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Improvement of Basmati rice varieties for resistance to blast and bacterial blight diseases using marker assisted backcross breeding

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ABSTRACT

Marker assisted backcross breeding was employed to incorporate the blast resistance genes, *Pi2* and *Pi54* and bacterial blight (BB) resistance genes *xa13* and *Xa21* into the genetic background of Pusa Basmati 1121 (PB1121) and Pusa Basmati 6. Foreground selection for target gene(s) was followed by arduous phenotypic and background selection which fast-tracked the recovery of recurrent parent genome (RPG) to an extent of 95.8% in one of the near-isogenic lines (NILs) namely, Pusa 1728-23-33-31-56, which also showed high degree of resemblance to recurrent parent, PB6 in phenotype. The phenotypic selection prior to background selection provided an additional opportunity for identifying the novel recombinants *viz.*, Pusa 1884-9-12-14 and Pusa 1884-3-9-175, superior to parental lines in terms of early maturity, higher yield and improved quality parameters. There was no significant difference between the RPG recovery estimated based on SSR or SNP markers, however, the panel of SNPs markers was considered as the better choice for background selection as it provided better genome coverage and included SNPs in the genic regions. Multi-location evaluation of NILs depicted their stable and high mean performance in comparison to the respective recurrent parents. The *Pi2* + *Pi54* carrying NILs were effective in combating a pan-India panel of *Magnaporthe oryzae* isolates with high level of field resistance in northern, eastern and southern parts of India. Alongside, the PB1121-NILs and PB6-NILs carrying BB resistance genes *xa13+Xa21* were resistant against *Xanthomonas oryzae pv. oryzae* races of north-western, southern and eastern parts of the country. Three of NILs developed in this study, have been promoted to final stage of testing during the *Kharif* 2015 in the Indian National Basmati Trial.

1. Introduction

Two major diseases of rice namely, rice blast caused by the heterothallic ascomycete *Magnaporthe oryzae* and bacterial blight (BB) disease caused by the gram negative bacterial pathogen *Xanthomonas oryzae pv. oryzae* (*Xoo*), are pervasive globally and impede rice productivity to the tune of 50–90% [1–6]. Breeding for resistance through incorporating broad spectrum resistance (R) genes is the most advocated strategy to circumvent these devastating diseases. However, the emerging pathogenic variants and dynamic biotypes among the fungal and bacterial populations engender serious and incessant threat to the resistant varieties carrying individual resistance genes [7,8]. Therefore, there is a necessity to develop durable resistant cultivars through pyramiding broad spectrum resistance genes [9]. Pyramiding of resistance genes to overcome blast and BB diseases in rice has been successfully demonstrated [10–14].

The efforts so far have led to the identification of ~100 major R genes and over 350 QTLs conferring partial to complete resistance to rice blast, among which 26 R genes (*Pib, Pita, Pi54, Pid2, Pi9, Pi2, Pizt, Pi36, Pi37, Pikm, Pi5, Pit, Pid3, pi21, Pish, Pb1, Pik, Pikp, Pia, Pi25, Pid3A4, Pi35, NLS1, Pikh, Pi54rh* and *Pi54of*) have been cloned [15,16].

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The blast resistance gene *Pi2* (formerly *Piz5*) was identified in *indica* rice cultivar 5173 and was mapped close to the centromere of chromosome 6 which possess nine NBS-LRR homologs. Four major blast resistance genes (*Pi2, Pi9, Pizt,* and *Piz*) from different rice cultivars were mapped to the same locus [17,18]. Further, it was deduced that the NBS4-*Pi2* is the candidate *Pi2* gene [19] which confers broad spectrum blast resistance against various *M. oryzae* isolates prevalent in the hot-spot locations for blast disease in India [20–22]. Another, broad spectrum blast resistance gene *Pi54* was identified in rice cultivar, Tetep and was mapped to long arm of chromosome 11 [23]. The gene *Pi54* belongs to NBS-LRR class of resistance genes whose expression is induced upon pathogen infection [24].

Similarly, a total of 39 genes conferring resistance to various strains of *Xoo*, including 28 dominant and 11 recessive genes have been identified and registered [25]. Among these, eight BB resistance genes namely, *Xa1*, *Xa3/Xa26, xa5*, *Xa10*, *xa13*, *Xa21*, *Xa26* and *Xa27* have been reportedly cloned [26–32], while the other eight genes (*Xa2*, *Xa4*, *Xa7*, *Xa30*, *Xa33, Xa38 and Xa39*) have been fine mapped [33–38]. The broad spectrum BB resistance gene *Xa21* introgressed from the wild species *Oryza longistaminata* is known to strongly impart resistance against BB races prevalent in Asia and Africa [39–42]. It has been mapped onto the long arm of chromosome 11 and found to possess LRR, transmembrane, juxtamembrane and intracellular kinase domains [31]. The interaction of *XA21*–Ax21 is one of the three well characterized PRR (Pattern recognition receptors) PAMP (Pathogen associated molecular patterns) interactions in the plant system [43]. PAMPs are essential for survival or pathogenicity of microbes and their mutation may compromise microbial fitness [44–45]. Therefore, *Xa21* is considered a good candidate for development of resistance in rice cultivars [46]. Another broad spectrum recessive gene *xa13,* governing BB resistance was mapped on chromosome 8 [30] and cloned using map based cloning approach [47]. The mutations in the promoter region of this gene govern resistance during host-pathogen interactions [47] by checking expression of Nodulin 3 protein and thereby limiting the disease incidence [48].

Basmati rice is a reverent legacy of India, renowned for its exceptionally superior cooking quality, appetizing taste and aroma. The landmark Basmati rice variety Pusa Basmati 1121 (PB1121) developed by ICAR - Indian Agricultural Research Institute (ICAR - IARI), has exceptionally high cooked kernel elongation ([49]. Another Basmati rice variety Pusa Basmati 6 (PB6) is known for its excellent cooking qualities and very strong aroma. These varieties together occupy more than 60% of the total area under Basmati rice cultivation and contribute nearly 60% of the total forex earning i.e., Rs. 29,300 crores (∼US$4.8 billion) [50], through export of Basmati rice from India. However, these varieties are highly susceptible to blast and BB. Managing diseases using the chemicals often leads to presence of pesticide residues in grains and thus raises concerns of food safety. Developing genetic resistance without hampering the grain quality standards is the most viable solution to this problem. Therefore, marker assisted backcross breeding (MABB), which ensures precise transfer of gene(s) of interest from a donor to a recurrent parent with minimum linkage drag and accelerated recurrent parent genome (RPG) and recurrent parent phenome (RPP) recovery, is the strategy of choice to rectify the specific defect of an otherwise excellent variety.

Conventional breeding augmented with marker assisted selection (MAS) has demonstrated a discernible progression in developing crop varieties with improvement towards the desired traits [51]. Several successful examples can be displayed across crops of using molecular markers for incorporating genes governing resistance against multiple biotic and abiotic stresses [13,14,52,53]. The utility of MAS in pyramiding genes for resistance to blast and BB from different donors into a common recurrent parent has been successfully demonstrated in various studies for introgressing blast and BB resistance genes in developing resistant cultivars [20,21,54,55].

In this study, we report pyramiding of two dominant blast R-genes namely, *Pi2* and *Pi54* and two BB R-genes, including one recessive (*xa13)* and one dominant *(Xa21)* gene in two widely grown Basmati rice varieties PB1121 and PB6 through MABB.

2. Materials and methods

2.1. Plant materials

Two Basmati rice varieties PB1121 and PB6 were used as recurrent parents in the current study. PB1121 is characterized by incredible grain and cooking quality characters with extra-long slender grains, exceptional grain length after cooking (20–25 mm), high elongation ratio and strong aroma. PB6 is another semi-dwarf high quality Basmati rice variety with excellent cooking and eating qualities and very strong aroma. The genotypes namely, Pusa 1602 with *Pi2*, Pusa 1603 carrying *Pi54*; SPS97 and Pusa 1460 possessing *xa13 + Xa21,* were used as donor parents in the MABB program. Pusa 1602 and Pusa 1603 are near isogenic lines (NILs) of a Basmati rice restorer, PRR78; while, SPS97 is a recombinant inbred line generated from a cross PB1121/IRBB60 and Pusa 1460 is a BB resistant NIL of the popular Basmati rice variety, Pusa Basmati 1. Further, the RPs PB1121 and PB6 possess Pusa Basmati 1 as one of the parents in their pedigree. All the breeding materials were developed using shuttle breeding system comprising of two locations, ICAR - IARI, New Delhi and Rice Breeding and Genetics Research Centre (RBGRC), Aduthurai [56].

