Marker-aided Incorporation of \textit{Xa38}, a Novel Bacterial Blight Resistance Gene, in PB1121 and Comparison of its Resistance Spectrum with \textit{xa13 + Xa21}

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Basmati rice is preferred internationally because of its appealing taste, mouth feel and aroma. Pusa Basmati 1121 (PB1121) is a widely grown variety known for its excellent grain and cooking quality in the international and domestic market. It contributes approximately USD 3 billion to India's forex earning annually by being the most traded variety. However, PB1121 is highly susceptible to bacterial blight (BB) disease. A novel BB resistance gene \textit{Xa38} was incorporated in PB1121 from donor parent PR114-\textit{Xa38} using a modified marker-assisted backcross breeding (MABB) scheme. Phenotypic selection prior to background selection was instrumental in identifying the novel recombinants with maximum recovery of recurrent parent phenome. The strategy was effective in delimiting the linkage drag to $<0.5$ mb upstream and $<1.9$ mb downstream of \textit{Xa38} with recurrent parent genome recovery up to 96.9% in the developed NILs. The NILs of PB1121 carrying \textit{Xa38} were compared with PB1121 NILs carrying \textit{xa13 + Xa21} (developed earlier in our lab) for their resistance to BB. Both NILs showed resistance against the Xoo races 1, 2, 3 and 6. Additionally, \textit{Xa38} also resisted Xoo race 5 to which \textit{xa13 + Xa21} was susceptible. The PB1121 NILs carrying \textit{Xa38} gene will provide effective control of BB in the Basmati growing region.

Harmonious combination of cooking quality characteristics and pleasant aroma is the uniqueness of Basmati rice. Intensive program on genetic improvement of Basmati rice has led to the development of a landmark rice variety, Pusa Basmati 1121 (PB1121), at ICAR-Indian Agricultural Research Institute (IARI), New Delhi. This is a medium duration high-yielding Basmati rice variety characterized by extra-long slender grains with very high kernel length after cooking (22–25 mm), high cooked kernel elongation ratio (2.5), intermediate amylose content, high volume expansion after cooking (>4 times) and strong aroma\textsuperscript{1}. These unique properties of PB1121 have attracted consumers worldwide. PB1121 currently occupies 1.35 million hectares, which is nearly 70% of the area under Basmati cultivation in India and contributes approximately 4 million tons to Basmati rice production annually. With the recognition of PB1121 as Basmati during 2008, the forex earning of the country has risen from USD 0.67 million to USD 4.5 billion in 2014–2015\textsuperscript{2}, in which the contribution of PB1121 is about 65%. This variety has not only revolutionized the international Basmati rice trade but also improved the livelihood of millions of Basmati farmers. However, major weakness of PB1121 is its susceptibility to a number of diseases such as to bacterial blight (BB)\textsuperscript{3}, blast\textsuperscript{3} and bakanae. The BB disease is caused by a gram-negative bacteria, \textit{Xanthomonas oryzae pv. oryzae} (Xoo), which severely constrains rice productivity and quality. Xoo breaches the plant epidermis and multiplies in the xylem vessels which lead to disease symptoms. The pathogen produces various virulence

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factors including EPS, extracellular enzyme, iron chelating siderophores and the type III-secretion dependent effectors, which are collectively essential for virulence and to counterattack plant’s immune response termed as PAMP-triggered immunity. Xoo populations are highly diversified in terms of mode of action through virulence factors. Mondal et al. characterized 780 Xoo isolates collected from thirteen states of India and grouped them into six races based on their reaction to 12 near isogenic lines (NILs). To date, 30 races of Xoo have been documented globally.

Utilizing the host plant resistance is an effective means to manage this pathogen in rice. As of now, 41 BB resistance genes have been reported among which 8 have been cloned and characterized. However, four genes namely, Xa4, Xa8, xa13 and Xa21 have been reported to be effective against the Xoo isolates prevalent in Basmati growing regions of India. Furthermore, the combination of genes xa13 + Xa21, xa5 + xa13 + Xa21 and Xa4 + xa5 + xa13 + Xa21 were reported to be equally effective and has provided greater degree of resistance as compared to monogenic lines. Therefore, xa13 + Xa21 have been widely used in the Basmati rice improvement program which resulted in development of several BB resistant genotypes such as Improved Pusa Basmati 1, Pusa 1718 and Pusa 1728. Extensive utilization of these genes over a long period of time, may lead to resurgence of pathogen with virulence genes that can overcome this combination. Therefore, deployment of diverse genes is essential to prolong the durability of resistance genes and to counter the development of virulent races of the pathogen.

