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ORIGINAL PAPER

Development and evaluation of near-isogenic lines for major blast resistance gene(s) in Basmati rice

Apurva Khanna[1] · Vinay Sharma[2] · Ranjith K. Ellur[1] · Asif B. Shikari[3] · S. Gopala Krishnan[1] · U. D. Singh[4] · G. Prakash[4] · T. R. Sharma[5] · Rajeev Rathour[6] · Mukund Variar[7] · S. K. Prashanthi[8] · M. Nagarajan[9] · K. K. Vinod[9] · Prolay K. Bhowmick[1] · N. K. Singh[5] · K. V. Prabhu[1] · B. D. Singh[10] · Ashok K. Singh[1]

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Abstract

Key message **A set of NILs carrying major blast resistance genes in a Basmati rice variety has been developed. Also, the efficacy of pyramids over monogenic NILs against rice blast pathogen *Magnaporthe oryzae* has been demonstrated.**

Abstract Productivity and quality of Basmati rice is severely affected by rice blast disease. Major genes and QTLs conferring resistance to blast have been reported only in non-Basmati rice germplasm. Here, we report incorporation of seven blast resistance genes from the donor lines DHMASQ164-2a (*Pi54, Pi1, Pita*), IRBLz5-CA (*Pi2*), IRBLb-B (*Pib*), IRBL5-M (*Pi5*) and IRBL9-W (*Pi9*) into the genetic background of an elite Basmati rice variety Pusa Basmati 1 (PB1). A total of 36 near-isogenic lines (NILs) comprising of 14 monogenic, 16 two-gene pyramids and six three-gene pyramids were developed through marker-assisted backcross breeding (MABB). Foreground, recombinant and background selection was used to identify the plants with target gene(s), minimize the linkage drag and increase the recurrent parent genome (RPG) recovery (93.5–98.6 %), respectively, in the NILs. Comparative analysis performed using 50,051 SNPs and 500 SSR markers revealed that the SNPs provided better insight into the RPG recovery. Most of the monogenic NILs showed comparable performance in yield and quality, concomitantly, Pusa1637-18-7-6-20 (*Pi9*), was significantly superior in yield and stable across four different environments as compared to recurrent parent (RP) PB1. Further, among the pyramids, Pusa1930-12-6 (*Pi2*+*Pi5*) showed significantly higher yield and Pusa1633-7-8-53-6-8 (*Pi54*+*Pi1*+*Pita*) was superior in cooking quality as compared to RP PB1. The NILs carrying gene *Pi9* were found to be the most effective against the concoction of virulent races predominant in the hotspot locations for blast disease. Conversely, when analyzed under artificial inoculation, three-gene pyramids expressed enhanced resistance as compared to the two-gene and monogenic NILs.

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✉ Ashok K. Singh
aks_gene@yahoo.com

[1] Division of Genetics, Indian Agricultural Research Institute, New Delhi 110012, India

[2] Department of Bioscience and Biotechnology, Banasthali University, Banasthali 304022, Rajasthan, India

[3] Division of Genetics and Plant Breeding, SKUAST, Srinagar 191121, Jammu and Kashmir, India

[4] Division of Plant Pathology, IARI, New Delhi 110012, India

[5] National Research Centre on Plant Biotechnology, New Delhi 110012, India

[6] ACHBB, CSKHPKV, Palampur 176062, Himachal Pradesh, India

[7] Central Rainfed Upland Rice Research Station- CRRI, Hazaribagh 825301, Jharkhand, India

[8] Agricultural Research Station, Mugad, UAS, Dharwad 580007, Karnataka, India

[9] Rice Breeding and Genetics Research Centre, IARI, Aduthurai 612101, Tamil Nadu, India

[10] School of Biotechnology, Banaras Hindu University, Varanasi 221005, Uttar Pradesh, India

Introduction

Rice (*Oryza sativa* L.) is a major staple food worldwide and more than half of the world population depends on it for its calorie requirements. More than 90 % of the world's rice is produced and consumed in Asia (Khush 2005). Globally, India ranks first with ~44 mha area under rice cultivation with an annual production of ~144 million tons of paddy rice (Ellur et al. 2013). India has huge varietal diversity and varied rice growing ecologies. The Indo-Gangetic region of north-western India is bestowed with the Basmati rice that has incredible grain and cooking qualities, characterized by high kernel length after cooking, high elongation ratio, pleasant aroma and excellent taste. Basmati rice is cultivated on ~2 million ha area with an annual production of ~7 million tons of milled rice. There is massive demand for Basmati rice in the international market and annually ~4 million tons of milled rice valued at US$ 4.6 billion is exported (APEDA 2011). Basmati rice has several production constraints including biotic stresses, among which rice blast caused by the ascomycete *Magnaporthe oryzae* is the most severe causing yield losses of up to 90 % and reducing the grain and cooking quality. Fungicides worth ~Rs. 222 crores were used during 2010–2011 on rice crop to control blast disease (Kumar et al. 2013). However, fungicide application is not a sustainable, viable and bio-safe option for managing the disease.

The most eco-friendly and sustainable approach to manage rice blast is to develop resistant cultivars. Previous studies have identified and mapped ~100 different blast resistance genes (R-genes) and more than 350 QTLs, of which 23 resistance genes, viz., *Pib*, *Pita*, *Pi54*, *Pid2*, *Pi9*, *Pi2*, *Pizt*, *Pi36*, *Pi37*, *Pikm*, *Pi5*, *Pit*, *Pid3*, *pi21*, *Pish*, *Pb1*, *Pik*, *Pikp*, *Pia*, *Rbr2*, *Pi25*, *Pid3A4* and *Pi35* have been cloned and functionally validated (Lv et al. 2013; Fukuoka et al. 2014). The identification of R-gene donors and linked molecular markers has enabled incorporation of blast resistance genes into the genetic backgrounds of elite *indica* rice variety CO39 and the parental lines of some rice hybrids using marker-assisted backcross breeding (MABB) (Yanoria et al. 2010; Hittalmani et al. 2000; Singh et al. 2012). Incorporation of major genes for resistance has been considered as the most conducive strategy for management of blast disease. However, the variable nature of the pathogen belies the single gene resistance rendering it susceptible during the course of commercial utilization. Therefore, there is an exigency for pyramiding the genes for attaining durable resistance (Hittalmani et al. 2000; Singh et al. 2012).

The major source for blast R-genes is the set of 24 International standard blast monogenic differentials developed in the background of a *japonica* type Chinese variety, Lijiangxintuanheigu (LTH) under the IRRI-Japan Collaborative Research Project (Tsunematsu et al. 2000). However, the use of non-Basmati rice genotypes as source of blast resistance genes for improving Basmati rice varieties leads to severe impairment of the quality characteristics of the derived lines. Therefore, the present study aimed at developing a set of NILs carrying major blast resistance gene(s) in the genetic background of Pusa Basmati 1 (PB1), the first semidwarf, high-yielding and photoperiod-insensitive, superior quality Basmati rice variety that was released during 1989, which laid the foundation for Basmati revolution in India.

Materials and methods

Plant materials and strategy used in MABB program

Five independent MABB schemes were initiated using PB1 as recurrent parent (RP) and five different donor parents (DPs) to develop a set of monogenic and pyramided NILs for blast resistance genes in the genetic background of PB1. The DP DHMASQ164-2a was a doubled haploid line derived from the cross HPU741/Tetep and harbored the genes *Pi1*, *Pi54* and *Pita*; while DPs IRBL9-W, IRBL5-M, IRBLb-B and IRBLz5-CA carrying *Pi9*, *Pi5*, *Pib* and *Pi2*, respectively, were obtained from the IRRI-standard set of 24 monogenic lines developed in the genetic background of LTH.

From each of the backcross schemes, a single F_1 plant with confirmed hybridity was backcrossed with PB1 to generate BC_1F_1s and the subsequent generations were handled as per the MABB scheme presented in Fig. 1. The scheme comprised of a four-step selection strategy in each backcross generation: (1) foreground selection for the target genes using gene-based/linked markers; (2) recombinant selection using markers flanking the respective target genes; (3) a two-phase background selection using 85–109 polymorphic markers, 40–50 of these markers (half set of the total polymorphic markers) were employed in BC_1F_1, and the remaining markers and the markers heterozygous in BC_1F_1 were used in BC_2F_1 to select 10 plants fixed for recurrent parent alleles at the maximum number of loci to enhance recurrent parent genome (RPG) recovery; and (4) stringent phenotypic selection for agro-morphological traits, grain, cooking quality and sensory evaluation for aroma to accelerate the recurrent parent phenome (RPP) recovery. Similarly, the BC_3F_1 plants with maximum RPG and RPP were identified from each of the MABB schemes and advanced to BC_3F_6 families homozygous for the target genes.

Simultaneously, the superior BC_3F_1 plant identified from the cross PB1/DHMASQ164-2a//PB1*3 was selfed to generate three two-gene pyramids, viz,

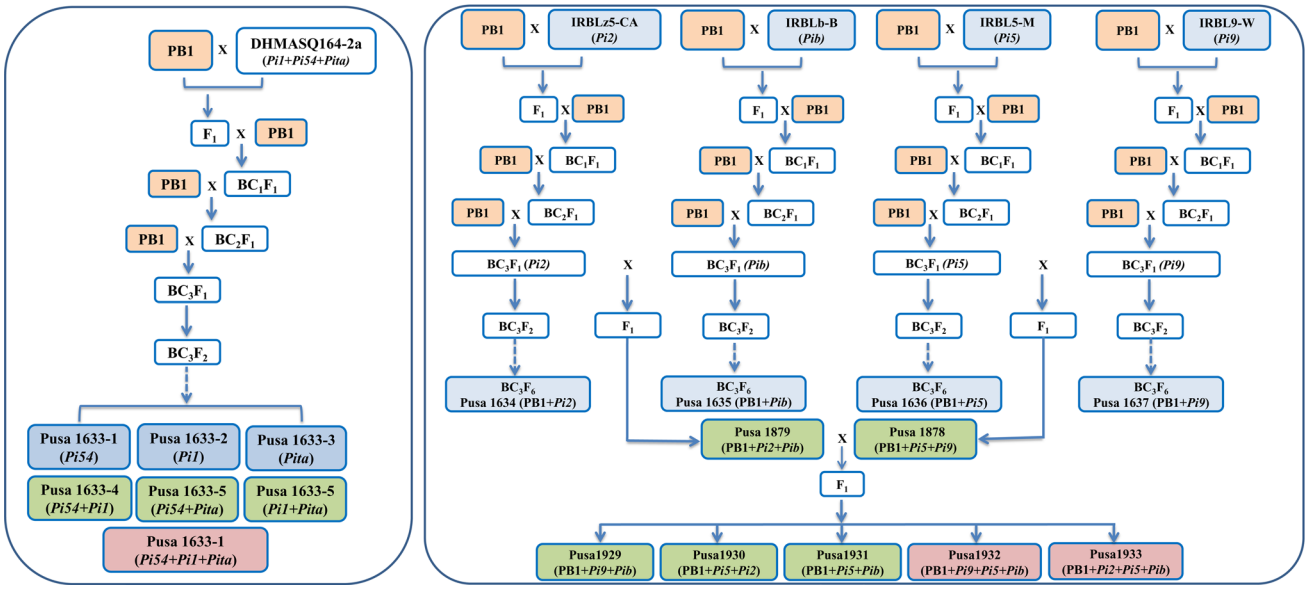

Fig. 1 Scheme of marker-assisted backcross breeding used in the current study

Pusa1633-4 (*Pi54+Pi1*), Pusa 1633-5 (*Pi54+Pita*), Pusa 1633-6 (*Pi1+Pita*) and one three-gene combination, viz, Pusa1633-7 (*Pi54+Pi1+Pita*). Concurrently, two additional crosses between the best BC_3F_1s, i.e., (1) PB1/IRBLz5-Ca//PB1*3 and PB1/IRBLb-B//PB1*3; and (2) PB1/IRBL5-M//PB1*3 and PB1/IRBL9-W//PB1*3 were attempted to generate intercross F_1s carrying blast resistance genes *Pi2+Pib* and *Pi5+Pi9*, respectively, in heterozygous condition. The plants homozygous for the genes *Pi2+Pib* (Pusa1879) and *Pi5+Pi9* (Pusa1878) were identified in respective F_2 populations and intercrossed. The F_1 thus obtained was selfed to produce an F_2 population wherein three additional two-gene pyramids, viz, *Pi9+Pib* (Pusa 1929), *Pi2+Pi5* (Pusa 1930) and *Pi5+Pib* (Pusa 1931); and two three-gene pyramids, viz. *Pi9+Pib+Pi5* (Pusa 1932) and *Pi2+Pi+Pi5* (Pusa 1933), carrying the target genes in homozygous condition were identified through marker-assisted foreground selection (Fig. 1).