2.2. Marker assisted backcross breeding strategy

Six crosses were attempted, namely, (1) PB1121/Pusa1602; (2) PB1121/Pusa1603; (3) PB1121/SPS97; (4) PB6/Pusa1602, (5) PB6/Pusa1603 and (6) PB6/Pusa1460 and designated as Pusa 1716, Pusa 1717, Pusa 1718, Pusa 1726, Pusa 1727 and Pusa 1728, respectively. The F_1s were analyzed for hybridity using the markers AP5659-5, RM206, xa13prom and pTA248 linked to the resistance genes *Pi2, Pi54, xa13* and *Xa21,* respectively (Supplementary Table S1). The gene positive plants were backcrossed with respective recurrent parents. At each backcross generation foreground selection was carried out for selecting the target gene, phenotypic selection to recuperate the RPP and background selection for accelerating the RPG recovery. The best BC_3F_1 plants from each of the combinations were selfed to generate BC_3F_2 populations and plants homozygous for the target genes were isolated. Further, selected plants were advanced till BC_3F_6 generation and two best families from each of the combinations were identified.

Concurrently, two intercrosses between the best BC_3F_1 plants *viz.,* (1) PB1121/Pusa1602//PB1121*3 and PB1121/Pusa1603//PB1121*3; and (2) PB6/Pusa1602//PB6*3 and PB6/Pusa1603//PB6*3 were made to obtain the intercross-F_1 plants carrying both the genes in heterozygous condition and were designated as Pusa 1883 (PB1121 + *Pi2* + *Pi54*) and Pusa 1884 (PB6 + *Pi2* + *Pi54*), respectively. The plants homozygous for both the blast resistance genes were isolated in F_2 population and advanced through pedigree method of selection. Finally, two best intercross-F_4 families from each of the combinations were identified.

2.3. Molecular marker assay

2.3.1. DNA extraction and PCR conditions

The leaf samples were collected from the field in liquid nitrogen and DNA extraction was carried out with modifications to the protocol of Murray and Thompson [57]. Total DNA was quantified

and the concentration was standardized to 20 ng. PCR reaction was assembled using 20 ng of template DNA, 1× PCR buffer {10 mM Tris–HCl (pH 8.4); 50 mM KCl, Invitrogen, Life Technologies, Brazil}, 5 pmol of each primer, 0.05 mMdNTPs (Bangalore Genei Pvt. Ltd., India), 1.8 mM $MgCl_2$ and 1 U of Taq DNA polymerase (Invitrogen, Life Technologies, Brazil). The PCR was executed using Veriti thermal cycler (Applied Biosystems) following the program: initial denaturation for 5 min at 94 °C; total of 35 cycles for denaturation at 94 °C for 30 s; annealing at 55 °C for 30 s; extension at 72 °C for 1 min; and final extension for 7 min at 72 °C. The amplified product was resolved using 3.5–4% Metaphor™ Agarose gel electrophoresis and visualized on ultraviolet transilluminator (Gel Doc™ XR + Imager, Bio-Rad Laboratories Inc., U.S.A). The setup included a positive and negative control for each reaction.

2.3.2. Foreground selection

The plants were analyzed to confirm the presence of target genes using gene based/linked markers. Foreground selection for the blast resistance genes, *Pi2* and *Pi54* was conducted using the gene linked markers, AP5659-5 [58] and RM206 [23]; and the bacterial blight resistant genes *xa13* and *Xa21* were evaluated using the gene based markers xa13prom [59] andpTA248 [60], respectively (Supplementary Table S1).

2.3.3. Background selection

Parental polymorphism survey was conducted between the recurrent and donor parents using a panel of 500 microsatellite markers spanning uniformly across the rice genome. The primer sequences for SSR markers were obtained from the Gramene SSR marker resource (www.gramene.org) and were synthesized from Sigma Technologies Inc., USA. The background selection was conducted with polymorphic markers spanning evenly across the genome.

2.3.4. Background analysis using SNP markers

At the final stage of the MABB program, a background recovery analysis was performed on the selected pyramids and their respective recurrent parents using a set of 50,051 SNP markers. SNP genotyping was conducted using a 50 K Axiom® 2.0 assay in GeneTitan® instrument. This SNP array constituted of 22,216 SNPs derived from 4695 single copy rice genes common to rice and wheat, 25,995 SNPs from 14,965 single copy genes unique to rice, 1216 SNPs located in 194 cloned agronomically important rice genes, and 624 SNPs based in multi-copy rice genes. The SNP markers were distributed uniformly across rice genome [61]. The RPG recovery based on SSR and SNP markers was calculated using the formula, RPG (%) = (R + 1/2H) × 100/P, wherein, R: number of markers homozygous for recurrent parent allele, H: number of markers which remained heterozygous, P: total number of polymorphic markers used in the background selection.

2.4. Screening for blast resistance

2.4.1. Evaluation for disease resistance under artificial epiphytotics

The NILs and pyramids were screened for blast resistance under artificial conditions using a set of thirty six *M. oryzae* isolates. The protocol of Bonman et al. [62] was followed and the experiment was conducted in three replications. The plastic trays were filled with autoclaved soil and seeds were germinated in a greenhouse maintained at 27–30 °C till three leaf emergence. The recurrent parents, PB1121 and PB6 along with standard susceptible check Lijiangxin Tuan Heigu (LTH) was used as negative control and the donor parents, Pusa 1602 and Pusa 1603 were used as positive control. The test entries were then shifted to the blast inoculation chambers maintained at 26–27 °C with relative humidity of 90–92%. The inoculum comprising ~5×10^4 conidia per mL was mixed with 0.02% Tween 20 and used for inoculating the test entries. After inoculation, seedlings were kept under dark conditions for 24 h. During the process high moisture content was maintained for promoting the fungal growth. Blast disease score was recorded after seven days according to the standard Bonman's scale [62].

2.4.2. Evaluation for disease resistance under field conditions in Uniform Blast Nursery

The NILs and pyramids were screened for their reaction to blast consecutively for two years under Uniform Blast Nursery (UBN) at two hot spot locations *viz.*, Hazaribagh-Jharkhand (eastern India), and Malan-Himachal Pradesh (north western India). Additionally, the pyramided lines carrying the blast resistance genes *Pi2 + Pi54* were evaluated at two more locations in south India namely, Pattambi–Kerala and Gudalur–Tamil Nadu. The test genotypes were planted individually in a 50 cm row with 10 cm spacing on a uniform raised bed nursery. The susceptible checks were planted after every five entries and on the borders to maximize the disease incision. The disease score was recorded on the 0–9 scale of Standard Evaluation Scale (SES) of IRRI [63].

2.5. Screening for bacterial blight resistance

The pyramids along with the recurrent parents (PB1121 and PB6) and donor parents (SPS97 and Pusa 1460) were evaluated for BB resistance. The plants were inoculated with the bacterial suspension at a density of 10^9 cells/ mL at maximum tillering stage using 'IARI-Kaul' and 'IARI-Ludhiana' isolates of *Xoo,* multiplied from single spore culture and maintained at Division of Plant Pathology, ICAR - IARI, New Delhi. The pyramids were also evaluated for BB resistance using 13 location specific *Xoo* isolates. Five leaves in each plant were inoculated with *Xoo* isolates through the clip inoculation method [64]. The lesion length was measured 21 days after inoculation adopting the SES scale [63]. The leaf with lesion area 1–5% was scored 1, 6–12% was given the score 3, 13–25% was scored 5, 26–50% was given the score 7 and 51–100% was scored 9. The lines with the score of 1, 3, 5, 7 and 9 were considered highly resistant, resistant, moderately resistant, moderately susceptible and highly susceptible, respectively.

2.6. Evaluation for agro-morphological, grain and cooking quality parameters

A set of backcross derived lines along with the respective recurrent parents PB1121 and PB6 were evaluated for agronomic traits in randomized complete block design (RCBD) with three replications. The data were recorded on five plants from each of the entries for the characters namely, 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). Further, the lines were also analyzed for the grain dimension and quality parameters *viz.*, kernel length before cooking (KLBC), kernel breadth before cooking (KBBC) and length/breadth ratio (L/B), kernel length after cooking (KLAC), kernel breadth after cooking (KBAC), aroma and alkali spreading value (ASV).

