a widely suggested strategy to widen the resistance spectrum of genotypes as well as to attain durable resistance. In view of the above, the current study was aimed at pyramiding two major blast resistance genes *Pi9* and *Pita* into the genetic background of Pusa Basmati 1 (PB1) through marker assisted selection.

Materials and methods

Pyramiding strategy

The Pusa Basmati 1 – near isogenic lines (NILs), Pusa 1637-18-7-6-20 (*Pi9*) and Pusa 1633-3-8-8-16-1 (*Pita*) possessing the recurrent parent genome (RPG) recovery of 95.6% and 98.6%, respectively were intercrossed to pyramid the blast resistance genes *Pi9+Pita*. The F_1s were subjected to test of hybridity using the markers AP5659-5/NBS2Pi9 and YL155/YL87 linked to the blast resistance genes *Pi9* and *Pita*, respectively. A F_1 plant confirmed for hybridity was selfed to generate F_2 population. A two-step foreground selection was adopted wherein in the first step, each of the plants in F_2 population was screened with marker AP5659-5 to identify plants homozygous for the gene *Pi9*. Then, these plants were subjected to foreground selection for *Pita* using a dominant

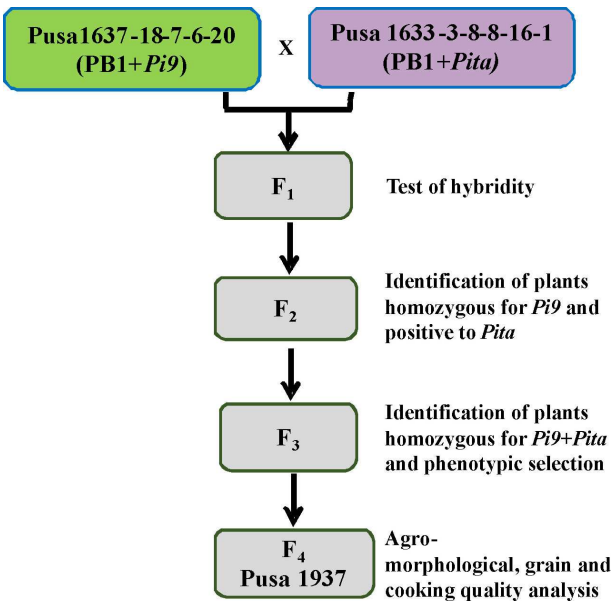

Fig. 1. Marker assisted selection scheme followed for pyramiding the blast resistance genes *Pi9* and *Pita* in the genetic background of PB1

marker YL155/YL87. All the plants homozygous for *Pi9* and positive for *Pita* were advanced to F_3 generation. Ten plants in each of the selected $F_{2:3}$ families were subjected to foreground selection using YL155/YL87 to identify families homozygous for *Pita.* The plants homozygous for *Pi9+Pita* within each family were subjected to phenotypic selection for recurrent parent phenotype. Top ten plants selected from different families were further evaluated for their agronomic performance, grain and cooking quality in randomized complete block design with three replications. A schematic representation of the procedure used for development of the material is shown in Fig. 1.

DNA extraction and PCR conditions

The leaf samples were collected from the field and DNA extraction was carried out following the procedure of Murray and Thompson (1980). The PCR reaction of 10µl volume was setup using 1µl of the template DNA, 1µl of 10× PCR buffer (10 mM Tris, pH 8.4, 50 mM KCl, 1.8 mM $MgCl_2$); 1µl of 5 pmol of each primer, 1 µl of 0.05 mM dNTPs (Bangalore Genei Pvt. Ltd., India) and 0.2 µl of 0.5 U of *Taq* DNA polymerase (Bangalore Genei Pvt. Ltd., India), the remaining volume was made up using nuclease free water. The PCR reaction was performed using the following program: initial denaturation for 5min at 94°C; total of 35 cycles for denaturation at 94°C for 30 sec; annealing at 55°C for 30 sec; extension at 72°C for 1 min; and final extension for 7 min at 72°C. The amplified product was resolved using 2% Metaphor™ agarose gel electrophoresis and visualized under ultraviolet transilluminator (Gel Doc™ XR+ Imager, Bio-Rad Laboratories Inc., U.S.A).

Foreground selection

The plants were confirmed for the presence of genes *Pi9* and *Pita* at each generation using gene linked markers AP5659-5/NBS2Pi9 (Fjellstrom et al. 2004 and Qu et al. 2006) and YL155/YL87 (Jia et al. 2002), respectively. The details of markers and their locations have been provided in Table 1.