Molecular marker analysis

DNA extraction and PCR

The genomic DNA was extracted with slight modification to the protocol of Murray and Thompson (1980). PCR of 10 µl volume was set up using 20–30 ng template DNA, 5 pmol of each primer, 0.05 mM dNTPs (MBI, Fermentas, Lithuania, USA), 10 × PCR buffer (10 mM Tris, pH 8.4, 50 mM KCl, 1.8 mM $MgCl_2$) and 0.5 U of *Taq* DNA polymerase (Bangalore Genei Pvt. Ltd., India). The PCR program was as follows: initial denaturation at 94 °C for 5 min; denaturation in subsequent 35 cycles at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 1 min; and final extension at 72 °C for 7 min. The amplification products were resolved using Metaphor™ agarose gel electrophoresis.

Foreground selection

Foreground selection for the genes *Pita*, *Pi9* and *Pib* was carried out using the gene-based markers YL155/YL87, NBS2*Pi9* and Pibdom, respectively. The selection for the genes *Pi1, Pi54, Pi5*, *Pib*, *Pi9* and *Pi2* was conducted using gene-linked markers RM224, RM206, C1454, RM208, AP5659-5 and AP4007, respectively, as mentioned in the Supplemental Table 1.

Background selection

The RP PB1 and the DPs were surveyed for polymorphic markers using 500 microsatellite (SSR) markers distributed evenly over the whole genome. The primer sequences for SSR markers were obtained from the Gramene SSR marker resource (http://www.grameme.org), and the primers were custom synthesized by Sigma Technologies Inc., USA. The background selection was carried out with a minimum of six evenly spaced polymorphic markers on each chromosome (Supplemental Table 2).

The genomic similarity between the RP PB1, and the NILs Pusa 1637-18-7-6-20, Pusa 1637-12-8-20-5, Pusa 1636-12-9-8-17 and Pusa 1636-12-9-12-4 was determined using the panel of 500 SSR markers (RMs, HVSSRs and

RGNMS) and a panel of 50,051 SNP markers. The objective of this study was to assess the efficiency of SSR and SNP markers in determining the extent of RPG recovery in the NILs. SNP genotyping was based on Axiom® 2.0 SNP assay using a rice 50 K SNP array in GeneTitan® Instrument. This array comprised 50,051 SNPs distributed uniformly across rice genome; 22,216 of these SNPs were derived from 4695 single copy rice genes common to rice and wheat; 25,995 SNPs were from 14,965 single copy genes unique to rice; 1216 SNPs were located in 194 cloned agronomically important rice genes; and 624 SNPs were based in multi-copy rice genes.

Screening for blast resistance

Evaluation for disease resistance under artificial epiphytotics

The NILs were evaluated for blast resistance under artificial inoculation at two locations, viz, IARI, New Delhi and CSKHPKV, Palampur using thirteen different *M.oryzae* isolates as per the protocol of Bonman et al. (1986). The seedlings of the susceptible checks PB1, LTH, CO39 and the five DPs (resistant checks) were grown in plastic trays filled with fertile soil in a greenhouse at 27–30 °C. The seedlings were inoculated at three-leaf stage by spraying 50 ml of spore suspension (~5×10^4 conidia ml^{-1}), and incubated in growth chambers for 24 h in dark at 26–27 °C. The seedlings were sprayed with water after every 6–7 h to maintain moisture for 4–5 days to facilitate the penetration by the fungus. The disease reaction was scored after 7 days of inoculation using the Bonman's scale: 0, seedlings completely free from infection; 1, seedlings with lesion length less than 0.5 mm; 2, seedlings with lesion size of 0.5–1 mm; 3, seedlings with lesions of 1–3 mm with a gray spot called blast eye in the center; 4, seedlings with spindle-shaped lesions of 3 mm; and 5, lines having lesions merging with each other leading to damage of more than half of the leaf. The lines with scores of 0, 1 and 2 were considered as resistant, those with score 3 were treated as moderately resistant, lines with score of 4 were regarded as moderately susceptible and those with score 5 were taken to be susceptible to the disease.

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

The NILs were also screened in UBN at two hot spot locations, viz, Hazaribagh, Jharkhand in eastern India, and Malan, Himachal Pradesh in north-western India. A 50-cm row each of the NIL along with the RP and the five DPs was planted in a raised nursery bed with a row spacing of 10 cm. To ensure uniform spread of disease, a row of susceptible check was planted after every five rows as well as on the borders. The disease evaluation was done on 0–9 Standard Evaluation Scale of IRRI (SES 1996). The NILs with 0–3 score were considered as resistant, those with the score of 4–5 were regarded as moderately resistant, lines having a score of 6–7 were treated as moderately susceptible and those with 8–9 score were considered to be susceptible.

Evaluation for agro-morphological, grain and cooking quality characteristics

The monogenic, digenic and trigenic NILs along with the RP PB1 were evaluated in a randomized complete block design (RCBD) and the data for agro-morphological traits, viz, days to 50 % flowering (DFF), plant height (PH), panicle length (PL), panicle number (PN), filled grains per panicle (FGP), spikelet fertility (%) (SF), thousand grain weight (TGW) and grain yield/plant (GY) were collected on five plants in each entry. To assay the grain characteristics, viz., kernel length before cooking (KLBC), kernel breadth before cooking (KBBC) and length/breadth ratio (L/B) and cooking quality characteristics, viz, kernel length after cooking (KLAC), kernel breadth after cooking (KBAC) and elongation ratio (ER) of ten grains from each entry were measured using e-vision Annadarpan (CDAC, Kolkatta). To study the cooking quality parameters, ten whole milled grains of each NIL and parental lines were soaked in 10 ml of distilled water for 30 min in test tubes and cooked in boiling water for 8–10 min. Thereafter, the tubes were removed from water bath and each sample was transferred to a Petri plate. The lids of the Petri plates were closed for 15–20 min till the temperature dropped down to room temperature.

Alkali spreading value (ASV) was tested by soaking six whole milled grains of each isogenic line and RP in 10 ml of 1.7 % KOH arranged at equal spacing in Petri plates. The Petri plates were kept at 30 °C for 24 h and, thereafter, the grains were individually scored on a scale of 1–7 (Little et al. 1958). For testing aroma, one gram of milled rice was soaked in 10 ml of 1.7 % KOH at room temperature in covered Petri plates for 10 min and sensory evaluation was done. The samples were scored on 0–3 scale, where 0 represents non-scented, 1 denotes mild scented, 2 stands for scented and 3 indicates strongly scented (Sood and Siddiq 1978). Amylose content of the NILs and parental lines was estimated using the protocol of Juliano (1971).

Multi-location evaluation of monogenic NILs

The NILs along with the RP PB1 were evaluated in a randomized complete block design (RCBD) with two replications

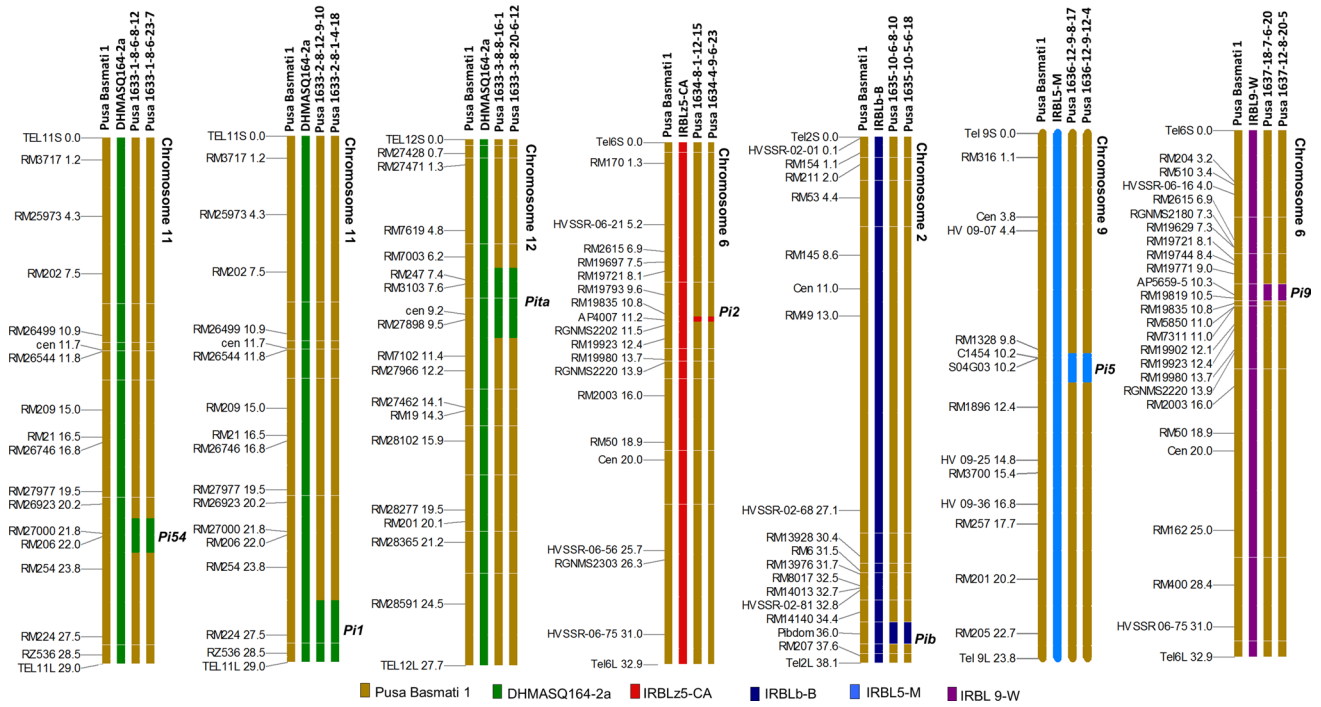

Fig. 2 GGT representing the RPG recovery on carrier chromosomes and the size of linkage drag

at four locations, viz, Karnal (Haryana); Nagina (Uttar Pradesh); Urlana (Haryana) and New Delhi. Yield data were subjected to location-wise RCBD analysis. The Bartlett's test was conducted to test the homogeneity of error variances using the software Cropstat 7.2 prior subjecting to combined analysis. Further, GGE biplot based stability analysis was conducted using the software Genstat v.12.