Ten milled grains from each entry were soaked using 10 mL of distilled water for 30 min in 50 mL test tubes, which were then kept in water bath having boiling water. After which they were cooked in boiling water for 8–10 min. The tubes were thereafter removed and each sample was poured in glass petri-dishes (50 × 17 mm). The dishes were covered with lids and allowed to cool for 15–20 min until they reached room temperature. The readings for aroma were recorded on 0–3 scale, 0: non scented, 1: mild scented, 2: scented, 3: strongly scented [65]. Further, the grain dimension parameters were recorded using ten milled/cooked grains for each entry using

Table 1
Number of plants produced and the estimated recovery of recurrent parent genome obtained in each of the backcross generations.

Cross	Gene(s)	No. of BGS markers	BC_1F_1			BC_2F_1			BC_3F_1		
			No.	No. of gene positive F_1s	RPG_{max} (%)	No.	No. of gene positive F_1s	RPG_{max} (%)	No.	No. of gene positive F_1s	RPG_{max} (%)
Pusa 1716	*Pi2*	60	85	39	77.5	76	36	84.16	82	40	90.83
Pusa 1717	*Pi54*	62	74	35	75.8	84	41	86.29	75	39	91.12
Pusa 1718	*xa13 + Xa21*	38	92	23	80.26	108	26	88.15	94	24	90.79
Pusa 1726	*Pi2*	58	68	36	79.31	74	35	89.65	89	48	91.37
Pusa 1727	*Pi54*	56	74	34	80.35	79	42	89.28	74	39	93.75
Pusa 1728	*xa13 + Xa21*	24	106	27	77.08	98	27	87.5	91	22	91.66
Pusa 1883	*Pi2 + Pi54*	–	–	–	–	–	–	–	–	–	–
Pusa 1884	*Pi2 + Pi54*	–	–	–	–	–	–	–	–	–	–
Mean		–	–	–	75.00	–	–	89.58	–	–	93.75

Pusa 1716—PB1121/Pusa1602; Pusa 1717—PB1121/Pusa1603; Pusa 1718—PB1121/Pusa1460; Pusa 1726—PB6/Pusa1602; Pusa 1727—PB6/Pusa1603; Pusa 1728—PB6/Pusa 1460; Pusa 1883—P1716/P1717; Pusa 1884—P1726/P1727; BGS—background selection; RPG_{max}—maximum recurrent parent genome recovery.

e-vision Annadarpan (CDAC, Kolkatta). To test the ASV, six whole milled grains were soaked in 10 mL of 1.7% KOH in petri-plates and were incubated at 30 °C for 24 h and scoring of individual grains was done using 1–7 scale [66].

2.7. Multi-location evaluation of pyramids

One best NIL representing each of the gene pyramiding combinations along with the recurrent parents PB1121 and PB6 and a check variety Pusa Basmati 1 were evaluated under National Basmati trial conducted by All India Coordinated Rice Improvement Project (AICRIP) at eight locations in Basmati growing region of the country *viz.,* New Delhi, Gurdaspur, Ludhiana, Rauni, Kaparthula, Kaul, Nagina and Modipuram during *Kharif* 2014. The design adopted was RCBD with three replications with recommended agronomic practices. The yield data was subjected to location wise RCBD analysis and further it was subjected to GGE-biplot based stability analysis using the software Genstat v.12 (VSN International Ltd, UK).

3. Results

3.1. Development of PB1121 and PB6 NILs for blast resistance genes

Details of progenies handled under each generation, RPG recovery and the number of selected plants among these crosses are given in Table 1. The donor parents, particularly for the blast resistance genes, were significantly inferior in cooking quality characteristics such as KLAC, kernel elongation ratio and aroma when compared to the recurrent parents. Therefore, the major challenge was to incorporate the target gene with minimum undesirable donor segments in the derived NILs. The gene positive plants were subjected to stringent phenotypic selection for agro-morphological, grain, cooking quality and aroma traits for hastening the RPP recovery. Foreground selections at BC_1F_1 generation resulted in 39 and 36 plants respectively for PB1121 and PB6 carrying *Pi2* gene, while for *Pi54*, 35 plants in the background of PB1121 and 34 plants in the background of PB6 were selected. The selections were subjected to phenotypic selection for morphological and grain dimension characters. Best 10 plants in each of the combinations were subjected to background selection using 60 and 62 polymorphic markers between PB1121/Pusa1602 and PB1121/Pusa1603; and 58 and 56 polymorphic markers between PB6/Pusa1602 and PB6/Pusa1603, respectively. Among the cross combinations, the maximum RPG recovery at BC_1F_1 stage ranged between 75.8–80.35%. The selected BC_1F_1 plants were backcrossed with the respective recurrent parents to generate BC_2F_1s that were further subjected to foreground, phenotypic and background selections. The maximum RPG recovery among BC_2F_1s ranged between 84.16–89.65%. The process was repeated to generate BC_3F_1 population from the selected BC_2F_1s, and the selection process at this stage realized the maximum RPG recovery to the tune of 90.79–93.75%. The best BC_3F_1 plant from each of the combinations was selected and selfed to generate BC_3F_2 populations. The plants homozygous for respective target genes were identified and forwarded till BC_3F_6 generation through pedigree based selection. Finally, two families in each of the combinations were selected i.e., Pusa 1716-12-4-29-12 and Pusa 1716-32-2-37-2 carrying the gene *Pi2;* and Pusa 1717-12-19-40-3 and Pusa 1717-20-3-38-7 carrying the gene *Pi54* in the genetic background of PB1121 with the RPG_{SSR} recovery of 93.3, 92.5, 94.4 and 93.5%, respectively; the NILs Pusa 1726-10-22-13-6 and Pusa 1726-37-56-24-8 carrying the gene *Pi2* and; the NILs Pusa 1727-23-16-27-4 and Pusa 1727-28-45-63-3 carrying the gene *Pi54* in the genetic background of PB6 with the RPG_{SSR} recovery of 94.0, 94.8, 95.5 and 94.6%, respectively.

Simultaneously, the best BC_3F_1s were intercrossed to generate the pyramids carrying the blast resistance genes *Pi2* and *Pi54.* Two gene positive intercross F_1s were selfed and plants homozygous for both the genes were isolated and advanced through pedigree method of selection. At intercross F_4 generation, two PB1121-NILs *viz.,* Pusa 1883-19-9-408 and Pusa 1883-28-16-360; and two PB6-NILs such as, Pusa 1884-26-15-28, Pusa 1884-36-9-16 carrying *Pi2 + Pi54* with the RPG_{SSR} recovery of 94.4, 93.8, 95.6 and 94.9%, respectively were isolated. Additionally, two PB6-NILs, Pusa 1884-9-12-14 and Pusa 1884-3-9-175 possessing an advantage of earliness, grain yield and higher kernel length after cooking over PB6 were also identified through phenotypic selection. These NILs possessed RPG recovery of 95.2 and 95.8%, respectively. A representative picture depicting the distribution of polymorphic markers across all the rice chromosomes, which were used for background selection is presented in Supplementary Fig. S1 and S2.

Concurrently, for the transfer of BB resistance genes *xa13* and *Xa21* into PB1121 and PB6, a set of 38 and 24 polymorphic markers between PB1121/SPS97 and PB6/P1460, respectively were used in background selection (Table 1). Two BC_3F_6 PB1121-NILs, Pusa 1718-14-2-150 and Pusa 1718-82-16-6 and two PB6-NILs *viz.,* Pusa 1728-23-33-31-56 and Pusa 1728-14-10-22-20 carrying BB resistance genes *xa13+Xa21* with RPG_{SSR} recovery of 92.1, 94.7, 95.8 and 93.8% were isolated.

3.2. Comparative background genome analysis of gene pyramided NILs

The PB1121 and PB6 NILs carrying blast or BB resistance genes were subjected to background analysis by computing the RPG recovery using a panel of 50,051 SNPs as well as with SSRs and the results are presented in Table 2. The RPG_{SNP} ranged from 84.10% in Pusa1718-82-16-6 to 96.80% in Pusa 1728-14-10-22-20. While,

Table 2
Comparative background analysis of gene pyramided NILs using SSR and SNP markers.