Background selection

A total of 179 microsatellite markers polymorphic between the recurrent parent PB1 and the donor parents DHMASQ164-2a/IRBL9-W were used for the background analysis of the derived lines. The marker sequences were obtained from the Gramene marker database (http://www.grameme.org) and the primers were custom synthesized by Sigma Technologies Inc., USA.

Table 1. Details of the markers used for foreground selection of the blast resistance genes *Pi9* and *Pita*

Gene	Marker	Forward Primer	Reverse Primer	Chr.	Linkage distance	Reference
Pita	YL155/ YL87	5'AGCAGGTTATAAGCTAGGCC 3'	5' CTACCAACAAGTTCATCAAA 3'	12	Gene based	Jia et al., 2004
Pi9	AP5659-5	5'CTCCTTCAGCTGCTCCTC 3'	5'TGATGACTTCCAAACGGTAG 3'	6	0.05 cM	Fjellstrom et al., 2006
	NBS2-Pi9 195-1	5'TTGCTCCATCTCCTCTGTT 3'	5'ATGGTCCTTTATCTTTATTG 3'	6	Gene based	Qu et al., 2005

Evaluation for blast resistance under artificial epiphytotics

The gene pyramids along with their parental lines were evaluated for blast resistance using three *M. oryzae* isolates *viz.,* Mo-nwi-kash 1, Mo-nwi- lon2 and Mo-ei-ran1 collected from three different locations in India namely, Anantnag (Jammu and Kashmir); Lonavala (Maharashtra), and Ranchi (Jharkhand), respectively. The blast screening was performed following the protocol elaborated by Bonman et al. (1986). The sowing of the gene pyramided lines along with the parental lines and the susceptible checks PB1 and CO 39 was undertaken in plastic trays filled with fertile soil. The seedlings were grown till three leaf stage under greenhouse condition maintained at 27-30°C. Thereafter, the pots were shifted to the blast inoculation chamber maintained at 26-27°C with 90-92% relative humidity. The inoculum of each isolate was prepared with spore concentration of ~5×10^4 conidia per milliliter. Prior to inoculation 0.02% tween 20 was added to inoculum to facilitate the spore adhesion to the plant surface. The pots were maintained under dark conditions for 24 hours post inoculation which was followed by intermittent spraying of water using the atomizer for the next seven days. The scoring was performed as per the Bonman's scale of 0 to 5.

Evaluation for blast resistance under field conditions in Uniform Blast Nursery (UBN)

The resistance to blast in the gene pyramids was evaluated under natural blast epiphytotic conditions at two hot spot locations *viz.,* Hazaribagh-Jharkhand (Eastern India) and Malan- Himachal Pradesh (North-western India). On the raised beds of 50 cm length and 10 cm wide were prepared and the test entries were individually planted in each row along with the susceptible checks (Co39 and LTH) after every five rows as well as on the border rows of each bed. The blast disease was scored following the Standard Evaluation Scale of IRRI on 0-9 scale (SES 1996).

Evaluation for agro-morphological, grain and cooking quality parameters

The gene pyramids were evaluated for agro-morphological, grain and cooking quality traits in a randomized complete block design (RCBD) with three replications. Data on five plants in each of the replications was recorded for various traits including days to 50% flowering (DFF), plant height (PH), panicle length (PL), filled grains per panicle (FGP), spikelet fertility (%) (SF), thousand grain weight (TGW) and grain yield (GY). The grain and cooking quality parameters *viz*., kernel length before cooking (KLBC), kernel breadth before cooking (KBBC), length/breadth ratio (L/B), Kernel length after cooking (KLAC), Kernel breadth after cooking (KBAC), Kernel elongation ratio (KER) were recorded on ten grains form each entry using e-vision Annadarpan (CDAC, Kolkatta). Sensory evaluation of cooked grains was conducted and aroma was scored on 0-3 scale (Sood and Siddiq 1978). The alkali spreading value (ASV) of each of the entries was estimated using 1.7% KOH (Little et al. 1958).