Results

Development of NILs carrying blast resistance genes in the genetic background of PB1

For the development of seven monogenic NILs, each carrying a single distinct blast resistance gene in the genetic background of PB1, the DPs DHMASQ164-2a (*Pi54*, *Pi1*, *Pita*), IRBLz5-CA (*Pi2*), IRBLb-B (*Pib*), IRBL5-M (*Pi5*), and IRBL9-W (*Pi9*) were used in MABB program (Fig. 1). Foreground selection was carried out using the markers RM206, RM224, YL155/YL87, AP4007, Pibdom/RM208, C1454 and Nbs2Pi9/AP5659-5 for the genes *Pi54*, *Pi1*, *Pita, Pi2*, *Pib, Pi5* and *Pi9*, respectively (Supplemental Table 1; Supplemental Fig. 1). The details of number of plants/lines developed in each generation along with RPG recovery are presented in Supplemental Table 2.

In the cross PB1/DHMASQ164-2a, the flanking markers RM27000 located 0.2 mb upstream and RM254 located 3.42 mb downstream of the gene *Pi54*; the marker RM254 positioned at 0.90 mb upstream of the gene *Pi1*; and the marker RM7003 placed at 6.2 mb upstream and RM7102 at 11.4 mb downstream of the gene *Pita* were used in recombinant selection (Fig. 2). The plants positive for the blast resistance genes *Pi1*, *Pi54* and *Pita* and also fixed for the RP alleles at the flanking markers were subjected to background selection. Out of 85 genome-wide polymorphic SSR markers identified for background selection, a set of 43 markers distributed uniformly across the genome were employed during BC_1F_1 generation, which revealed the maximum RPG recovery of 78.4 %. During BC_2F_1 generation, background selection with the remaining 43 markers and the markers that were heterozygous in the BC_1F_1 generation revealed the maximum RPG recovery of 85.6 %. Further, among the BC_3F_1 plants, ten plants with RPG recovery ranging from 90.6 to 93.1 % were identified and were subjected to stringent phenotypic selection for agro-morphological, grain and cooking quality characters. Finally, one best plant with RPG recovery of 92.7 % and maximum RPP recovery was identified and selfed to generate BC_3F_2 population. The plants homozygous for individual genes, viz, *Pi54*, *Pi1* and *Pita*; two-genes, viz, *Pi54+Pi1*, *Pi54+Pita* and *Pi1+Pita*; and three-genes combination, *Pi54+Pi1+Pita* were identified and advanced through pedigree method of selection. The monogenic NILs Pusa 1633-1-8-6-8-12 (RPG recovery of 98.6 %) and Pusa 1633-1-8-6-23-7 (RPG recovery of 95.7 %) carrying

blast resistance genes *Pi54*; Pusa 1633-2-8-12-9-10 (RPG recovery of 98.6 %) and Pusa 1633-2-8-1-4-18 (RPG recovery of 95.7 %) carrying *Pi1* gene; and Pusa 1633-3-8-8-16-1 (RPG recovery of 98.6 %) and Pusa 1633-3-8-20-6-12 (RPG recovery of 94.5 %) carrying blast resistance gene *Pita* were isolated. The two-gene pyramids namely, Pusa 1633-4-8-26-5-4 and Pusa 1633-4-8-48-12-7 carrying blast resistance genes *Pi54*+*Pi1*; Pusa 1633-5-8-26-5-4 and Pusa 1633-5-8-48-12-7 possessing *Pi54*+*Pita*; Pusa 1633-6-8-54-8-2 and Pusa 1633-6-8-87-3-2 harboring *Pi1*+*Pita*; and also three-gene pyramids namely, Pusa 1633-7-8-53-6-8 and Pusa 1633-7-8-67-4-12 carrying blast resistance genes *Pi54*+*Pi1*+*Pita,* showed RPG recovery ranging from 96.7 to 98.4 % based on SSR marker-based background selection (Table 5).

Similarly, for the four crosses generated between the RP PB1 and DPs IRBLz5-CA(*Pi2*), IRBLb-B (*Pib*), IRBL5-M (*Pi5*) and IRBL9-W (*Pi9*), foreground selection was carried out using the respective gene-linked molecular markers. The foreground positive BC_1F_1 plants were subjected to recombinant selection using the markers, viz, RM19793 located 0.8 mb upstream and RM19835 which is 0.4 mb downstream the gene *Pi2* on the chromosome 6; the markers RM14140 located 0.7 mb upstream and RM213 located 1.68 mb downstream the gene *Pib* on chromosome 2; the markers RM1328 and RM1896 flanking the gene *Pi5* in an interval of 2.59 mb on chromosome 9; the markers RM19771 and RM19819 flanking the gene *Pi9* on chromosome 6 with an interval of 1.5 mb, were used for recombinant selection to reduce the linkage drag (Fig. 2). The BC_1F_1 plants fixed for the RP alleles of the markers flanking the target genes were subjected to background selection. In BC_1F_1 generation, half of the polymorphic markers, viz, 34 polymorphic markers in the cross PB1/IRBLz5-CA; 33 markers in the cross PB1/IRBLb-B, 53 polymorphic markers in the cross PB1/IRBL5-M and 53 polymorphic markers in the cross PB1/IRBL9-W spanning uniformly across the entire genome were used for background selection. The remaining half of the polymorphic markers in each along with the markers that remained heterozygous during BC_1F_1 generation were used in the BC_2F_1 generation of each of the backcross programs (Supplemental Table 2). The BC_3F_1 plants with superior RPG recovery were subjected to stringent phenotypic selection for agro-morphological, grain, cooking quality and aroma traits for improved RPP recovery. Finally, the NILs Pusa 1634-8-1-12-15 (RPG recovery 94.5 %) and Pusa 1634-4-9-6-23 (RPG recovery 95.0 %) carrying the gene *Pi2*, Pusa 1635-10-6-8-10 (RPG recovery 94.0 %) and Pusa 1635-10-5-6-18 (RPG recovery 93.5 %) having the gene *Pib*, Pusa 1636-12-9-8-17 (RPG recovery 96.0 %) and Pusa 1636-12-9-12-4 (RPG recovery 95.4 %) with *Pi5*, and Pusa 1637-18-7-6-20 (RPG recovery 95.6 %) and Pusa 1637-12-8-20-5 (RPG recovery 95.5 %) possessing the gene *Pi9* were developed. The detailed information on number of plants generated in the backcross generations, and selected by foreground, recombinant and background selections are presented in Supplemental Table 2. Two- and three-gene pyramids involving genes *Pi2*, *Pib*, *Pi5* and *Pi9* in different combinations, viz, Pusa 1879-4-8-6 and Pusa 1879-4-14-6 carrying *Pi2*+*Pib*; and Pusa 1878-5-16-4 and Pusa 1878-8-12-7 harboring *Pi5*+*Pi9*; Pusa 1929-4-8 and Pusa 1929-6-3 having *Pi9* + *Pib*; Pusa 1930-6-4 and Pusa 1930-12-6 possessing *Pi2*+*Pi5*; Pusa 1931-6-7 and Pusa 1931-7-2 carrying *Pi5*+*Pib*; and three-gene pyramids, viz, Pusa 1932-7-6 and Pusa 1932-12-4 possessing *Pi9*+*Pi5*+*Pib* and Pusa 1933-4-6 and Pusa 1933-64-3 carrying blast resistance genes *Pi2*+*Pi5*+*Pib*, showed RPG recovery ranging from 93.0 to 96.7 % (Table 5).

Comparison between SSR and SNP-based similarity between the NILs and the RP

A comparison of genomic similarity between monogenic NILs and RP PB1 based on 500 SSR markers (including polymorphic as well as monomorphic markers) and 50,051 SNP markers (including both polymorphic as well as monomorphic markers) revealed that the percent similarity between the recurrent parent PB1 and the monogenic NILs, viz, Pusa 1636-12-9-8-17, Pusa 1636-12-9-12-4, Pusa 1637-18-7-6-20 and Pusa 1637-12-8-20-5 was 99.3, 98.9, 99.2, 98.7 %, respectively, based on SSR markers, while the percent similarity was 94.76, 97.95, 90.74, and 97.18 %, respectively, based on SNP assay (Supplemental Fig. 4).

Evaluation of monogenic NILs for agronomic performance

Two best BC_3F_6 isogenic lines carrying each of the seven blast resistance genes along with the RP PB1 were evaluated for yield and yield-related traits at IARI, New Delhi during *Kharif* 2013 (Table 1). The NILs were at par with RP for all the agro-morphological traits. However, Pusa 1636-12-9-8-17, Pusa 1637-12-8-20-5, Pusa 1635-10-5-6-18 and Pusa 1637-18-7-6-20 showed significant increase in panicle length, panicle number, spikelet fertility and thousand grain weight, respectively.

Multi-location evaluation of monogenic NILs for yield performance

All the NILs along with the RP PB1 were also evaluated at four locations, viz, Karnal (Haryana), Nagina (Uttar Pradesh); Urlana (Haryana); and IARI (New Delhi) during *Kharif* 2013. Location-wise RCBD analysis of data was performed and it

Table 1 Agronomic performance of the monogenic NILs of PB1 carrying different blast resistance gene