Recently, a novel BB resistance gene Xa38 was identified in Oryza nivara and mapped on to the long arm of chromosome 4. It was further fine mapped to a region of 38.4 kb and an InDel marker based on the putative candidate gene LOC_Os04g53050-1 was developed, which co-segregated with the BB resistance. It is expected that deployment of Xa38 in rice varieties may help in checking the breakdown of resistance to Xoo. Therefore, the present study was undertaken to transfer Xa38 into the elite Basmati rice variety PB1121 through marker assisted backcross breeding (MABB) and to study its resistance spectrum in comparison to the combination of genes xa13 + Xa21 in the genetic background of PB1121.

Materials and Methods

Marker assisted breeding scheme. PR114-Xa38, an introgression line generated from a cross PR114/Oryza nivara carrying the BB resistance gene Xa38 was used as the donor parent and PB1121 was used as the recurrent parent in a MABB (Fig. 1). Individual F1 plant generated from the cross PB1121/PR114-Xa38 (designated as Pusa 1927), confirmed for hybridity using the Xa38 linked marker-Os04g53050-1 was backcrossed with the recurrent parent PB1121 to generate BC1F1 seeds. Foreground selection followed by phenotypic and background selection was carried out to identify the plants with maximum recovery for recurrent parent phenotype (RPP) and genome (RPG). The best BC1F1 plant was further backcrossed to generate BC2F1 seeds. The BC2F1 plants were also subjected to foreground, phenotypic and background selection to identify the plant with maximum recovery for RPG and RPP. The superior plant was advanced to BC2F2 generation and foreground selection was repeated in order to identify the plants homozygous for Xa38. Further, these plants were advanced via pedigree based phenotypic selection to obtain Xa38 introgressed near isogenic lines (NILs) of PB1121.

Molecular Marker Analysis. DNA from each of the leaf sample was extracted using the modified protocol of Murray and Thompson. PCR reaction was carried out using 20 ng of template DNA, 1x PCR buffer [10 mM Tris–HCl (pH 8.4); 50 mM KCl], 5 pmol of each primer, 0.05 mM dNTPs, 1.8 mM MgCl2 and 1U of Taq DNA polymerase (Invitrogen, Life Technologies, Brazil). The PCR was carried out in a G-Storm thermal cycler with the following program: 1) initial denaturation at 94 °C for 5 min; 2) 35 cycles for denaturation for 30 s at 94 °C, annealing for 30 s at 56 °C, extension for 1 min at 72 °C; and 3) final extension at 72 °C for 7 min. The amplified products were resolved on a 1.5% agarose gel and visualized under UV light.

Figure 1. Marker assisted backcross breeding scheme adopted for introgression of Xa38 in Pusa Basmati 1121.
product was resolved in a gel electrophoresis using 3.5% Metaphor™ Agarose gel and visualized on GelDoc™ XR (Bio-Rad Laboratories Inc., USA).

Foreground selection was carried out using the Xa38 linked molecular marker Os04g53050-1. For foreground selection, 628 genome-wide SSR markers were used in parental polymorphism survey between the parental lines PB1121 and PR114-Xa38, and a total of 70 polymorphic markers spanning uniformly across the genome were selected. Recombinant selection was carried out using the markers RM5511 and RM17523 flanking the BB resistance gene Xa38 on chromosome 4.

Screening for resistance to BB. The NILs, along with the checks PB1121 and PR114-Xa38, were grown in field conditions till maximum tillering stage. Different races of Xoo maintained as single spore culture was used to generate the BB suspension with a density of 10^9 cells/mL. Top five leaves from each of the entry were inoculated with Xoo isolate through the clip inoculation method. After 21 days of inoculation, the BB lesion length was measured using the disease assessment scheme as adopted by Mondal et al., wherein, lesion length of <5 cm was considered resistant, 5–10 cm was considered moderately resistant, 10–15 cm was considered moderately susceptible and more than 15 cm was considered highly susceptible reaction. Additionally, the resistance spectrum of Pusa1927 (PB1121 + Xa38) was compared with Pusa1718 (PB1121 + xa13 + Xa21) using a set of six races of Xoo.