Results

Pyramiding of blast resistance genes Pi9 and Pita

The monogenic-NILs of PB1, namely, Pusa 1637-18-7-6-20 and Pusa 1633-3-8-8-16-1 carrying *Pi9* and *Pita,* respectively were intercrossed to generate 2-gene pyramids. Eight F_1 plants were subjected for testing the hybridity using the gene linked markers AP5659-5 and YL155/YL87. One F_1 plant confirmed for hybridity was advanced to generate F_2 seeds. The F_2 population comprising of 450 plants were subjected to foreground selection using the marker AP5659-5 and a total of 109 plants were found to be homozygous for *Pi9*. These 109 plants were further subjected to foreground selection using the dominant marker YL155/YL87. Among which 78 plants were found to be positive (either homozygous or heterozygous) for *Pita.* Ten seeds from each of these families were germinated in propots and subjected to foreground analysis using

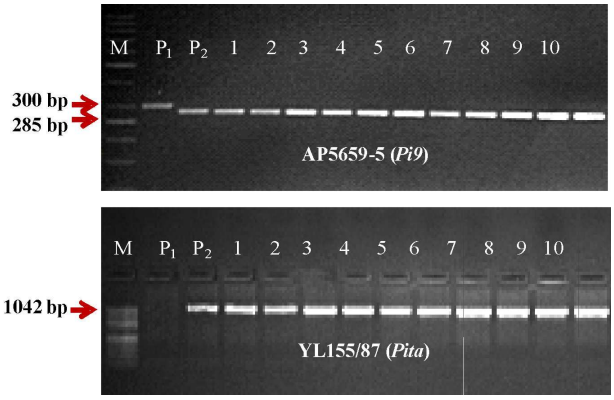

Fig. 2. Foreground selection of pyramids for blast resistance genes *Pi9* and *Pita*. Lane P_1 - Pusa Basmati 1, P_2 – IRBL9-W, P_3- DHMASQ-164a, 1 to 10 - Pusa 1937-8-1, Pusa 1937-33-2, Pusa 1937-51-3, Pusa 1937-74-4, Pusa 1937-82-5, Pusa 1937-91-6, Pusa 1937-138-7, Pusa 1937-148-8, Pusa 1937-160-9 and Pusa 1937-182-10

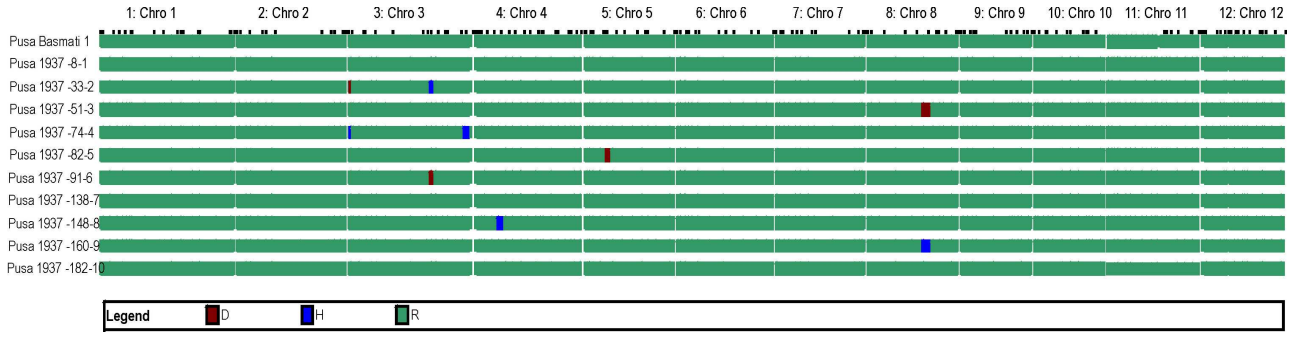

Fig. 3. Graphical representation of the gene pyramids *vis-a-vis* recurrent parent Pusa Basmati 1

the marker YL155/YL87, among which a total of 24 families were found to be homozygous for the gene *Pita*. Further, these 24 families homozygous for both the genes were subjected to phenotypic selection for agro-morphological, grain and cooking quality traits and ten best families were identified. A representative gel picture of foreground selection using the *Pi9* and *Pita* gene linked marker, AP5659-5 and YL155/YL87, respectively is presented in Fig 2. The background analysis revealed the RPG recovery ranging from 99.1 % in Pusa 1937-33-2 to complete recovery in Pusa 1937-8-1, Pusa 1937-138-7 and Pusa 1937-182-10 (Fig. 3).