Genotypes	Genes	DFF (days)	PH (cm)	PL (cm)	PN	NFG	SF (%)	TGW (g)	RPG (%)
Pusa 1633-1-8-6-8-12	*Pi54*	104 ± 0.6*	130.37 ± 2.97	29.06 ± 0.31	13.33 ± 1.51	143.87 ± 3.20	87.11 ± 1.31	22.92 ± 0.58	98.6
Pusa 1633-1-8-6-23-7	*Pi54*	106 ± 0.6	126.60 ± 8.17	28.89 ± 3.25	13.13 ± 0.85	133.23 ± 0.65	81.12 ± 4.85*	23.29 ± 0.35	95.7
Pusa 1633-2-8-12-9-10	*Pi1*	101 ± 1.0*	131.27 ± 6.33	28.34 ± 0.42	12.53 ± 0.78	134.33 ± 2.99	86.47 ± 1.05	22.89 ± 0.55	98.6
Pusa 1633-2-8-1-4-18	*Pi1*	105 ± 1.0	128.83 ± 2.55	30.13 ± 0.76	10.33 ± 2.91	148.27 ± 2.69	88.37 ± 1.00	24.37 ± 0.28	95.7
Pusa 1633-3-8-8-16-1	*Pita*	103 ± 1.0*	136.13 ± 3.31	28.33 ± 0.42	11.27 ± 1.00	156.33 ± 5.35	86.77 ± 2.97	22.95 ± 0.23	98.6
Pusa 1633-3-8-20-6-12	*Pita*	106 ± 1.0	135.50 ± 5.50	30.30 ± 0.53	12.20 ± 1.01	151.20 ± 10.47	85.83 ± 2.83	22.7 ± 0.46	94.5
Pusa 1634-8-1-12-15	*Pi2*	102 ± 1.0*	131.20 ± 1.64	28.95 ± 0.31	12.73 ± 0.19	135.27 ± 3.76	84.27 ± 1.19	24.31 ± 0.18	94.5
Pusa 1634-4-9-6-23	*Pi2*	107 ± 1.0	130.67 ± 1.01	27.93 ± 1.45	14.33 ± 0.64	156.23 ± 2.80*	86.13 ± 0.83	22.12 ± 0.37	95.0
Pusa 1635-10-6-8-10	*Pib*	105 ± 0.6	131.47 ± 4.81	29.17 ± 0.91	12.37 ± 0.55	151.63 ± 8.96*	85.00 ± 3.30	20.42 ± 0.70*	94.0
Pusa 1635-10-5-6-18	*Pib*	102 ± 0.6*	132.00 ± 5.57	28.43 ± 2.44	11.93 ± 0.39	168.10 ± 8.61	91.48 ± 2.99*	22.33 ± 0.58	93.5
Pusa 1636-12-9-8-17	*Pi5*	102 ± 0.6*	139.27 ± 3.9	31.63 ± 1.29*	11.73 ± 1.11	151.03 ± 9.11	87.13 ± 0.35	22.9 ± 0.20	96.0
Pusa 1636-12-9-12-4	*Pi5*	107 ± 1.0	133.97 ± 1.26	28.85 ± 2.12	11.73 ± 0.22	138.43 ± 11.24	86.63 ± 1.47	24.19 ± 0.34	95.4
Pusa 1637-18-7-6-20	*Pi9*	107 ± 0.6	130.47 ± 0.83	29.18 ± 2.03	12.80 ± 1.25	136.37 ± 7.36	86.83 ± 0.51	25.62 ± 0.07*	95.6
Pusa 1637-12-8-20-5	*Pi9*	108 ± 0.6*	123.13 ± 7.8*	29.34 ± 1.70	14.73 ± 0.41*	136.23 ± 4.67	86.96 ± 0.8	22.93 ± 0.15	95.5
Pusa Basmati 1	–	106 ± 1.0	133.13 ± 2.89	29.06 ± 1.5	11.47 ± 1.25	140.53 ± 3.21	86.67 ± 1.56	23.37 ± 0.77	
CD (0.05)		1.59	7.55	1.98	2.33	11.21	3.72	1.66	

* $P < 0.05$

Table 2 Yield performance in RCBD at four locations

Sl. no.	Genotypes	Genes	Yield (kg/ha)				
			Karnal	Nagina	New Delhi	Urlana	Mean
1	Pusa 1633-1-8-6-8-12	*Pi54*	4066 ± 200.75	5117 ± 424.26	7440 ± 1018.23	7550 ± 608.11	6043 ± 1673.4
2	Pusa 1633-1-8-6-23-7	*Pi54*	3474 ± 118.09	5625 ± 294.63	6820 ± 84.85	6290 ± 721.25	5552 ± 1393
3	Pusa 1633-2-8-12-9-10	*Pi1*	4392 ± 897.46	4792 ± 294.63	6520 ± 282.84	6530 ± 466.69	5558 ± 1122.73
4	Pusa 1633-2-8-1-4-18	*Pi1*	3925 ± 118.09	4479 ± 1325.83	7280 ± 848.53	7050 ± 212.13	5683 ± 1709.55
5	Pusa 1633-3-8-8-16-1	*Pita*	4701 ± 791.18*	4688 ± 736.57	7160 ± 311.13	7520 ± 395.98	6017 ± 1490.52
6	Pusa 1633-3-8-20-6-12	*Pita*	4885 ± 350.43*	5000 ± 589.26	7180 ± 311.13	7200 ± 565.69	6066 ± 1246.86
7	Pusa 1634-8-1-12-15	*Pi2*	4142 ± 283.41	4896 ± 124.69	7400 ± 197.99	7850 ± 565.69	6072 ± 1247.6
8	Pusa 1634-4-9-6-23	*Pi2*	4509 ± 259.79	4583 ± 589.26	7140 ± 395.98	7300 ± 452.55	5883 ± 1546.57
9	Pusa 1635-10-6-8-10	*Pib*	3925 ± 507.77	5208 ± 589.26	7560 ± 141.42	7510 ± 282.84	6051 ± 1773.93
10	Pusa 1635-10-5-6-18	*Pib*	4225 ± 732.14	4458 ± 294.63	7440 ± 141.42	7450 ± 593.97	5893 ± 1457.73
11	Pusa 1636-12-9-8-17	*Pi5*	4693 ± 165.32	4255 ± 294.63*	6860 ± 1187.94	6600 ± 692.96	5602 ± 1743.61
12	Pusa 1636-12-9-12-4	*Pi5*	4609 ± 991.93	5417 ± 176.78	7760 ± 395.98	7800 ± 636.4	6396 ± 1727.6
13	Pusa 1637-18-7-6-20	*Pi9*	4534 ± 377.88	5833 ± 147.31	8580 ± 622.25	8000 ± 608.11	6737 ± 1731.67*
14	Pusa 1637-12-8-20-5	*Pi9*	4392 ± 755.76	5208 ± 589.26	7220 ± 254.56	6530 ± 565.69	5838 ± 1494.42
15	Pusa Basmati 1	–	3524 ± 118.09	5521 ± 441.94	7500 ± 84.85	7110 ± 183.85	5914 ± 1685.15
	CD (0.05)		1172	1159	1188	1012	565

* $P < 0.05$

Table 3 Combined analysis of variance for yield evaluated in a randomized complete block design across four locations

Source	DF	Mean square
Environment	3	7391.45**
Replication within environment	4	36.79
Genotypes	14	7391.45**
Environment × genotype	42	31.47
Pooled error	56	28.01
Total	119	

** $P < 0.01$

was observed that most of the NILs were at par with RP PB1 for yield/ha at all the four locations, except for NILs Pusa 1633-3-8-8-16-1 and Pusa 1633-3-8-20-6-12 carrying *Pita* yielded significantly higher than the RP, and the NIL, Pusa 1636-12-9-8-17(*Pi5*), which was significantly inferior to RP PB1 in yield at Nagina, Uttar Pradesh (Table 2).

Bartlett's test revealed the homogeneity of error variances. Therefore, multi-location data were subjected to combined analysis. The differences among genotypes and those among the environments were highly significant, but the G × E interaction was non-significant (Table 3). The monogenic NIL Pusa 1637-18-7-6-20 (*Pi9*) yielded significantly higher (6737 kg/ha) than the RP PB1 (5914 kg/ha), while the other NILs performed at par with the RP.

Further, GGE biplot analysis for the trait yield/ha revealed that there exists two mega-environments: Karnal formed one mega-environment and the remaining three locations, viz., Urlana, New Delhi and Nagina, constituted the other mega-environment. The NIL Pusa 1633-3-8-8-16-1 (G5) was the best performer in the first mega environment and the NIL Pusa 1637-18-7-6-20 (G13) was the best performer in the second mega-environment (Supplemental Fig. 2).

Further, these NILs were ranked based on the "ideal genotype" across environments, wherein the ideal genotype is the one that has both high mean yield and high stability. In the current study, it was deduced that the genotype Pusa 1637-18-7-6-20 (G13) is the best genotype and is closest to the ideal genotype. Considering the mean yield and stability of the RP PB1, the performance of most of the NILs was either superior or equal to PB1. Although, the NILs Pusa 1636-12-9-8-17 (G11), Pusa 1633-2-8-12-9-10 (G3) and Pusa 1633-1-8-6-23-7 (G2) performed at par with PB1, they were relatively less stable (Fig. 3).

Evaluation of the monogenic NILs for grain and cooking quality parameters

The five DPs were non-aromatic, have lower amylose content, inferior grain and cooking quality traits (Fig. 4) than the RP PB1 (Table 4). However, all the NILs possessed intermediate amylose content, alkali spreading value of 7, hard gel consistency (25.5–27.8 mm) and strong aroma like PB1. Some of the NILs namely, Pusa 1634-8-1-12-15 (8.74 mm), Pusa 1634-4-9-6-23 (8.75 mm) and Pusa

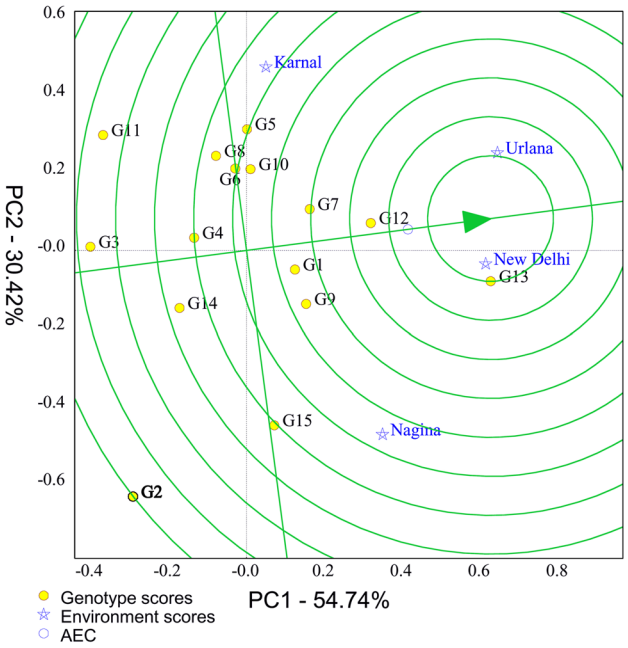

Fig. 3 Ranking of NILs and RP PB1 relative to an "ideal genotype" (represented by *blue round circle*). G1 -Pusa 1633-1-8-6-8-12, G2-Pusa 1633-1-8-6-23-7, G3-Pusa 1633-2-8-12-9-10, G4-Pusa 1633-2-8-1-4-18. G5-Pusa 1633-3-8-8-16-1, G6-Pusa 1633-3-8-20-6-12, G7-Pusa 1634-8-1-12-15, G8-Pusa 1634-4-9-6-23, G9-Pusa 1635-10-6-8-10, G10-Pusa 1635-10-5-6-18, G11-Pusa 1636-12-9-8-17, G12-Pusa 1636-12-9-12-4, G13-Pusa 1637-18-7-6-20, G14-Pusa 1637-12-8-20-5, G15- PB1 (color figure online)

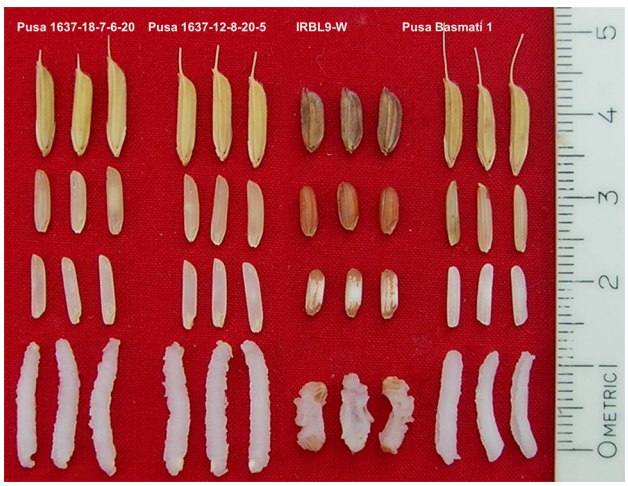

Fig. 4 Representative picture of grain and cooking quality characters of RP PB1, DP IRBL9-W and the monogenic NILs carrying blast resistance gene *Pi9*

1635-10-6-8-10 (8.68 mm) showed significantly superior KLBC as compared to PB1 (8.27 mm), while Pusa 1637-12-8-20-5 showed significantly superior kernel elongation ratio of 1.94 as against 1.79 in PB1 (Table 4). The representative picture of gel length of developed isogenic lines compared with the donor line DHMASQ164-2a is presented in the Supplemental Fig. 3, which illustrates the clear difference in gel consistency between the RP and the DP. The RP PB1 and the NILs had 28 mm of gel length, while the DPs DHMASQ164-2a, IRBLz5-CA, IRBLb-B, IRBL5-M and IRBL9-W had gel lengths of 55, 70, 67, 58 and 59 mm, respectively.