Genotypes	RPG	Genes	RPG_{SSR} (%)	RPG_{SNP} (%)	Difference#(%)
Pusa 1883-19-9-408	PB1121	*Pi2 + Pi54*	94.4	92.8	1.6
Pusa 1883-28-16-360	PB1121	*Pi2 + Pi54*	93.8	93.2	0.6
Pusa 1718-14-2-150	PB1121	*xa13 + Xa21*	92.1	90.5	1.6
Pusa 1718-82-16-6	PB1121	*xa13 + Xa21*	94.7	84.1	10.6*
Pusa 1884-36-9-16	PB6	*Pi2 + Pi54*	94.9	95.1	−0.2
Pusa 1884-26-15-28	PB6	*Pi2 + Pi54*	95.6	95.8	−0.2
Pusa 1728-23-33-31-56	PB6	*xa13 + Xa21*	95.8	95.6	0.2
Pusa 1728-14-10-22-20	PB6	*xa13 + Xa21*	93.8	96.8	−3.0
Mean			94.4	93.0	1.4

Two tailed critical $t = 2.31$; confidence limit (95%): upper: 4.73; lower: −1.93. *Significantly different estimates ($p > 0.05$). Pusa 1718–PB112/Pusa1460; Pusa 1728R—PB6/Pusa1460; Pusa 1883—P1716/P1717; Pusa 1884—P1726/P1727; RPG—Recurrent parent genome; RPG_{SSR}—recurrent parent genome recovery using SSR markers; RPG_{SNP}—recurrent parent genome recovery using SSR markers; #difference—RPG_{SSR}–RPG_{SNP}

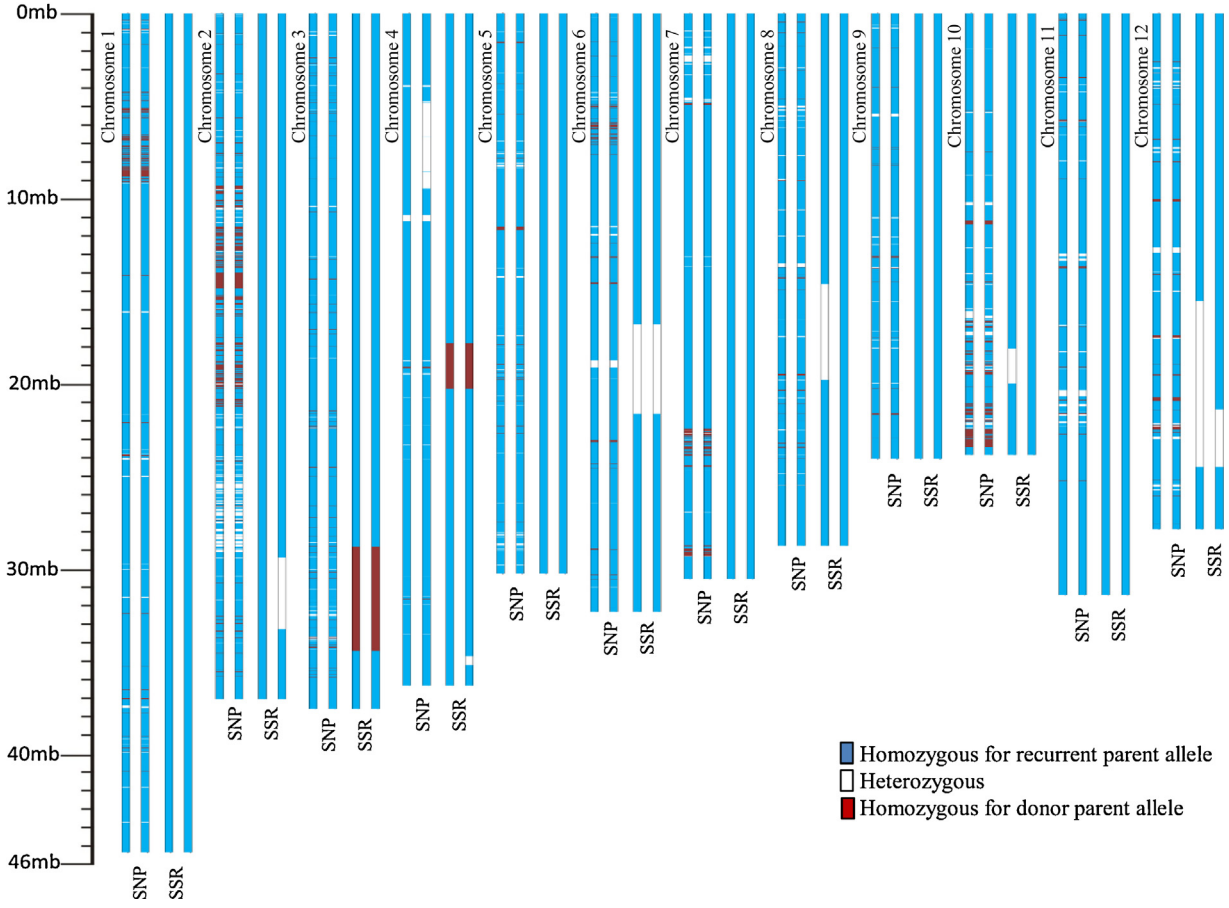

Fig. 1. Graphical representation of RPG recovery using SNP and SSR markers in PB1121-NILs carrying Pi2 + Pi54. In each of the chromosomes first bar represents the NIL Pusa 1883-28-16-360 and second bar represents the NIL Pusa 1883-19-9-408.

the RPG_{SSR} ranged from 92.10% in Pusa 1718-14-2-150 to 95.80% in Pusa 1728-23-33-31-56. Except for one NIL, Pusa 1728-14-10-22-20, there was a general high RPG values in case of SSR than SNP based assay. The range of over or under estimation of RPG recovery by SSR markers was however, statistically non-significant except for Pusa1718-82-16-6 at 95% confidence interval. The graphical representation for comparison of RPG recovery estimated using SNP and SSR markers in *Pi2 + Pi54* carrying NILs (Fig. 1).

3.3. Evaluation of PB1121-NILs for agro-morphological, grain and cooking quality traits

The data on evaluation for yield and yield related traits of two best monogenic NILs and pyramided lines carrying the blast or BB resistance genes in the genetic background of PB1121 are presented in Table 3. Overall performance of NILs was commensurate to the recurrent parent, PB1121 except few differences. There was no significant variation observed for GY and DFF. However, Pusa 1718-82-16-6 had shown slight reduction in PH as compared to PB1121 along with increased PL. Other NILs that showed desirable variation than PB1121 were Pusa 1883-28-16-360 for PN and PL, Pusa 1717-12-19-40-3 for FGP, SF and TGW, Pusa 1883-19-9-408 for FGP and SF. Pusa 1716-12-4-29-12 and Pusa 1716-32-2-37-2 were superior to PB1121 for PL and FGP, respectively.

The selected NILs, in general, showed comparatively similar grain and cooking quality traits as that of PB1121 (Table 4). There was no significant variation between PB1121 and Pusa 1716-12-4-29-12, Pusa 1883-28-16-360 and Pusa 1718-82-16-6. While ASV,

Table 3
Agro-morphological evaluation of PB1121-NILs.

NILs	Genes	DFF (days)	PH (cm)	PN	PL (cm)	FGP	SF (%)	TGW(g)	YLD (kg/ha)
Pusa 1716-12-4-29-12	*Pi2*	102	117.40	12.40	28.90*	105.06	86.45	28.69	5921.6
Pusa 1716-32-2-37-2	*Pi2*	103	117.60	12.90	28.30	106.40*	86.80	28.87	5924.5
Pusa 1717-12-19-40-3	*Pi54*	103	119.20	14.30	27.30	108.70*	88.12*	28.24*	5821.2
Pusa 1717-20-3-38-7	*Pi54*	104	117.70	14.20	28.30	97.90*	85.03	29.82	5918.4
Pusa 1883-19-9-408	*Pi2 + Pi54*	104	121.10	14.60	27.90	110.80*	89.14*	30.78	5821.4
Pusa 1883-28-16-360	*Pi2 + Pi54*	104	116.70	15.03*	29.08*	103.20	86.38	30.86	5790.2
Pusa 1718-14-2-150	*xa13 + Xa21*	103	118.80	14.30	28.20	97.30*	84.30	28.56	5793.7
Pusa 1718-82-16-6	*xa13 + Xa21*	103	110.70*	14.10	28.90*	102.40	85.56	30.37	5977.9
Pusa Basmati 1121	–	103	118.90	12.80	27.20	102.90	85.61	29.95	5872.3
CD(0.05)		2.00	2.74	2.21	1.67	2.27	2.31	1.40	177.4

DFF—days to 50% flowering; PH—plant height in cm; PN—number of panicles; PL—length of panicle in cm; FGP—number of filled grains per panicle; SF—spikelet fertility percentage; TGW—weight of 1000 grains in g; YLD—yield in kg ha^{-1}; * denotes genotypes that has significantly different ($p > 0.05$) trait values as compared to the recurrent parent, PB1121.

Table 4
Grain, cooking quality and recurrent parent genome recovery of PB1121-NILs.