Evaluation of pyramided lines for agronomic traits

Ten pyramids carrying blast resistance genes *Pi9*+*Pita* in the genetic background of PB1 were evaluated for agronomic performance along with the recurrent parent PB1 in a randomized complete block design (RCBD) with three replications. Most of the pyramided lines were found to be at par with the recurrent parent for all the agro-morphological traits except few variations (Table 2). The NIL Pusa 1937-8-1 was found to possess higher filled grains/panicle and yielded significantly higher than that of recurrent parent PB1. The NIL Pusa 1937-148-8 was dwarf with higher panicle number and significantly higher yield as compared to the recurrent parent PB1.

Evaluation of the two gene pyramids for cooking quality traits

The gene pyramids were also evaluated for grain and cooking quality traits. The NIL Pusa 1937-33-2 although possessed significantly smaller KLBC, due to higher KER, KLAC was at par with recurrent parent PB1. Pusa 1937-82-5 possessed significantly higher KLAC (14.78 mm), while the NIL Pusa 1937-160-9 possessed significantly low KLAC (13.97mm) as against PB1 (14.50mm). Further, all the pyramids were found to possess strong aroma like PB1 (Table 3).

Screening of the isogenic lines for blast resistance under artificial and natural epiphytotic conditions

The two gene pyramided lines were evaluated for their resistance to blast along with the respective NILs and the recurrent parent PB1 and susceptible checks CO39 and LTH. Three isolates namely, Mo-nwi-kash 1, Mo-nwi-lon2 and Mo-ei-ran1 were used to screen the gene pyramided lines under artificial inoculation conditions at IARI, New Delhi. The monogenic-NILs carrying the blast resistance gene, *Pi9* was found to be resistant

Table 2. Agronomic performance of the gene pyramided lines

Genotypes	DFF	PH	PL	PN	FG/P	SF%	TGW	SPY	Plot Yield
Pusa 1937-8-1	104.67 ± 0.58	107.37±3.42	33.00±1.63	18.67±1.25	145.67±10.27*	84.37±3.28	23.12 ± 0.30	23.60±0.83	59.64 ± 0.74*
Pusa 1937-33-2	104.67 ± 0.58	104.23±1.44	31.17±2.95	20.33±1.25	146.67±13.42*	82.02±3.22	23.06 ± 0.17	24.20±1.12	58.73 ± 0.70
Pusa 1937-51-3	104.00 ± 1.00*	106.47±3.39	30.43±1.94	24.33±1.25*	133.67±9.84	81.84±4.54	23.33 ± 0.22	23.20±2.16	58.42 ± 1.48
Pusa 1937-74-4	107.00 ± 1.00*	111.67±2.87	33.33±0.47	23.00±1.63	128.67±15.37	81.34±1.98	23.22 ± 0.23	20.73±0.54*	58.41 ± 0.53
Pusa 1937-82-5	105.67 ± 0.58	110.33±1.25	32.17±0.85	19.67±0.94	111.67±16.21	87.31±2.86	23.23 ± 0.12	23.23±0.54	59.49 ± 0.58
Pusa 1937-91-6	106.67 ± 0.58	97.33±4.03*	30.00±0.41	22.67±1.70	109.33±1.89	88.17±4.72	23.28 ± 0.17	26.20±0.65	58.77 ± 0.11
Pusa 1937-138-7	105.67 ± 0.58	110.67±4.92	31.00±0.82	21.33±0.47	117.67±5.73	85.39±4.77	23.07 ± 0.15	24.77±1.39	59.23 ± 0.35
Pusa 1937-148-8	104.67 ± 0.58	102.67±2.87*	32.33±1.7	24.00±0.82*	120.33±8.18	87.53±2.73	23.02 ± 0.24	23.47±0.53	59.79 ± 0.34*
Pusa 1937-160-9	106.67 ± 0.58	110.63±2.61	30.13±1.64	22.00±0.82	141.67±2.87	81.61±1.66	23.28 ± 0.17	23.43±0.68	58.13 ± 0.31
Pusa 1937-182-10	105.67 ± 0.58	111.97±2.81	29.93±1.59	23.67±2.05*	117.67±9.46	83.25±0.20	23.08 ± 0.26	24.37±0.60	59.14 ± 0.30
Pusa Basmati 1	105.67 ± 0.58	109.27±2.19	33.13±3.08	20.67±1.70	123.67±9.74	83.05±1.83	23.34 ± 0.34	24.53±0.97	58.34 ± 1.42
CD (0.05)	1.05	6.47	3.56	2.88	20.35	6.90	0.38	2.05	1.23