Assessment of agronomic, grain and cooking quality traits in pyramids

Data on yield and yield components of the pyramided lines are presented in (Table 5). The pyramids were found to be at par with the PB1 for all the agro-morphological traits with few exceptions such as Pusa 1879-4-14-6 possessed higher TGW, Pusa 1930-12-6 was significantly high yielding, Pusa 1633-7-8-53-6-8 possessed significantly higher KLAC and KER compared to RP PB1 (Tables 5, 6).

Evaluation of the NILs for blast resistance under artificial inoculation conditions

A total of 60 *M. oryzae* isolates were collected from different parts of the country and their virulence pattern was characterized using the 24 standard differentials developed at IRRI using the protocol described by Bonman et al. (1986). Based on the virulence pattern (Supplemental Table 3), thirteen diagnostic isolates for the seven blast resistance genes were identified to screen the NILs for blast resistance (Supplemental Table 4). All the fourteen monogenic NILs, sixteen two-gene pyramids and six three-gene pyramids along with their respective DPs, RP and susceptible check LTH were evaluated under artificial inoculation conditions for resistance to blast using respective diagnostic isolates.

The diagnostic isolates used for screening NILs carrying *Pi1*, *Pi54* and *Pita* included Mo-ni-53, Mo-ni-0052a, Mo-ni-0066a, Mo-nwi-53, Mo-nwi-lon2, Mo-ni-0067, Mo-ei-bara1 and Mo-ei-imp1. All the NILs showed resistant reaction against the isolates Mo-ei-bara1 and Mo-ei-imp1. However, the monogenic NILs possessing *Pi54* were resistant against all the isolates except for Mo-nwi-lon2, for which the *Pi1* carrying NILs were resistant. Therefore, pyramiding these genes was efficient in combating both the isolates (Table 7).

In the other case, considering the isolates Mo-ni-0037, Mo-ni-0067, Mo-ei-bara1 and Mo-ni-0052a, the monogenic NILs carrying *Pi2* were susceptible with the isolates Mo-ni-0037, Mo-ni-0067 and Mo-ei-bara1 and resistant against Mo-ni-0052a. The monogenic NILs carrying *Pib* were resistant against the isolates Mo-ni-0037 and Mo-ni-0067, but susceptible against Mo-ei-bara1 and

Table 4 Grain and cooking quality of the NILs carrying blast resistance genes

Genotypes	Genes	KLBC	KBBC	L/B	KLAC	ER	Amylose content (%)	ASV	GC	Aroma
Pusa 1633-1-8-6-8-12	*Pi54*	8.57 ± 0.31	1.59 ± 0.10	5.39 ± 0.34	15.36 ± 0.11*	1.79 ± 0.06	25.7	7	26	2
Pusa 1633-1-8-6-23-7	*Pi54*	8.43 ± 0.11	1.51 ± 0.06	5.58 ± 0.08*	14.39 ± 0.13*	1.71 ± 0.04	25.7	7	26	2
Pusa 1633-2-8-12-9-10	*Pi1*	8.38 ± 0.18	1.52 ± 0.04	5.51 ± 0.14	15.32 ± 0.25*	1.83 ± 0.07	25.0	7	26	2
Pusa 1633-2-8-1-4-18	*Pi1*	8.42 ± 0.06	1.58 ± 0.13	5.33 ± 0.27	15.05 ± 0.42*	1.79 ± 0.05	25.4	7	26	2
Pusa 1633-3-8-8-16-1	*Pita*	8.48 ± 0.31	1.63 ± 0.03	5.20 ± 0.17	14.94 ± 0.28	1.76 ± 0.09	26.0	7	28	2
Pusa 1633-3-8-20-6-12	*Pita*	8.41 ± 0.17	1.66 ± 0.02*	5.07 ± 0.08	15.23 ± 0.02*	1.81 ± 0.03	25.6	7	28	2
Pusa 1634-8-1-12-15	*Pi2*	8.74 ± 0.35	1.66 ± 0.06*	5.27 ± 0.13	14.85 ± 0.06	1.70 ± 0.07	23.9	7	26	2
Pusa 1634-4-9-6-23	*Pi2*	8.75 ± 0.11	1.57 ± 0.07	5.57 ± 0.20*	14.91 ± 0.08	1.7 ± 0.03	24.0	7	26	2
Pusa 1635-10-6-8-10	*Pib*	8.68 ± 0.05	1.62 ± 0.12	5.36 ± 0.19	14.86 ± 0.22	1.71 ± 0.02	23.4	7	27	2
Pusa 1635-10-5-6-18	*Pib*	8.23 ± 0.11	1.55 ± 0.14	5.31 ± 0.21	14.52 ± 0.04	1.76 ± 0.03	23.0	7	28	2
Pusa 1636-12-9-8-17	*Pi5*	8.36 ± 0.12	1.55 ± 0.13	5.39 ± 0.20	14.7 ± 0.17	1.76 ± 0.04	24.9	7	27	2
Pusa 1636-12-9-12-4	*Pi5*	8.16 ± 0.18	1.59 ± 0.07	5.13 ± 0.09	15.15 ± 0.18	1.86 ± 0.06	24.5	7	27	2
Pusa 1637-18-7-6-20	*Pi9*	8.30 ± 0.06	1.59 ± 0.21	5.22 ± 0.37	15.11 ± 0.4	1.82 ± 0.06	23.2	7	27	2
Pusa 1637-12-8-20-5	*Pi9*	8.12 ± 0.15	1.58 ± 0.04	5.14 ± 0.13	15.78 ± 0.47*	1.94 ± 0.07*	23.6	7	27	2
Pusa Basmati 1	–	8.27 ± 0.09	1.56 ± 0.06	5.30 ± 0.14	14.80 ± 0.10	1.79 ± 0.03	25.6	7	28	2
IRBL9-W	*Pi9*	5.37 ± 0.24*	2.41 ± 0.07*	2.23 ± 0.04*	9.20 ± 0.10*	1.71 ± 0.07	19.0	3	59	0
IRBL5-M	*Pi5*	5.21 ± 0.27*	2.27 ± 0.16	2.30 ± 0.06*	8.02 ± 0.19*	1.54 ± 0.09*	15.9	3	58	0
IRBLb-B	*Pib*	5.46 ± 0.02*	2.33 ± 0.13	2.43 ± 0.06*	9.52 ± 0.25*	1.74 ± 0.04	12.5	4	67	0
IRBLz5-CA	*Pi2*	5.52 ± 0.23*	2.32 ± 0.21	2.40 ± 0.17*	9.45 ± 0.54*	1.71 ± 0.07	19.0	3	70	0
DHMASQ164-2a	*Pi54+Pi1+Pita*	6.3 ± 0.1*	2.40 ± 0.18	2.63 ± 0.22*	9.23 ± 0.06*	1.47 ± 0.02*	21.7	4	55	0
CD (0.05)		0.3	0.08	0.26	0.4	0.09				

* $P < 0.05$

Grain shape: *extra long* >7.50 mm, *long* 6.61–7.50 mm, *medium* 5.51–6.60, *short* < 5.00 mm, *KLBC* kernel length before cooking, *KBBC* kernel breadth before cooking, *L/B ratio* length/breadth ratio, *KLAC* kernel length after cooking, *KBAC* kernel breadth after cooking, *ER* elongation ratio, *Amylose content* 9–20 %—low, 20–25 %—*intermediate*, 25–33 %—high; *ASV* alkali spreading value (1–2 high and 6–7 low), *GC* (gel consistency) 26–40 mm: *hard gel*, 41–60 mm: *medium gel*, 61–100 mm: *soft gel*, aroma: (0 absent, 1 mild, 2 strong and 3 very strong)