NILs	Genes	KLBC (mm)	KBBC (mm)	L/B	KLAC (mm)	KBAC (mm)	KER	ASV	RPG_{SSR} (%)
Pusa 1716-12-4-29-12	*Pi2*	8.62	1.62	5.32	18.79	2.50	2.18	7	93.3
Pusa 1716-32-2-37-2	*Pi2*	8.53	1.54*	5.54*	19.11*	2.56	2.24*	7	92.5
Pusa 1717-12-19-40-3	*Pi54*	8.35*	1.59	5.25	18.78	2.67	2.25*	7	94.4
Pusa 1717-20-3-38-7	*Pi54*	8.71	1.61	5.41	18.33	2.78*	2.10	7	93.5
Pusa 1883-19-9-408	*Pi2 + Pi54*	8.55	1.55*	5.52*	18.78	1.89*	2.20	6	94.4
Pusa 1883-28-16-360	*Pi2 + Pi54*	8.70	1.60	5.44	18.56	2.56	2.13	7	93.8
Pusa 1718-14-2-150	*xa13 + Xa21*	8.61	1.60	5.38	18.44	1.67*	2.14	7	92.1
Pusa 1718-82-16-6	*xa13 + Xa21*	8.56	1.63	5.25	18.78	2.56	2.19	7	94.7
Pusa Basmati 1121	–	8.59	1.62	5.30	18.44	2.33	2.15	7	
CD (0.05)		0.15	0.05	0.16	0.39	0.36	0.06		

KLBC—kernel length before cooking in mm; KBBC—kernel breadth before cooking in mm; L/B—ratio of kernel length and breadth; KLAC—kernel length after cooking in mm; KBAC—kernel breadth after cooking in mm; KER—kernel elongation ratio; ASV—alkali spreading value; RPG_{SSR}—recurrent parent genome recovery estimated using SSR markers; * denotes genotypes that has significantly different ($p > 0.05$) trait values as compared to the recurrent parent, PB1121.

which is an indicator of amylose content, showed no variation between lines, few NILs showed little deviation such as Pusa 1716-32-2-37-2 recorded slightly lower KBBC, better L/B ratio, KLAC and good KER. Similarly, Pusa 1716-32-2-37-2 showed low KBBC, KBAC and better L/B ratio. The Pusa 1717-12-19-40-3 recorded low KLBC and better KER than PB 1121. Further, Pusa 1883-19-9-408 and Pusa 1718-14-2-150 exhibited lower KBAC values.

3.4. Evaluation of PB6-NILs for agro-morphological, grain and cooking quality parameters

Phenotypic evaluation of NILs derived in the genetic background of PB6 are presented in Tables 5 and 6. The PB6-NILs were found to be at par for most of the agro-morphological traits with PB6 except for few. There was no significant variation recorded for Pusa1726-37-56-24-8 and Pusa1728-14-10-22-20 with PB6 for any of the traits. Two NILs namely, Pusa1884-9-12-14 and Pusa1884-3-9-175, showed significantly higher yield than PB6 coupled with significant reduction in flowering time for about fourteen days. Further, Pusa1884-3-9-175 exhibited significantly higher FGP (137.45) and SF (86.97%) than PB6. Pusa1884-36-9-16 depicted significantly higher PL (30.60 cm) and higher FGP (131.60). Pusa1727-28-45-63-3 showed significantly lower PH (99.4 cm), while Pusa1726-10-22-13-6 and Pusa1884-26-15-28 significantly better SF than the recurrent parent. Pusa1884-36-9-16 recorded better PL (30.6 cm).

Evaluation of PB6-NILs for grain and cooking quality revealed that, the NILs Pusa1727-23-16-27-4 and Pusa1884-36-9-16 had no significant deviation from PB6 for any of the traits studied. Further, ASV value of all the NILs were at par with that of PB6. Two NILs, Pusa1884-9-12-14 and Pusa1884-3-9-175 showed significantly better grain and cooking qualities than PB6 in terms of higher KLBC, KBBC and KLAC values. Further, the NILs Pusa1726-37-56-24-8 and Pusa1728-14-10-22-20 possessed significantly higher KLBC and L/B ratio than that of recurrent parent, PB6. Pusa1727-28-45-63-3 and Pusa1728-23-33-31-56 and Pusa1728-14-10-22-20 recorded significantly better KLAC, KBAC and KER, respectively, over PB6.

3.5. Multilocation evaluation of pyramids carrying Blast/BB resistance genes

There was no significant yield difference between NILs and recurrent parents and the national check, Pusa Basmati 1 at New Delhi (Table 7). Pusa1718-14-2-150 carrying *xa13 + Xa21* in the background of PB1121 yielded significantly superior at four locations (Gurdaspur, Rauni, Nagina and Modipuram), while it performed at par to PB1121 at remaining four test locations. The NIL Pusa1883-19-9-408 performed at par at most of locations tested while it was found significantly superior at Modipuram and significantly inferior at Nagina. Pusa1728-23-33-31-56 was found to be at par with PB6 in almost all locations except for Gurdaspur where it recorded significantly higher yield. However, Pusa1884-9-12-14 was found to be significantly superior to PB6 at four locations namely, Gurdaspur, Kaul, Nagina and Modipuram. Further, at Gurdaspur, all the NILs performed significantly superior to Pusa Basmati 1, while at Kapurthala and Nagina, Pusa1884-9-12-14 and Pusa1728-23-33-31-56 showed superior yield over Pusa Basmati 1. Similarly, Pusa1883-19-9-408 and Pusa1718-14-2-150 performed better than Pusa Basmati 1 at one more location, Modipuram.

GGE-biplot analysis of the yield performance of the gene pyramided lines revealed existence of four mega environments: Gurdaspur, Rauni, Kaul and Modipuram forming the largest mega-environment, New Delhi and Kapurthala forming the second mega-environment, followed by Ludhiana as the third mega-environment and Nagina forming the fourth mega-environment.

Table 5
Evaluation of PB6-NILs for yield and yield related traits.

NILs	Gene	DFF (days)	PH (cm)	NT	PL (cm)	FGP	SF (%)	TGW (g)	YLD (kg/ha)
Pusa 1726-10-22-13-6	*Pi2*	112	102.50	12.70	30.10	129.40	86.60*	23.31	5610.6
Pusa 1726-37-56-24-8	*Pi2*	112	103.90	13.10	29.30	124.90	85.72	23.31	5743.5
Pusa 1727-23-16-27-4	*Pi54*	113	101.90	12.40	29.10	126.50	82.65*	23.06	5567.0
Pusa 1727-28-45-63-3	*Pi54*	112	99.40*	13.70	29.10	123.50	85.05	23.24	5696.5
Pusa 1884-26-15-28	*Pi2+Pi54*	112	102.40	14.60	29.40	126.70	86.61*	23.15	5658.2
Pusa 1884-36-9-16	*Pi2+Pi54*	113	101.80	14.40	30.60*	131.60*	84.38	23.01	5696.1
Pusa 1884-9-12-14	*Pi2+Pi54*	99*	103.55	14.35	29.40	129.00	86.15	25.54	6946.6*
Pusa 1884-3-9-175	*Pi2+Pi54*	99*	102.60	13.95	29.80	137.45*	86.97*	25.40	6837.7*
Pusa 1728-23-33-31-56	*xa13+Xa21*	113	102.60	13.20	29.00	132.20*	85.62	23.06	5612.8
Pusa 1728-14-10-22-20	*xa13+Xa21*	113	104.00	13.90	28.80	128.70	86.39	23.11	5592.6
Pusa Basmati 6 (PB6)	–	113	102.70	13.50	29.60	122.70	85.40	23.25	5537.7
CD (0.05)		2.00	1.62	1.51	0.87	6.72	1.20	0.36	230.5

DFF—days to 50% flowering; PH—plant height in cm; N—number of productive tillers; PL—length of panicle in cm; FGP—number of fully grown grains per panicle; SF—spikelet fertility percentage; TGW—weight of 1000 grains in g; YLD—yield in kg ha^{-1}; * denotes genotypes that has significantly different ($p > 0.05$) trait values as compared to the recurrent parent, PB6.

Table 6
Grain, cooking quality and recurrent parent genome recovery of PB6- NILs.