Chr = Chromosome

Table 3. Grain and cooking quality of the pyramided lines carrying blast resistance genes

Genotypes	KLBC	KBBC	L/B	KLAC	KER	Aroma
Pusa 1937-8-1	8.29 ± 0.19	1.56 ± 0.02	5.32 ± 0.19	14.31 ± 0.08	1.73 ± 0.03	2
Pusa 1937-33-2	8.09 ± 0.06*	1.55 ± 0.03	5.23 ± 0.1	14.5 ± 0.06	1.79 ± 0.01*	2
Pusa 1937-51-3	8.18 ± 0.14	1.54 ± 0.02	5.31 ± 0.16	14.07 ± 0.11*	1.72 ± 0.03	2
Pusa 1937-74-4	8.31 ± 0.04	1.53 ± 0.01	5.42 ± 0.04	14.55 ± 0.09	1.75 ± 0.01	2
Pusa 1937-82-5	8.28 ± 0.2	1.52 ± 0.02	5.44 ± 0.17	14.78 ± 0.16*	1.79 ± 0.06*	2
Pusa 1937-91-6	8.14 ± 0.07	1.48 ± 0.02	5.51 ± 0.10	14.38 ± 0.05	1.77 ± 0.01	2
Pusa 1937-138-7	8.31 ± 0.03	1.5 ± 0.03	5.53 ± 0.09	14.44 ± 0.08	1.74 ± 0.02	2
Pusa 1937-148-8	8.44 ± 0.10	1.53 ± 0.02	5.53 ± 0.07	14.35 ± 0.08	1.70 ± 0.01	2
Pusa 1937-160-9	8.21 ± 0.06	1.59 ± 0.06*	5.18 ± 0.19*	13.97 ± 0.17*	1.70 ± 0.03	2
Pusa 1937-182-10	8.29 ± 0.01	1.52 ± 0.03	5.45 ± 0.11	14.48 ± 0.1	1.75 ± 0.01	2
Pusa Basmati 1	8.34 ± 0.10	1.52 ± 0.03	5.49 ± 0.06	14.5 ± 0.05	1.74 ± 0.02	2
CD (0.05)	0.23	0.06	0.27	0.21	0.05	

against all the three isolates tested. However, the monogenic-NIL carrying *Pita* was found to be resistant against the *M. oryzae* isolate Mo-nwi-lon2, while susceptible against the isolates Mo-nwi-kash 1 and Mo-ei-ran1. Further, all the gene pyramided lines carrying *Pi9+Pita* were found to be resistant against all the three isolates (Fig. 4a). Additionally, the gene pyramided lines were screened under natural epiphytotic conditions at two hot spot locations UBN-Malan and UBN-Hazaribag. At UBN-Malan, the monogenic-NILs carrying the blast resistance gene *Pita* were moderately resistant, while, the monogenic-NIL carrying *Pi9* and the pyramids carrying *Pi9+Pita* were found to be resistant. However, at UBN-Hazaribagh, monogenic-NILs carrying *Pi9* and *Pita* as well as pyramids carrying *Pi9+Pita* were highly resistant against blast disease (Fig. 4b) (Table 4).

Discussion

The Basmati rice production is severely hampered due to several biotic and abiotic stresses. Among the biotic stresses, rice blast is one of the major constraints leading to impairment of grain quality and substantial yield losses. However, ample efforts have been made to overcome the pathogen by introgressing major blast resistance genes into the genetic backgrounds of Basmati rice varieties. Nevertheless, the resistance imparted by single gene remains at risk of being compromised owing to the dynamic, diverse and erratic races and forms of *M. oryzae*. Therefore to withstand the pathogen population irrepressibly, pyramiding of the dominant blast resistance genes would be the most appropriate strategy.