Agromonic and Cooking Quality Evaluation. The NILs, along with the recurrent and donor parents, were evaluated for agronomic traits in randomized complete block design (RCBD) with three replications. Standard agronomic management practices were followed for raising the rice crop at ICAR-IARI, New Delhi. The data was 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 grain and cooking quality parameters such as kernel length before cooking (KLBC), kernel breadth before cooking (KBBC), length/breadth ratio (L/B), kernel length after cooking (KLAC), kernel breadth after cooking (KBAC) and aroma as described by Khanna et al.

Results

Development of PB1121-NILs carrying BB resistance gene Xa38. The F₁₀ generated from the cross PB1121/PR114-Xa38 were tested for hybridity using the marker Os04g53050-1. A single plant confirmed for hybridity was backcrossed to recurrent parent and a total of 468 BC₁F₁ seeds were produced, out of which 223 plants were found to be heterozygous for Xa38. All the heterozygous plants were subjected to phenotypic evaluation for agro-morphological traits. Ten superior plants, possessing relatively superior recovery for RPP were selected. The phenotypic selection for key Basmati traits such as KLAC and KBAC resulted in positive selection differential of 0.32 mm and 1.56 mm, respectively. Further, the background selection resulted in RPG recovery ranging from 70.6% to 79.29%. The best BC₁F₁ plant with RPG recovery of 79.29% was selected for further backcrossing with the recurrent parent PB1121 and a total of 198 BC₂F₁ seeds were produced. Foreground selection revealed that a total of 100 plants were heterozygous for Xa38, which were subjected to phenotypic selection for agronomic, grain and cooking quality traits. The selection differential was positive with a gain of 0.12 mm for KLAC and 0.36 mm for KBAC in the selected top ten superior BC₂F₁ plants. These plants when subjected to background selection using 29 markers which were heterozygous in the BC₁F₁ generation; the RPG recovery ranged from 83.6% to 88.6%.

The superior BC₂F₁ plant was selfed to produce 243 BC₃F₂ plants where 65 plants were homozygous for Xa38. Based on grain and cooking quality parameters, the selection differential was 0.29 mm in KLAC and 0.15 mm in KBAC. Furthermore, background selection with 16 markers remained heterozygous in BC₃F₁ generation, and the RPG recovery increased to the range of 88.57% to 92.86%. Finally, best plant was selected and advanced to BC₃F₃ generation. In ensuing BC₃F₃ generation, after phenotypic selection for the key Basmati traits KLAC and KBAC, the selection differential was 0.37 mm and 0.02 mm, respectively. The corresponding RPG recovery in this generation ranged from 93.18 to 94.70%. Repeating the same process in the next generation, BC₄F₄, the selection differential observed due to selection was 0.45 mm for KLAC and no gain observed for KBAC. The aroma score did not show significant variation between generations from that of PB1121 except in backcross generations (BC₂F₁ and BC₃F₃). The linkage drag was delimited to the region of <0.5 mb upstream and <1.9 mb downstream the BB resistance gene Xa38 (Fig. 2). Finally, ten best BC₃F₂ families were isolated and evaluated for agronomic and cooking quality characters. The number of plants generated during each of the backcross generations, the selection differential obtained due to phenotypic selection and the background recovery realized are presented in Table 1.

Evaluation of NILs for Agronomic and Cooking Quality Performance. All the NILs tested were found to be at par with the recurrent parent PB1121 with some exceptions such as NIL Pusa1927-17-16 which was found to mature 2 days later with slightly taller stature but possessed higher spikelet fertility than that of PB1121 (Table 2). Interestingly, six NILs, namely Pusa1927-19-21, Pusa1927-14-77, Pusa1927-8-86, Pusa1927-13-55, Pusa1927-45-72 and Pusa1927-62-2, were found to possess significantly higher spikelet fertility as compared to PB1121. The NIL Pusa1927-8-86 was found to be significantly superior in yield as compared to PB1121 owing to its better spikelet fertility. With respect to grain and cooking quality, all NILs were at par with the recurrent parent PB1121 (Table 3). However, three NILs Pusa1927-19-21, Pusa1927-14-77 and Pusa1927-17-16 were found to be significantly inferior, while NIL Pusa1927-75-56 was significantly superior as compared to the recurrent parent PB 1121 for the trait kernel length after cooking (Table 3 and Fig. 3).

Comparative Analysis of Resistance Spectrum of BB Resistance Genes, xa13 + Xa21 and Xa38. The resistance imparted by the BB resistance genes, xa13 + Xa21 and Xa38 was compared by subjecting...
the NILs Pusa1927 (PB1121 + Xa38) and Pusa1718 (PB1121 + xa13 + Xa21) against six different Xoo isolates. The disease reaction observed in the recurrent parent PB1121, Pusa1927 (PB1121 + Xa38) and Pusa1718 (PB1121 + xa13 + Xa21) is presented in Table 4 and Fig. 4.