NILs	Genes	KLBC (mm)	KBBC (mm)	L/B	KLAC (mm)	KBAC (mm)	KER	ASV	RPG_{SSR} (%)
Pusa 1726-10-22-13-6	*Pi2*	7.64	1.32*	5.79*	15.94	2.12	2.09	7	94.0
Pusa 1726-37-56-24-8	*Pi2*	7.72*	1.36	5.68*	16.11	2.21	2.09	7	94.8
Pusa 1727-23-16-27-4	*Pi54*	7.61	1.36	5.60	15.99	2.41	2.10	7	95.5
Pusa 1727-28-45-63-3	*Pi54*	7.59	1.36	5.58	16.56*	2.35	2.18	7	94.6
Pusa 1884-26-15-28	*Pi2 + Pi54*	7.72*	1.38	5.59	16.34	2.22	2.12	7	95.6
Pusa 1884-36-9-16	*Pi2 + Pi54*	7.50	1.34	5.60	15.90	2.36	2.12	7	94.9
Pusa 1884-9-12-14	*Pi2 + Pi54*	7.72*	1.44*	5.36	17.38*	2.44	2.25	7	95.2
Pusa 1884-3-9-175	*Pi2 + Pi54*	7.94*	1.45*	5.48	17.33*	2.33	2.18	7	95.8
Pusa 1728-23-33-31-56	*xa13 + Xa21*	7.17	1.38	5.20	15.86	2.52*	2.21	7	95.8
Pusa 1728-14-10-22-20	*xa13 + Xa21*	7.84*	1.34	5.85*	16.09	2.44	2.05*	7	93.8
Pusa Basmati 6 (PB6)	–	7.33	1.38	5.31	16.11	2.30	2.20	7	
CD(0.05)		0.32	0.05	0.33	0.29	0.22	0.15		

KLBC—kernel length before cooking in mm; KBBC—kernel breadth before cooking in mm; L/B—ratio of kernel length and breadth; KLAC—kernel length after cooking in mm; KBAC—kernel breadth after cooking in mm; KER—kernel elongation ratio; ASV—alkali spreading value; RPG_{SSR}—recurrent parent genome recovery estimated using SSR markers; * denotes genotypes that has significantly different ($p > 0.05$) trait values as compared to the recurrent parent, PB6.

Table 7
Multi-location yield performance of pyramids carrying BB and Blast resistance genes in the genetic background of PB1121 and PB6.

Genotypes	Genes	Grain yield (Kg ha^{-1})							
		NDL	GDP	LDH	RUN	KPT	KUL	NGN	MDP
Pusa 1883-19-9-408	*Pi2 + Pi54*	5237	4539[c]	3939	4559	4508	5237	2885[a]	5048[a,c]
Pusa 1718-14-2-150	*xa13 + Xa21*	5387	6044[a,c]	3745	5218[a]	4361	5592	4274[a]	5312[a,c]
Pusa 1884-9-12-14	*Pi2 + Pi54*	4463	4671[b,c]	3922	4572	4547[c]	5865[b]	5235[b,c]	4645[b]
Pusa 1728-23-33-31-56	*xa13 + Xa21*	5173	3328[b,c]	3960	4834	5056[c]	5019	3579[c]	4062
Pusa Basmati 1121	–	5067	4546	4199	4562	4498	5374	3339	4209
Pusa Basmati 6	–	5010	2658	4131	4270	4700	4746	3579	4139
Pusa Basmati 1	–	4832	2901	3724	4939	3937	5074	4193	4520
CD (0.05)		614	291	848	624	772	716	375	267

NDL—New Delhi; GDP—Gurdaspur; LDH—Ludhiana; RUN—Rauni; KPT—Kaparthula; KUL—Kaul; NGN—Nagina; MDP—Modipuram

[a] Pusa Basmati 1121 NILs that showed better performance over Pusa Basmati 1121.

[b] Pusa Basmati 6 NILs that showed better performance over Pusa Basmati 6.

[c] Gene pyramided lines that showed better performance over Pusa Basmati 1.

The NIL Pusa1718-14-2-150 was the best performer in first mega environment (Fig. 2). A comparison plot based on "ideal genotype" revealed Pusa1718-14-2-150 as the best performer as well stable across locations as compared to its recurrent parent PB1121. Based on the position of PB6, it can be deduced that the PB6-NILs Pusa1728-23-33-31-56 and Pusa1884-9-12-14 were comparatively superior performing and stable across environments (Supplementary Fig. S3).

3.6. Screening of the NILs for blast resistance

Screening of blast gene introgressed NILs under artificial epiphytotics using a total of 35 *M. oryzae* isolates revealed that NILs and the donor parent carrying blast resistance gene *Pi2* were resistant against 32 isolates and susceptible for 3 isolates namely, *Mo*-ni-15, *Mo*-ni-67 and *Mo*-ei-bara1, depicting a resistance spectrum of 91.0% (Fig. 3; Supplementary Table S2). However, the NILs carrying *Pi54* were resistant only against ten isolates, but susceptible against 25 isolates, depicting a resistance spectrum of 29%. Interestingly, *Pi54* was found to impart resistance against all three isolates that compromised *Pi2* gene. Further, during two consecutive years of field screening for blast resistance, the monogenic-NILs carrying *Pi2* were found highly resistant at both the locations UBN-Malan and UBN-Hazaribagh, while, the NILs carrying *Pi54* were highly susceptible at UBN-Malan and moderately resistant at UBN-Hazaribagh.

The pyramided lines carrying *Pi2 + Pi54* in the background of PB1121 and PB6 exhibited resistance against all 35 isolates, showing a 100% resistance spectrum. Further, these pyramids were found to be highly resistant to blast disease at UBN-Malan, UBN-

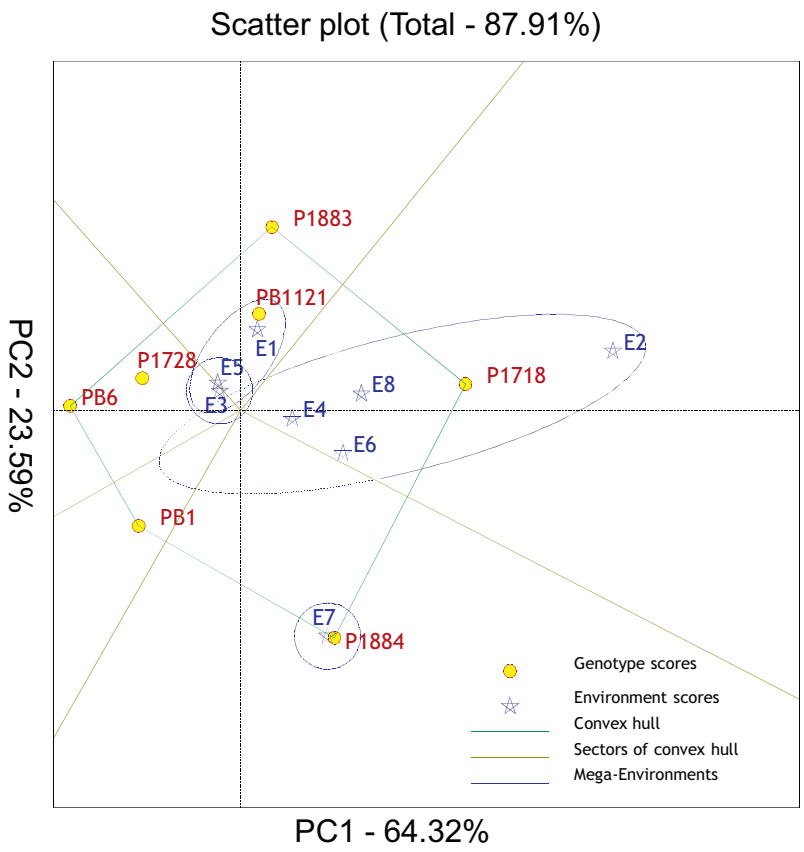

Fig. 2. Scatter diagram of GGE-biplot analysis depicting the mega environments and the performance of pyramids across 8 locations. E1-New Delhi, E2-Gurdaspur, E3-Ludhiana, E4-Rauni, E5-Kaparthula, E6-Kaul, E7-Nagina and E8-Modipuram; P1718—Pusa 1718-14-2-150 (P B1121 + *xa13* + *Xa21*); P1728—Pusa 1728-23-33-31-56 (PB6 + *xa13* + *Xa21*); P1883- Pusa 1883-19-9-408 (PB1121 + *Pi2* + *Pi54*); P1884- Pusa 1884-9-12-14 (PB6 + *Pi2* + *Pi54*).