Among several blast resistance genes known, *Pi9* has been reported to be the most effective resistance gene followed by *Pita* across various locations of the Indian sub-continent (Khanna et al. 2015; Imam et al. 2013; Thakur et al. 2013).Therefore, in the current study, the gene pyramids harboring both the dominant blast resistance genes *Pi9+Pita* were developed in the genetic background of PB1 through marker assisted selection. Since, the two monogenic-

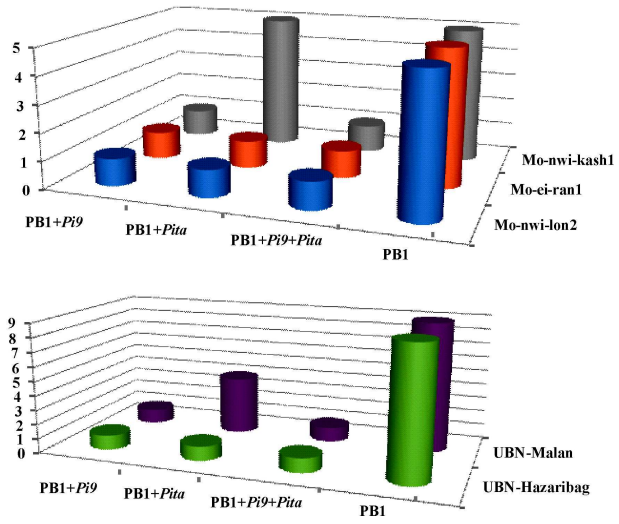

Fig. 4. Blast disease score of PB1-NILs under (a) artificial inoculation conditions; (b) natural epiphytotic conditions

Table 4. Reaction to blast under artificial and natural conditions

S.No.	Entry	Genes	Artificial inoculation conditions (AIC) (0-5 Scale)			Natural inoculation conditions (NIC) (0-9 Scale)	
			Mo-nwi-kash 1	Mo-nwi-lon2	Mo-ei-ran1	UBN-Malan	UBN-Hazaribagh
1	Pusa 1637-18-7-6-20	*Pi9*	1	1	1	1	1
2	Pusa 1633-3-8-8-16-1	*Pita*	5	1	5	4	2
3	Pusa 1937-8-1	*Pi9+Pita*	1	1	1	1	1
4	Pusa 1937-33-2	*Pi9+Pita*	1	1	1	1	1
5	Pusa 1937-51-3	*Pi9+Pita*	1	1	1	1	1
6	Pusa 1937-74-4	*Pi9+Pita*	1	1	1	1	1
7	Pusa 1937-82-5	*Pi9+Pita*	1	1	1	2	1
8	Pusa 1937-91-6	*Pi9+Pita*	1	1	1	1	1
9	Pusa 1937-138-7	*Pi9+Pita*	1	1	1	1	1
10	Pusa 1937-148-8	*Pi9+Pita*	1	1	1	2	1
11	Pusa 1937-160-9	*Pi9+Pita*	1	1	1	2	1
12	Pusa 1937-182-10	*Pi9+Pita*	1	1	1	1	1
13	PB1	-	5	5	5	9	8

Under AIC: Score 0 to 2 = resistant, 3 = moderately resistant and 4 to 5 = susceptible; Under NIC: Score 0 to 3 = resistant, 4 to 5 = moderately resistant, 6 = moderately susceptible and 7 to 9 = susceptible

NILs carrying *Pi9* and *Pita* in the genetic background of PB1 were developed through marker assisted backcross breeding, pyramids carrying *Pi9+Pita* generated in the current study had minimum background variation. Also, during the course of pyramiding rigorous phenotypic selection for agro-morphological traits was carried out at each generation to ensure maximum recurrent parent phenome recovery. Alongside the agronomic traits, assessment of cooking quality traits augmented the recovery of pyramided lines with at par or superior performance over recurrent parent PB1. Efficiency of phenotypic selection in the marker assisted backcross breeding for incorporation of biotic stress resistance genes have been successfully demonstrated by various researchers (Joseph et al. 2004; Gopalakrishnan et al. 2008; Basavaraj et al. 2010; Ellur et al. 2015; Singh et al. 2011, 2012a, 2012b, 2013 and 2015).

In the earlier studies (Khanna et al. 2015, Imam et al. 2013 and Thakur et al. 2013) as well as in the current study, it has been unequivocally demonstrated that *Pi9* is the most effective blast resistance gene followed by *Pita*. However, large scale deployment of *Pi9* may reduce the effectiveness of genes. Therefore, in order to enhance the spectra and durability of resistance, pyramiding of genes conferring resistance to different isolates is widely advocated. The pyramided lines carrying both the blast resistance genes *Pi9* and *Pita* in the genetic background of PB1, showed broad spectrum resistance under artificial and natural conditions, which may be attributed to the effective exclusion of multiple lineages of *M. oryzae* prevalent in nature (Gnanamanickam et al. 2000). The pyramids generated in the current study would serve as impeccable donor lines in blast resistance breeding of Basmati rice and can also be released as improved varieties after required testing.

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