Table 5 Agronomic performance of pyramided lines

Genotypes	Genes	DFF	PH	PN	PL	FG/P	SF %	TGW	Yield (kg/ha)
Pusa 1633-4-8-26-5-4	*Pi54+Pi1*	106 ± 0.6	112.17 ± 3.01	14.33 ± 1.53	30.83 ± 1.04	144.86 ± 3.07	87.73 ± 3.73	22.64 ± 2.7	6966.67 ± 814.45
Pusa 1633-4-8-48-12-7	*Pi5+Pi1*	106 ± 1.0	111.67 ± 2.04	13.33 ± 1.53	28.80 ± 2.02	135.76 ± 6.26	92.32 ± 2.00	22.59 ± 1.23	5944.44 ± 335.55
Pusa 1633-5-8-26-5-4	*Pi54+Pita*	107 ± 0.6	111.97 ± 2.06	13.67 ± 1.53	30.60 ± 0.4	134.51 ± 10.67	88.77 ± 3.88	23.14 ± 0.79	6822.22 ± 234.13
Pusa 1633-5-8-48-12-7	*Pi54+Pita*	107 ± 1.0	112.17 ± 4.74	14.00 ± 1.00	30.07 ± 1.4	138.05 ± 5.01	88.09 ± 6.07	22.61 ± 0.63	6622.22 ± 467.06
Pusa1633-6-8-54-8-2	*Pi1+Pita*	108 ± 0.6	110.07 ± 2.67	15.00 ± 1.00	29.33 ± 0.29	133.15 ± 4.36	82.61 ± 1.70*	23.67 ± 1.76	7011.11 ± 407.34
Pusa 1633-6-8-87-3-2	*Pi1+Pita*	106 ± 0.6	112.4 ± 3.14	14.67 ± 1.53	26.67 ± 1.15	128.00 ± 4.78	86.63 ± 6.6	20.63 ± 1.79	6433.33 ± 120.19
Pusa 1879-4-8-6	*Pi2+Pib*	105 ± 0.6	110.4 ± 4.48	13.67 ± 2.08	29.67 ± 0.70	157.08 ± 4.61*	84.85 ± 4.05	22.48 ± 2.47	6855.55 ± 476.48
Pusa 1879-4-14-6	*Pi2+Pib*	106 ± 0.6	111.27 ± 0.81	13.33 ± 1.15	30.7 ± 1.47	158.33 ± 11.93*	89.1 ± 6.25	24.84 ± 0.15*	6688.89 ± 320.30
Pusa 1930-6-4	*Pi5+Pi2*	105 ± 2.3	110.47 ± 2.53	16.00 ± 1.00	31.00 ± 2.50	141.67 ± 12.42	82.02 ± 3.56	22.38 ± 0.2	7455.55 ± 258.92
Pusa 1930-12-6	*Pi5+Pi2*	105 ± 0.6	110.27 ± 1.10	15.00 ± 3.00	29.67 ± 2.08	133.67 ± 10.6	88.49 ± 1.59	23.39 ± 0.52	8255.55 ± 781.97*
Pusa 1931-6-7	*Pi5+Pib*	105 ± 1.0	112.37 ± 4.07	15.00 ± 1.00	32.00 ± 2.65	129.67 ± 2.08	86.86 ± 2.69	23.45 ± 0.56	7088.89 ± 516.76
Pusa 1931-7-2	*Pi5+Pib*	105 ± 1.5	112.07 ± 4.08	11.67 ± 0.58	33.67 ± 1.53	132.00 ± 7.21	87.76 ± 2.99	23.35 ± 0.5	6722.22 ± 167.77
Pusa 1878-5-16-4	*Pi5+Pi9*	105 ± 2.0	110.43 ± 3.5	11.00 ± 2.00	30.07 ± 1.29	143.00 ± 4.58	85.22 ± 5.51	21.73 ± 1.89	6822.22 ± 216.88
Pusa 1878-8-12-7	*Pi5+Pi9*	106 ± 1.0	112.1 ± 1.35	13.00 ± 2.00	30.35 ± 2.48	140.67 ± 5.86	90.5 ± 4.95	22.61 ± 3.41	6900.00 ± 251.66
Pusa 1929-4-8	*Pi9+Pib*	105 ± 1.0	113.37 ± 0.90	12.67 ± 2.08	28.1 ± 2.05	131.33 ± 2.08	87.95 ± 1.08	23.68 ± 0.43	6933.33 ± 290.59
Pusa 1929-6-3	*Pi9+Pib*	103 ± 1.5*	111.83 ± 0.29	12.00 ± 2.00	28.93 ± 1.37	131.67 ± 4.51	92.77 ± 3.70	23.95 ± 0.30*	6888.89 ± 483.43
Pusa 1633-7-8-53-6-8	*Pi54+Pi1+Pita*	106 ± 0.6	109.5 ± 1.28	13.33 ± 1.15	31.17 ± 0.55	131.71 ± 2.72	86.5 ± 1.57	23.25 ± 0.30	6769.33 ± 213.49
Pusa 1633-7-8-67-4-12	*Pi54+Pi1+Pita*	105 ± 0.6	108.27 ± 1.08	13.00 ± 1.00	29.93 ± 0.59	126.33 ± 1.93*	84.18 ± 1.26	22.27 ± 0.61	6769.33 ± 213.49
Pusa 1932-7-6	*Pi9+Pi5+Pib*	107 ± 0.6	113.3 ± 1.67	12.67 ± 1.53	29.27 ± 1.14	133.83 ± 4.54	86.41 ± 4.07	23.92 ± 1.17	6833.33 ± 578.31
Pusa 1932-12-4	*Pi9+Pi5+Pib*	106 ± 0.6	112.5 ± 0.50	15.33 ± 0.58	30.50 ± 0.46	133.67 ± 4.89	83.53 ± 4.22*	22.32 ± 1.85	7266.67 ± 296.27
Pusa 1933-4-6	*Pi2+Pi5+Pib*	108 ± 0.6	109.3 ± 0.61	13.67 ± 0.58	29.37 ± 0.75	130.03 ± 2.24	87.86 ± 4.46	21.17 ± 1.42	7155.56 ± 750.06
Pusa 1933-64-3	*Pi2+Pi5+Pib*	106 ± 0.6	112.37 ± 0.71	14.00 ± 1.00	30.87 ± 1.10	135.03 ± 3.84	81.63 ± 2.90*	22.66 ± 1.37	6988.89 ± 757.43
PB1	–	106 ± 0.6	111.15 ± 2.88	14.00 ± 1.00	31.52 ± 1.38	136.91 ± 5.43	90.62 ± 2.68	21.48 ± 0.91	6911.11 ± 356.42
CD (0.05)		1.65	4.16	3.63	2.47	10.02	6.51	2.44	755.61

* $P < 0.05$

Table 6 Grain and cooking quality of pyramided lines

Genotypes	Genes	KLBC	KBBC	L/B	KLAC	KER	Aroma	RPG (%)
Pusa 1633-4-8-26-5-4	*Pi54+Pi1*	8.01 ± 0.26*	1.48 ± 0.02	5.4 ± 0.16	14.45 ± 0.38	1.80 ± 0.04	2	95.6
Pusa 1633-4-8-48-12-7	*Pi54+Pi1*	8.15 ± 0.19	1.49 ± 0.01	5.47 ± 0.15	14.69 ± 0.31	1.80 ± 0.01	2	96.4
Pusa 1633-5-8-26-5-4	*Pi54+Pita*	8.3 ± 0.09	1.46 ± 0.05	5.69 ± 0.15	14.50 ± 0.10	1.75 ± 0.01	2	96.6
Pusa 1633-5-8-48-12-7	*Pi54+Pita*	8.22 ± 0.16	1.48 ± 0.03	5.54 ± 0.10	14.55 ± 0.26	1.77 ± 0.06	2	95.4
Pusa1633-6-8-54-8-2	*Pi1+Pita*	8.27 ± 0.19	1.6 ± 0.02	5.16 ± 0.10	14.42 ± 0.22	1.74 ± 0.02	2	97.4
Pusa 1633-6-8-87-3-2	*Pi1+Pita*	8.30 ± 0.10	1.58 ± 0.03	5.24 ± 0.16	14.7 ± 0.01	1.77 ± 0.02	2	96.8
Pusa 1879-4-8-6	*Pi2+Pib*	8.17 ± 0.19	1.5 ± 0.03	5.46 ± 0.04	14.6 ± 0.17	1.79 ± 0.04	2	94.3
Pusa 1879-4-14-6	*Pi2+Pib*	8.38 ± 0.1	1.48 ± 0.04	5.87 ± 0.26*	14.31 ± 0.21	1.71 ± 0.02	2	94.1
Pusa 1930-6-4	*Pi5+Pi2*	8.40 ± 0.07	1.52 ± 0.04	5.52 ± 0.14	14.53 ± 0.2	1.73 ± 0.01	2	95.2
Pusa 1930-12-6	*Pi5+Pi2*	8.34 ± 0.06	1.55 ± 0.02	5.38 ± 0.09	14.44 ± 0.14	1.73 ± 0.01	2	96.3
Pusa 1931-6-7	*Pi5+Pib*	8.33 ± 0.12	1.54 ± 0.10	5.41 ± 0.31	14.6 ± 0.25	1.75 ± 0.01	2	94.9
Pusa 1931-7-2	*Pi5+Pib*	8.24 ± 0.10	1.48 ± 0.10	5.60 ± 0.46	14.19 ± 0.25	1.72 ± 0.01	2	94.5
Pusa 1878-5-16-4	*Pi5+Pi9*	8.42 ± 0.38	1.46 ± 0.04	5.76 ± 0.10	14.73 ± 0.3	1.75 ± 0.05	2	95.5
Pusa 1878-8-12-7	*Pi5+Pi9*	8.39 ± 0.43	1.49 ± 0.07	5.83 ± 0.57	14.6 ± 0.28	1.74 ± 0.06	2	95.8
Pusa 1929-4-8	*Pi9+Pib*	8.22 ± 0.10	1.49 ± 0.04	5.83 ± 0.23	14.48 ± 0.32	1.76 ± 0.02	2	93.5
Pusa 1929-6-3	*Pi9+Pib*	8.07 ± 0.29*	1.48 ± 0.10	5.82 ± 0.26	14.19 ± 0.3	1.76 ± 0.04	2	95.4
Pusa 1633-7-8-53-6-8	*Pi54+Pi1+Pita*	8.38 ± 0.10	1.64 ± 0.02*	5.1 ± 0.12	15.07 ± 0.59*	1.80 ± 0.09*	2	96.7
Pusa 1633-7-8-67-4-12	*Pi54+Pi1+Pita*	8.16 ± 0.45	1.46 ± 0.04	5.61 ± 0.42	14.28 ± 0.21	1.75 ± 0.08	2	95.9
Pusa 1932-7-6	*Pi9+Pi5+Pib*	8.28 ± 0.02	1.57 ± 0.03	5.28 ± 0.11	14.54 ± 0.07	1.75 ± 0.05	2	94.5
Pusa 1932-12-4	*Pi9+Pi5+Pib*	8.10 ± 0.11	1.59 ± 0.05	5.11 ± 0.19	14.55 ± 0.22	1.79 ± 0.02	2	93.7
Pusa 1933-4-6	*Pi2+Pi5+Pib*	8.29 ± 0.10	1.56 ± 0.02	5.3 ± 0.09	14.78 ± 0.02	1.81 ± 0.01*	2	93.0
Pusa 1933-64-3	*Pi2+Pi5+Pib*	8.25 ± 0.13	1.60 ± 0.01	5.15 ± 0.07	14.79 ± 0.01	1.78 ± 0.02	2	94.5
PB1	–	8.49 ± 0.26	1.52 ± 0.02	5.44 ± 0.13	14.61 ± 0.29	1.72 ± 0.06	2	–
CD (0.05)		0.35	0.08	0.39	0.42	0.07	–	–

* $P < 0.05$

Mo-ni-0052a. Further, the monogenic NILs carrying *Pi5* were resistant against Mo-ei-bara1, but susceptible against Mo-ni-0037 and Mo-ni-0067 and Mo-ni-0052a. Therefore, pyramiding of the diverse genes was necessary to build resistance against all the four isolates. The pyramids carrying *Pi2+Pib* were susceptible against Mo-ei-bara1; *Pi2+Pi5* were susceptible to Mo-ni-0037 and Mo-ni-0067; *Pib+Pi5* were susceptible against Mo-ni-0052a, but resistant against other isolates. However, the three-gene pyramids carrying *Pi2+Pib+Pi5* were resistant against all the isolates (Table 7).

Evaluation of NILs for blast resistance in Uniform Blast Nursery

The NILs were also evaluated for blast resistance under natural conditions in Uniform Blast Nursery (UBN) at two hotspot locations, viz., Malan (Himachal Pradesh) and Hazaribagh (Jharkhand). The RP PB1 was found to be highly susceptible at both the locations. At Malan, Pusa 1637-18-7-6-20, Pusa 1637-12-8-20-5 and the DP IRBL9-W carrying the gene *Pi9* were highly resistant. However, the monogenic NILs with the gene *Pi2* or *Pita* was moderately resistant (score 4–5); the monogenic NILs carrying *Pib* or *Pi54* were moderately susceptible (score 6–7); and the monogenic NILs carrying *Pi5* or *Pi1* were susceptible (score 8). The two- and three-gene pyramids evaluated under UBN showed varied reaction pattern. Among the two-gene pyramids, *Pi5+Pi9* and *Pib+Pi9* showed resistant reaction with the score 0. While the combinations *Pi54+Pita, Pi1+Pita*, *Pi2+Pib* and *Pi2+Pi5* were moderately resistant with the score of 4. The remaining two-gene combinations *Pi54+Pi1, Pi5+Pib* were moderately susceptible with score 6. The NILs carrying three-gene combinations, viz., *Pi9+Pib+Pi5*, *Pi2+Pib+Pi5* and *Pi54+Pita+Pi1* were highly resistant with score 0–1.

The screening of NILs in UBN at Hazaribagh showed a different disease reaction pattern as compared to that of UBN-Malan. All the NILs carrying *Pi9* or *Pita* showed resistant reaction (score 0), but the lines carrying *Pi54, Pi1*, *Pi5, Pi2* or *Pib* were moderately resistant (score 4–5). Further, all the pyramids were found resistant to blast disease except for the combinations *Pi5+Pib* and *Pi54+Pi1*, which showed moderate resistance.