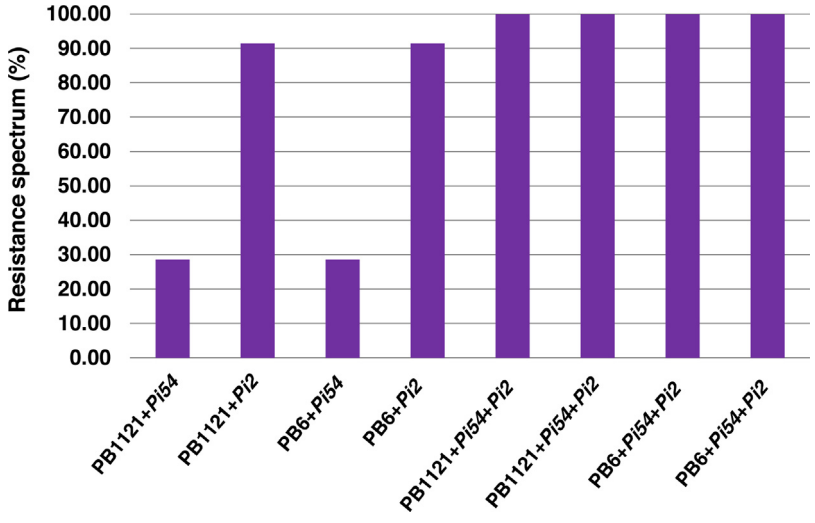

Fig. 3. Per cent of isolates to which NILs carrying blast resistance genes *Pi2* and *Pi54* or in combination in the genetic background of PB1121 and PB6, showed resistance reaction.

Hazaribagh and moderately resistant in two locations of Southern India *viz.,* Pattambi–Kerala and Gudalur–Tamil Nadu (Fig. 4).

3.7. Screening of the NILs for BB resistance

A total of 12 isolates were used for screening the PB1121 NILs carrying BB resistance genes *xa13* + *Xa21*, among which the NILs were completely resistant against eight isolates *viz.,* Kaul-IARI, Ludhiana-IARI, Kaul, Hyderabad, Aduthurai, Cuttack, Karjat and Raipur, while moderately resistant against four isolates namely, Pattambi, Faizabad, Chiplima and Nellore (Fig. 5a). Similarly, 9 isolates were used to screen the PB6 NILs possessing BB resistance. The NILs were highly resistant against seven isolates *viz.,* Kaul-IARI, Ludhiana-IARI, Kaul, Hyderabad, Cuttack, Karjat and Raipur, while depicted moderate resistance with a score of 5 against two isolates namely, Pattambi and Chiplima (Fig. 5b).

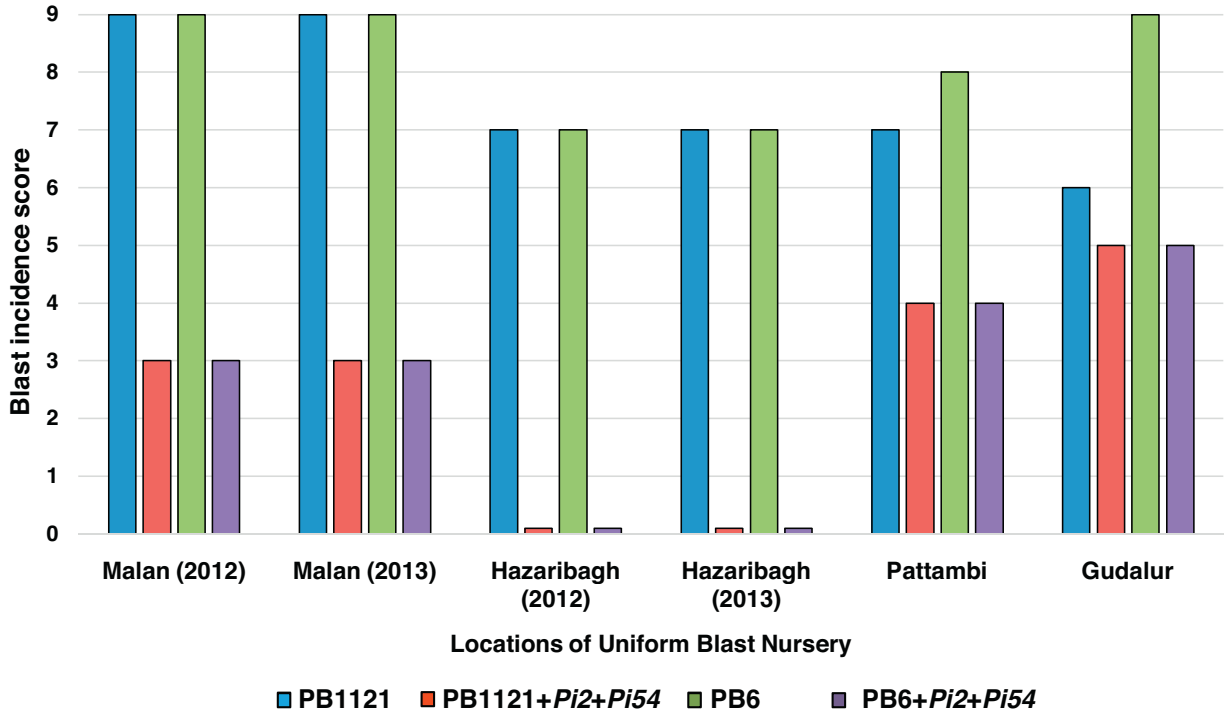

Fig. 4. Reaction of pyramids carrying blast resistance genes *Pi2* and *Pi54* in the genetic background of PB1121 and PB6 against the blast disease in various blast hotspot locations.

4. Discussion

Basmati rice is renowned worldwide for its unique grain quality, aroma and palatability. Its cultivation in Indian sub-continent is threatened by several diseases, the most severe ones being blast and BB. The present study, demonstrates successful incorporation of genetic resistance to these diseases using MABB in two most widely cultivated Basmati rice varieties, PB1121 and PB6 that account for 65–70% of the total Basmati rice export of the country.

The donor parents, Pusa 1602 and Pusa 1603 carrying blast resistance genes *Pi2* and *Pi54,* respectively, were NILs of an aromatic long grain Basmati quality restorer, PRR78, a male parent of an aromatic superfine grain aromatic rice hybrid PusaRH10 [67]. Both, Pusa 1602 and Pusa 1603 were relatively inferior as compared to recurrent parents in grain and cooking quality traits such as KLAC, KER and aroma. Therefore, recovering grain and cooking quality traits of recurrent parents was particularly challenging in these crosses. Hence, the major focus of the study was to incorporate the target gene(s) with minimum undesirable donor segments in the derived NILs. Therefore, marker-assisted foreground selection for target gene(s) followed by arduous phenotypic selection for agronomic performance, grain and cooking quality traits in addition to background selection in each backcross generation accelerated the recovery of agronomic, grain and cooking quality traits in NILs. However, the donor parent for BB resistance i.e., Pusa1460, a NIL of a widely grown Basmati rice variety Pusa Basmati 1, carrying genes *xa13* and *Xa21,* had excellent grain and cooking quality traits [55]. Therefore, recovering grain and cooking quality traits in BB resistant NILs of PB1121 and PB6 was relatively easy. It was apparent from the study that when donor parents had reasonably good grain and cooking quality and share common genomic regions with recurrent parent with respect to quality traits, the recovery of Basmati quality traits in the NILs was relatively easier, which has been a major challenge in our earlier studies where donor parents for blast and BB resistance came from diverse non-Basmati sources [20,21,22,54,55].

Background selection using microsatellite markers polymorphic between donor parents and recurrent parents providing genome-wide coverage was effective in hastening the RPG recovery. Although, the parental polymorphism survey was conducted using 500 markers with genome-wide distribution, but the per cent polymorphism between donor parents and recurrent parents was relatively low with similarity of >87% in case of blast gene donors (Pusa 1602 and Pusa 1603) and recipients (PB1121 and PB6); while it was >92% in case of BB resistance gene donors (SPS97 and Pusa 1460) and the recipient genomes. Pusa Basmati 1, the original background genome of Pusa 1460 was involved in the pedigree of both the recurrent parents, PB1121 and PB6 [56], therefore, high level of similarity was observed among the recurrent and donor parents. This proved that the strategy of selecting donors with Basmati background was vital in quick recovery of the RPG in these crosses.