Table 7 Blast disease score of pyramided lines under artificial epiphytotic conditions using diagnostic isolates

Genotype	Genes	Mo-ni-53	Mo-ni-0052a	Mo-ni-0066a	Mo-nwi-lon2	Mo-nwi-kash 1	Mo-ni-0067	Mo-ei-bara1	Mo-ei-imp1	Mo-nwi-53
Pusa 1633-1-8-6-8-12	*Pi54*	0	0	0	4	3	1	2	2	–
Pusa 1633-1-8-6-23-7	*Pi54*	0	0	0	4	3	1	2	1	–
Pusa 1633-2-8-12-9-10	*Pi1*	4	4	4	2	2	4	2	2	0
Pusa 1633-2-8-1-4-18	*Pi1*	4	4	4	2	2	4	2	2	0
Pusa 1633-3-8-8-16-1	*Pita*	4	4	4	4	3	4	2	0	0
Pusa 1633-3-8-20-6-12	*Pita*	4	4	4	4	3	4	2	0	0
Pusa 1633-4-8-26-5-4	*Pi54+Pi1*	0	0	0	0	2	0	0	1	0
Pusa 1633-4-8-48-12-7	*Pi54+Pi1*	0	0	0	0	2	0	0	1	0
Pusa 1633-5-8-26-5-4	*Pi54+Pita*	0	0	0	3	3	0	0	0	0
Pusa 1633-5-8-48-12-7	*Pi54+Pita*	0	0	0	3	3	0	0	0	0
Pusa1633-6-8-54-8-2	*Pi1+Pita*	4	3	3	0	0	3	0	0	0
Pusa 1633-6-8-87-3-2	*Pi1+Pita*	4	3	3	0	0	3	0	0	0
Pusa 1633-7-8-53-6-8	*Pi54+Pi1+Pita*	0	0	0	1	0	0	0	0	0
Pusa 1633-7-8-67-4-12	*Pi54+Pi1+Pita*	0	0	0	0	0	0	0	0	0

Genotype	Genes	Mo-nwi–kash 1	Mo-ni-0060	Mo-ei-ran1	Mo-ei-bara1	Mo-ni-0037	Mo-ni-0067	Mo-ei-imp1	Mo-nwi-53	Mo-nwi-35	Mo-nwi-lon2
Pusa 1637-18-7-6-20	*Pi9*	0	1	0	3	0	0	0	2	0	0
Pusa 1637-12-8-20-5	*Pi9*	0	0	0	3	0	0	0	1	0	0
Pusa 1635-10-6-8-10	*Pib*	3	4	4	3	1	0	4	3	1	2
Pusa 1635-10-5-6-18	*Pib*	3	4	4	3	1	0	4	3	1	2
Pusa 1636-12-9-8-17	*Pi5*	3	4	4	0	3	4	0	0	0	2
Pusa 1636-12-9-12-4	*Pi5*	3	4	4	2	3	4	0	0	2	2
Pusa 1878-5-16-4	*Pi5+Pi9*	0	0	0	1	1	1	0	0	0	0
Pusa 1878-8-12-7	*Pi5+Pi9*	0	0	0	1	1	1	0	0	0	0
Pusa 1929-4-8	*Pi9+Pib*	0	0	0	–	0	0	0	0	0	0
Pusa 1929-6-3	*Pi9+Pib*	0	0	0	–	0	0	0	0	0	0
Pusa 1931-6-7	*Pi5+Pib*	3	4	3	2	1	1	1	2	1	2
Pusa 1931-7-2	*Pi5+Pib*	3	4	3	2	1	1	1	2	1	2
Pusa 1932-7-6	*Pi9+Pib+Pi5*	0	0	0	0	0	0	0	0	0	0
Pusa 1932-12-4	*Pi9+Pib+Pi5*	0	0	0	0	0	0	0	0	0	0

Genotype	Genes	Mo-ni-53	Mo-ni-0052a	Mo-ni-0066a	Mo-ni-0037	Mo-ni-0067	Mo-ei-bara1	Mo-nwi-53	Mo-ei-imp1	Mo-nwi-35	Mo-nwi-lon2
Pusa 1634-8-1-12-15	*Pi2*	0	2	2	4	4	3	0	1	1	1
Pusa 1634-4-9-6-23	*Pi2*	0	1	0	4	4	3	2	1	1	1
Pusa 1635-10-6-8-10	*Pib*	4	4	4	1	0	3	2	4	1	2
Pusa 1635-10-5-6-18	*Pib*	4	4	4	1	0	3	1	4	1	2
Pusa 1636-12-9-8-17	*Pi5*	4	4	4	3	4	0	3	0	0	2
Pusa 1636-12-9-12-4	*Pi5*	4	4	4	3	4	2	3	0	2	2
Pusa 1931-6-7	*Pi5+Pib*	4	4	4	1	1	2	2	1	1	1
Pusa 1931-7-2	*Pi5+Pib*	4	4	4	1	1	2	2	1	1	1
Pusa 1930-6-4	*Pi2+Pi5*	1	1	1	2	2	1	1	1	1	1
Pusa 1930-12-6	*Pi2+Pi5*	1	1	1	2	2	1	1	1	1	1
Pusa 1929-4-8-6	*Pi2+Pib*	0	1	1	0	0	3	1	1	0	1
Pusa 1929-4-14-6	*Pi2+Pib*	0	1	1	0	0	3	1	1	0	1
Pusa 1933-4-6	*Pi2+Pib+Pi5*	0	0	0	0	0	0	0	0	0	0
Pusa 1933-64-3	*Pi2+Pib+Pi5*	0	0	0	0	0	0	0	0	0	0

Table 7 continued

Genotype	Genes	Mo-ni-53	Mo-ni-0052a	Mo-ni-0066a	Mo-nwi-53	Mo-nwi-lon2	Mo-nwi–kash 1	Mo-ni-0060	Mo-ei-ran1	Mo-ni-0037	Mo-ni-0067	Mo-ei-bara1	Mo-ei-imp1	Mo-nwi-35
IRBLz5-CA	*Pi2*	0	0	0	0	1	3	4	4	4	4	3	0	1
IRBLb-B	*Pib*	4	4	4	1	2	3	4	4	1	0	3	4	1
IRBL5-M	*Pi5*	4	4	4	3	2	3	4	4	3	4	0	0	0
IRBL9-W	*Pi9*	4	4	4	0	0	0	0	0	0	0	3	0	0
DHMAS Q164-2a	*Pi54+Pi1 +Pita*	0	0	0	0	0	3	–	4	0	0	0	0	0
LTH	–	5	5	5	4	4	5	5	5	5	5	4	4	4
PB1	–	5	5	5	5	5	4	4	5	5	5	5	4	5

Discussion

Basmati rice production is severely constrained due to several diseases, of which blast is considered as the most notorious leading to yield losses and deterioration of quality. Blast resistance breeding program in Basmati rice has been severely handicapped by the non-availability of blast resistance sources in the Basmati germplasm. Therefore, the present study aimed to develop a set of seven NILs with seven major broad-spectrum blast resistance genes in the genetic background of PB1 using monogenic lines carrying different blast resistance genes in the genetic background of a non-Basmati rice variety LTH and DHMASQ164-2a as donors through MABB. Foreground selection using the gene-based/linked markers for the target blast resistance genes ensured the successful incorporation of the target genes.

The RP PB1 is known for its excellent grain and cooking quality and pleasant aroma. However, the DPs possessed small and bold grain, black hull, brown pericarp, less elongation on cooking and non-aromatic grains. Therefore, it was a challenging task to reconstruct the PB1-NILs with Basmati grain and cooking quality characters and resistance to blast. In view of this, the recombinant and background selections were augmented with stringent phenotypic selection for recovery of the RPP, including agro-morphological, grain, cooking quality and aroma traits.

The recombinant and background selection played a vital role in identifying the NILs with minimum donor segment in the genomic region flanking the target gene and maximum RPG recovery. However, NILs Pusa 1633-3-8-8-16-1 and Pusa 1633-3-8-20-6-12 harbored a large donor segment (5.2 mb) between the markers RM7003 and RM7102 located 6.2 mb upstream and 11.4 mb downstream of the gene *Pita,* respectively. Since, the gene *Pita* is located adjacent to the centromeric region, which shows reduced crossing over because of heterochromatinization; the donor segment surrounding *Pita* could not be reduced any further. However, there was no undesirable phenotypic effect of the donor segment on the performance of the NILs having *Pita*.

Background selection using polymorphic markers providing genome-wide coverage has been extensively used to hasten the RPG recovery in MABB (Basavaraj et al. 2010; Chen et al. 2008). However, it was challenging to complete the analysis using entire set of polymorphic markers in limited time to utilize the information for further backcrossing. Therefore, a two-step background selection strategy was used in this study, which reduced the genotyping work and successfully accelerated the RPG recovery in the NILs in the range of 93.0–98.6 % in just three backcrosses despite the fact that the DPs used in the present study were genetically diverse as compared to RP PB1 and possessed undesirable agronomic features and grain and cooking quality traits. Further, the phenotypic selection among the plants with maximum RPG recovery, hastened the recovery of RPP. The efficacy of background selection combined with phenotypic selection in recovering RPP in rice has been demonstrated earlier (Joseph et al. 2004; Gopalakrishnan et al. 2008; Singh et al. 2011, 2012, 2013).

In a comparative study for estimating the similarity between the RP and the developed NILs using SSRs and the SNP markers, it was deduced that the background analysis using a set of 500 genome-wide SSR markers overestimated the percent similarity between NILs and RP PB1 when compared to genome-wide 50,051 SNP markers. Additionally, SNP markers provided better insights of the donor segments still present in the NILs. Furthermore, the background analysis using SNP assay was almost 300 times cost effective (US$ 84/genotype with 50,051 SNPs data points) as against use of SSR markers (US$ 70/genotype with approximately 85–110 data points) and, therefore, use of SNPs would be a better choice for the background selection in MABB.

The comparison of yield performance of NILs and RP based on multi-location evaluation unequivocally proved

that the blast resistance genes had no yield penalty in the NILs, which has been a concern in case of isogenic lines developed for rust resistance in wheat (Ortelli et al. 1996; Singh et al. 2011; Latter et al. 1998). Further, GGE Biplot analysis revealed that the NIL Pusa 1637-18-7-6-20 (*Pi9*) is the best genotype across environments. The comparison plot depicted the NILs scattering across the concentric circles away from the point of ideal genotype. However, in comparison to the RP PB1, it is clear that except NILs Pusa 1636-12-9-8-17 (G11), Pusa 1633-2-8-12-9-10 (G3) and Pusa 1633-1-8-6-23-7 (G2), all other NILs were either superior to or similar to RP, demonstrating the efficiency of MABB program in recovering RPG and RPP.

Artificial inoculation with respective diagnostic isolates confirmed the potentiality of individual blast resistance genes in the genetic background of PB1. Under natural epiphytotic condition in UBN at Malan (north-western India) and Hazaribagh (Eastern India), the isogenic lines carrying the gene *Pi9* were found to be consistently resistant across two years of testing. Multi-location testing of genotypes carrying *Pi9* had earlier indicated its effectiveness against the prevalent pathotypes of Eastern India (Variar et al. 2009; Imam et al. 2014). However, from the present study, it can be deduced that *Pi9* is the most effective gene followed by *Pi2* and *Pita* not only in the eastern part of India, but also effective against predominant races prevalent in the northern part of India, which form geographical indication (GI) area for Basmati cultivation. The effectiveness of *Pi9* over *Pi2* has also been earlier reported by Imam et al. (2014). However, other genes were resistant in one location and moderately resistant/moderately susceptible at another location indicating the effectiveness of different blast resistance genes against the location-specific predominant races of blast pathogen.

The monogenic NILs developed in the genetic background of RP PB1 have been bred and selected to be nearly identical in height, maturity, plant type, grain and cooking quality traits and other characteristics among themselves and also in relation to RP PB1, they are similar in appearance and genetic makeup, but have different genes for resistance to blast disease. Therefore, these NILs may be used to create a multiline variety by mechanically mixing their seeds, which would offer farmers a way to tame outbreaks of blast disease, thus stabilizing Basmati rice production. The concept of multiline has been well demonstrated in management of wheat rust diseases earlier (Luthra and Rao 1979) and more recently transgenic multiline variety for controlling powdery mildew of wheat has been developed (Brunner et al. 2012).

Pyramiding of diverse genes conferring resistance to different isolates has been advocated as the most promising strategy for attaining resistance against the concoction of races of the pathogen present in the natural conditions. The pyramids carrying multiple R-genes tend to exclude various races of the pathogen by effective complementation (Gnanamanickam et al. 2000). Therefore, developing the pyramided lines is pivotal from the perspective of resistance stabilization owing to the hypervariability of the blast pathogen population structure (Casela et al. 1995). The artificial inoculation using diagnostic blast isolates has unambiguously proved that three-gene pyramids developed in the present study were much more effective than that of two-gene pyramids and monogenic NILs, in imparting broad-spectrum resistance to diverse isolates of blast pathogen through effective complementation.

Conclusion

The study has led to the development of first-ever set of NILs carrying major blast resistance genes in the genetic background of a Basmati rice variety PB1. These NILs are potential candidates for release as a variety after the required testing for replacing PB1, which is highly susceptible to blast disease and still occupies substantial area under cultivation. Additionally, these NILs can be used as donor lines for blast resistance and as a component of a multiline in Basmati breeding program. The deployment of blast-resistant NILs and multilines may substantially reduce the fungicide use in Basmati rice cultivation and thus alleviate the problem of fungicide residue in the grain, which is a concern in the domestic as well as global market. These are also excellent genetic materials for functional genomics to understand the molecular mechanisms and pathways underlying the resistance governed by the respective blast resistance genes without the effect of background noise.

Author contribution statement AKS conceptualized the idea and supervised all the experiments; AKS, AK, ABS were involved in development of NILs; AK, RKE, ABS, UDS, GP, RR, MV, SKP performed blast phenotyping of the NILs; NKS developed the affymetrix chip and helped in SNP genotyping; AKS, GKS, AK, RKE, ABS, MN, KKV managed field trials during offseason; AKS, AK, RKE, ABS, GKS, PKB conducted the field trials; AKS, RKE, AK did the statistical analysis and formulated the manuscript; BDS, AKS, KVP, TRS and VS critically read the manuscript and provided valuable inputs; all authors read and approved the final manuscript.

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Conflict of interest None of the authors have any conflict of interest associated with this study.

References

APEDA (2011) www.apeda.gov.in. Accessed 20 Nov 2011

Basavaraj SH, Singh VK, Singh A, Singh A, Singh A, Anand D, Yadav S, Ellur RK, Singh D, Gopala Krishnan S, Nagarajan M, Mohapatra T, Prabhu KV, Singh AK (2010) Marker-assisted improvement of bacterial blight resistance in parental lines of Pusa RH10, a superfine grain aromatic rice hybrid. Mol Breed 26(2):293–305

Bonman JM, Vergel de Dios TI, Khin MM (1986) Physiologic specialization of *Pyricularia oryzae* in the Philippines. Plant Dis 70:767–769

Brunner S, Stirnweis D, Diaz Quijano C, Buesing G, Herren G, Parlange F, Barret P, Tassy C, Sautter C, Winzeler M, Keller B (2012) Transgenic *Pm3* multilines of wheat show increased powdery mildew resistance in the field. Plant Biotechnol J 10:398–409

Casela CR, Alexandre SF, Zeller KA, Levy M (1995) Pathotype variation in the sorghum anthracnose fungus: a phylogenetic perspective for resistance breeding. In: Leslie JF, Frederiksen RA (eds) Disease analysis through genetics and molecular biology: interdisciplinary bridges to improved sorghum and millet crops. Iowa State University Press, Ames, Iowa, pp 257–276

Chen S, Xu CG, Lin XH, Zhang Q (2008) Improving bacterial blight resistance of '6078', an elite restorer line of hybrid rice, by molecular marker-assisted selection. Plant Breed 120(2):133–137

Ellur RK, Singh AK, Gupta HS (2013) Enhancing Rice Productivity in India: Aspects and Prospects. In: Shetty PK, Ayyappan S, Swaminathan MS (eds) Climate change and sustainable food security. National Institute of Advanced Studies, IISC, Bangalore and Indian Council of Agricultural Research, New Delhi, pp 99–132

Fukuoka S, Yamamoto S, Mizobuchi R, Yamanouchi U, Ono K, Kitazawa N, Yasuda N, Fujita Y, Nguyen TTT, Koizumi S, Sugimoto K, Matsumoto T, Yano M (2014) Multiple functional polymorphisms in a single disease resistance gene in rice enhance durable resistance to blast. Sci Rep 4:4550

Gnanamanickam SS, Babujee L, Priyadarisini VB, Dayakar BV, Leenakumari D, Sivaraj R, Levy M, Leong SA (2000) Lineage-exclusion resistance breeding: pyramiding of blast resistance genes for management of rice blast in India. In: Tharreau D, Lebrun MH, Talbot NJ and Notteghem JL (eds) Advances in rice blast research, developments in plant pathology, vol 15, pp 172–179

Gopalakrishnan S, Sharma RK, Rajkumar KA, Joseph M, Singh VP, Singh AK, Bhat KV, Singh NK, Mohapatra T (2008) Integrating marker assisted background analysis with foreground selection for identification of superior bacterial blight resistant recombinants in Basmati rice. Plant Breed 127:131–139

Hittalmani S, Parco A, Mew TV, Zeigler RS, Huang N (2000) Fine mapping and DNA marker-assisted pyramiding of the three major genes for blast resistance in rice. Theor Appl Genet 100(7):1121–1128

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

Joseph M, Gopalakrishnan S, Sharma RK, Singh AK, Singh VP, Singh NK, Mohapatra T (2004) Combining bacterial blight resistance and Basmati quality characteristics by phenotypic and molecular marker assisted selection in rice. Mol Breed 13:377–387

Juliano BO (1971) A simplified assay for milled rice amylose. Cereal Sci Today 16:334–338

Khush GS (2005) What it will take to Feed 5.0 billion rice consumers in 2030. Plant Mol Biol 59:1–6

Kumar MKP, Gowda DKS, Moudgal R, Kumar NK, Gowda KTP, Vishwanath K (2013) Impact on fungicides on rice production in India. In: Nita M (ed) Fungicides-showcases of integrated plant disease management from around the world. ISBN: 978-953-51-1130-6, InTech, doi:10.5772/51009. http://www.intechopen.com/books/fungicides-showcases-of-integrated-plant-disease-management-from-around-the-world/impact-of-fungicides-on-rice-production-in-india

Latter BDH, McIntosh RA, Ellison FW, Brennan PS, Fisher J, Hollamby GJ, Rathjen AJ, Wilson RE (1998) Grains yields of near-isogenic lines with added genes for stem rust resistance. In: Miller TE, Koebner RMD (eds) Proceedings of the 7th international wheat genetics symposium. Institute for Plant Science Research, Cambridge, UK, pp 901–906

Little RR, Hilder GB, Dawson EH (1958) Differential effect of dilute alkali on 25 varieties of milled white rice. Cereal Chem 35:111–126

Luthra JK, Rao MV (1979) Multiline cultivars—How their resistance influence leaf rust disease in wheat. Euphytica 28(1):137–144

Lv Q, Xu X, Shang J, Jiang G, Pang Z, Zhou Z, Wang J, Liu Y, Li T, Li X, Xu J, Cheng Z, Zhao X, Li S, Zhu L (2013) Functional Analysis of *Pid3-A4*, an ortholog of rice blast resistance gene *Pid3* revealed by allele mining in common wild rice. Phytopathology 103(6):594–599

Murray HG, Thompson WF (1980) Rapid isolation of high molecular weight DNA. Nucl Acids Res 8:4321–4325

Ortelli S, Winzeler H, Winzeler M, Fried PM, Nösberger J (1996) Leaf rust resistance gene *Lr9* and winter wheat yield reduction: I yield and yield components. Crop Sci 36:1590–1595

SES (1996) Standard evaluation system for rice. International Rice Research Institute, Manila, Philippines, p 56

Singh AK, Gopala Krishnan S, Singh VP, Prabhu KV, Mohapatra T, Singh NK, Sharma TR, Nagarajan M, Vinod KK, Singh D, Singh UD, Chander S, Atwal SS, Seth R, Singh VK, Ellur RK, Singh A, Anand D, Khanna A, Yadav S, Goel N, Singh A, Shikari AB Singh A, Marathi B (2011) Marker assisted selection: a paradigm shift in Basmati breeding. Indian J Genet 71(2) special Issue: 1–9

Singh VK, Singh A, Singh SP, Ellur RK, Choudhary V, Sarkel S, Singh D, Gopala Krishnan S, Nagarajan M, Vinod KK, Singh UD, Rathore R, Prashanthi SK, Agrawal PK, Bhatt JC, Mohapatra T, Prabhu KV, Singh AK (2012) Incorporation of blast resistance into "PRR78", an elite Basmati rice restorer line through marker assisted backcross breeding. Field Crops Res 128:8–16

Singh VK, Singh A, Singh SP, Ellur RK, Singh D, Gopala Krishnan S, Bhowmick PK, Nagarajan M, Vinod KK, Singh UD, Mohapatra T, Prabhu KV, Singh AK (2013) Marker-assisted simultaneous but stepwise backcross breeding for pyramiding blast resistance genes *Pi2* and *Pi54* into an elite Basmati rice restorer line PRR78. Plant Breed 132(5):486–495

Sood BC, Siddiq EA (1978) A rapid technique for scent determinations in rice. Indian J Genet 38:2268–2271

Tsunematsu H, Yanoria MJT, Ebron LA, Hayashi N, Ando I, Kato H, Imbe T, Khush GS (2000) Development of monogenic lines of rice for blast resistance. Breed Sci 50:229–234

Variar M, Vera Cruz CM, Carrillo MG, Bhatt JC, Sangar RBS (2009) Rice blast in India and strategies to develop durably resistant cultivars. In: Xiaofan W, Valent B (eds) Advances in genetics, genomics and control of rice blast disease. Springer, New York, pp 359–374

Yanoria T, Koide Y, Fukuta Y, Imbe I, Kato H, Tsunematsu H, Kobayashi N (2010) Development of near-isogenic lines of Japonica-type rice variety Lijiangxintuanheigu as differentials for blast resistance. Breed Sci 60:629–638