Augmenting marker assisted foreground selection with phenotypic selection prior to background selection proved pivotal in isolating the genotypes with target trait and agronomic and grain and cooking quality traits on par with recurrent parents with limited backcrosses. This is discernible from the high RPG recovery estimates starting from BC_1F_1 generation (78.25%) and progressing rapidly to 87.75% in BC_2F_1 and to 92.02% in BC_3F_1, ultimately realizing a RPG recovery around 94.5% by the end of BC_3F_6 generation and intercross F_4 generation (Table 1). Phenotypic selection for the agronomic, grain and cooking quality parameters prior to the background selection also provided an added advantage of identifying the novel recombinants *viz.,* Pusa 1884-9-12-14 and Pusa 1884-3-9-175 possessed desirable attributes such as of 14 days earliness, significant increased yield and improved quality parameters such as higher KLBC and KLAC over the recurrent parent, PB6. These lines would be of great value to the farmers as they will be able to harvest the crop early with minimum inputs and realize higher profit. The effectiveness of phenotypic selection in MABB has been demonstrated earlier [20,21,22,54,55]. In addition it reduced the cost and time needed in background selection using SSR markers,

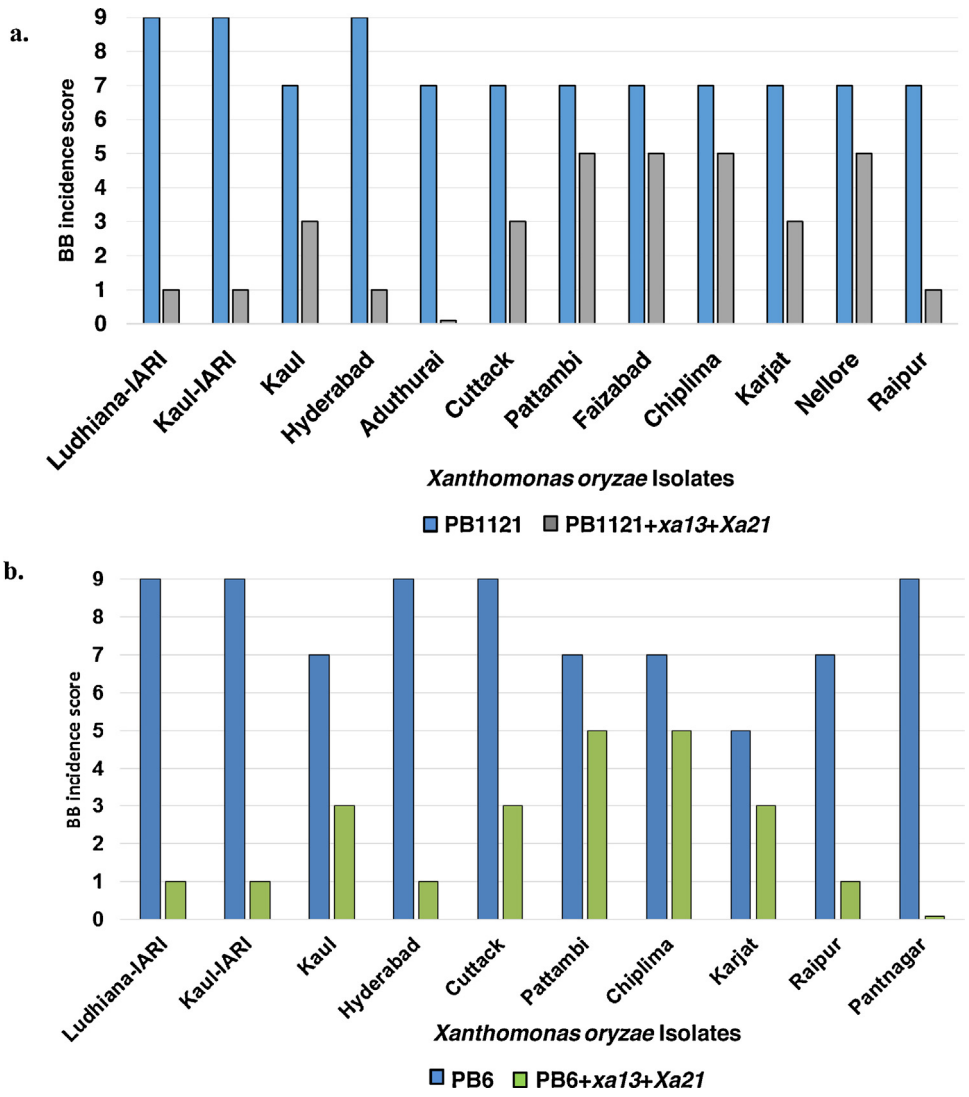

Fig. 5. Reaction of pyramids carrying BB resistance genes *xa13* and *Xa21* against various BB isolates.

as only top ten plants selected based on phenotype were subjected to background selection.

The comparative RPG recovery analysis using SSRs and SNPs in general, revealed no significant difference in estimated RPG recovery in NILs compared to respective recurrent parents. However, in a specific case of a NIL Pusa 1718-82-16-6, the estimated difference between the RPG_{SSR} and RPG_{SNP} recovery (10.6%), fell beyond the upper confidence limit, indicating an overestimation of RPG recovery by SSR over SNP. This may be due to improper coverage of polymorphic SSRs as the per cent polymorphism observed between the parental lines PB1121 and SPS97 was comparatively low. Here, it is pertinent to mention that most of the random SSRs are located in the non-coding regions. Consequently, the recovery of RPG estimated based on SSR markers may not have functional significance in terms of phenotypic performance of NILs in relation to recurrent parents. While, the SNP markers used in this study were widely distributed throughout the genome including the functional rice genes and thus may be the ideal choice for precise estimation of functional RPG recovery and gaining better insights into the introgression of donor segments in the developed NILs [20].

In agronomic performance and key Basmati quality traits, the blast and BB resistant NILs of PB1121 and PB6 and the gene pyramided lines were in general similar to recurrent parents. However, some of the lines showed improved performance, which was further confirmed by the multi-location evaluation of selected pyramided NILs namely, Pusa 1718-14-2-150, Pusa 1883-19-9-408, Pusa 1728-23-33-31-56 and Pusa 1884-9-12-14, which showed either at par performance or in some cases superior to the respective recurrent parents. This indicated that there was no yield penalty due to pyramiding of resistance genes. Multi-location testing however did show a few cross over interactions that can be attributed to G x E interaction. However, this needs to be established by multi-season testing at same location. Stability analysis with GGE-biplot unequivocally demonstrated that the pyramid Pusa 1718-14-2-150 is superior and highly stable genotype as compared to its recurrent parent, PB1121. Further, all the developed NILs were similar to or superior to their respective background cultivar for mean performance and stability.

When the NILs for blast resistance, both monogenic carriers and the pyramided lines, were challenged by a pan-India panel of rice blast isolates under an artificial epiphytotic screen, the pyramided lines exhibited 100% incompatibility reaction, implying that the gene pyramided lines stand a better chance to survive under disease epidemics. It was interesting to note that there were isolates that were specifically virulent to either *Pi2* or *Pi54*. Therefore, the pyramided lines displayed broad spectrum resistance to all the isolates through effective complementation suggesting that deployment of pyramids would maximize the durability of resistance genes.

Further, incomplete spectrum of resistance by either of the blast resistant genes, indicated their ineffectiveness in long-term resistance and monogenic carrier genotypes would compromise their resistance leading to 'boom and bust' cycle. The field survival reaction of the gene pyramided NILs was proved very effective against blast disease at UBN at Hazaribagh and Malan, located respectively at eastern and north-western India. Among the genes pyramided, efficacy of gene *Pi2* in the eastern and northern parts of the country has already been reported [20,68,69]. Multiple lineages consisting of multiple pathotypes in each of the lineages have been reported [70]. The combination of *Pi2 + Pi54* was not only effective in northern and eastern part of the country, but also effective in southern parts of the country such as Pattambi, Kerala and Gudalur, Tamil Nadu. Similarly, the PB1121-NILs and PB6-NILs carrying bacterial blight resistance genes *xa13 + Xa21* were resistant under artificial epiphytotic conditions against the *Xoo* isolates prevalent in the north-western, southern and eastern parts of the country.

The gene pyramided NILs generated in the current study are the potential candidates for release as cultivars in the blast and BB affected Basmati growing regions of the country consisting of the states of Jammu & Kashmir, Himachal Pradesh, Punjab, Haryana, Delhi, Western UP and Uttarakhand, which has been proposed to be earmarked as Geographical Indication (GI) area for Basmati rice. Based on testing in the National Basmati Trials during Kharif 2014, three NILs namely, Pusa1718-14-2-150, Pusa1728-23-33-31-56 and Pusa1884-9-12-14 have been promoted to the final year of testing before the varietal release. Leaf blast, neck blast and BB diseases in Basmati rice accounts for nearly 40% of pesticide use. This can be substantially reduced by adopting varieties with genetic resistance and thereby reducing the cost of cultivation while improving environment and food safety.

Conflict of interest

No conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.plantsci.2015.08.020.

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