The recurrent parent PB1121 was found to be highly susceptible to all the six Xoo races, while Pusa1927 (PB1121 + Xa38) and Pusa 1718 (PB1121 + xa13 + Xa21) were found to be highly resistant against Xoo races 1, 2, 3 and 6. However, for race 5, Pusa1718 (PB1121 + xa13 + Xa21) was found to be moderately resistant to moderately susceptible with the lesion length ranging from 9.37 ± 0.26 cm to 10.30 ± 0.55 cm, while NIL Pusa1927 (PB1121 + Xa38) was highly resistant with the lesion length ranging from 0.77 ± 0.15 cm to 3.83 ± 0.64 cm. However, both Pusa1718 and Pusa1927 were found to be susceptible against race 4 of Xoo.

Discussion
Basmati is the specialty rice of India, acclaimed worldwide for its unprecedented cooking quality characters and appealing aroma. Being susceptible to most of the diseases and pests including BB disease, the most popular Basmati cultivar in India, PB1121, which enjoys an export contribution of about 60–70%, often entreats agro-management practices that include use of chemical pesticides. Clean production of Basmati rice under eco-friendly environments is a prime target for producing export standard rice. Arming the cultivars with in-built resistance is therefore inevitable in contemporary Basmati breeding. Several genes conferring resistance to diseases such as BB and blast have already been deployed in Basmati cultivars3,22,28.

Table 1. Number of plants produced, selection differential and recurrent parent genome recovery in backcross generations. Δd – Selection differential, the mean deviation of the selected population from the base population.

| Generation | No. of plants | Foreground Selection | Traits | Phenotypic Selection for quality traits | Background Selection |
|------------|---------------|----------------------|--------|----------------------------------------|---------------------|
|            |               | No. of plants selected | PB1121 | Range before selection | Range in selected top ten plants | Δd | Progressive Δd | RPG recovery (%) |
| BC1F1      | 468           | 223                  | KLAC (mm) | 18.56 | 11.26 to 15.65 | 14.89 to 15.65 | 0.32 | 0.32 | 70.60–79.29 |
|            |               |                      | KLBC (mm) | 8.65 | 6.78 to 7.98 | 7.11 to 7.98 | 1.56 | 1.56 |          |
|            |               |                      | Aroma  | 2 | 0 to 2 | 1 to 2 | – | – |          |
| BC2F1      | 198           | 100                  | KLAC (mm) | 18.41 | 15.00 to 17.20 | 16.82 to 17.24 | 0.12 | 0.44 | 83.60–88.57 |
|            |               |                      | KLBC (mm) | 8.65 | 7.12 to 8.72 | 8.21 to 8.72 | 0.36 | 1.92 |          |
|            |               |                      | Aroma  | 2 | 1 to 2 | 2 | – | – |          |
| BC2F2      | 243           | 65                   | KLAC (mm) | 18.45 | 17.18 to 18.25 | 17.95 to 18.25 | 0.29 | 0.73 | 88.57–92.86 |
|            |               |                      | KLBC (mm) | 8.75 | 7.83 to 8.79 | 8.25 to 8.79 | 0.15 | 2.07 |          |
|            |               |                      | Aroma  | 2 | 2 | 2 | – | – |          |
| BC2F3      | 145           | 10                   | KLAC (mm) | 18.54 | 17.25 to 18.82 | 17.95 to 18.82 | 0.37 | 1.10 | 93.18–94.70 |
|            |               |                      | KLBC (mm) | 8.68 | 8.56 to 8.78 | 8.64 to 8.76 | 0.02 | 2.09 |          |
|            |               |                      | Aroma  | 2 | 2 | 2 | – | – |          |
| BC2F4      | 25            | 10                   | KLAC (mm) | 18.66 | 17.65 to 19.16 | 18.22 to 19.16 | 0.45 | 1.55 | 93.18–96.92 |
|            |               |                      | KLBC (mm) | 8.74 | 8.26–8.74 | 8.42 to 8.74 | 0.00 | 2.09 |          |
|            |               |                      | Aroma  | 2 | 2 | 2 | – | – |          |
Aroma is a by additive, dominance and epistatic gene action. Hence, progressive response to selection in later generation was uted to the number of genes governing them. The trait KLAC and KLBC are under polygenic control governed

### Table 2. Agronomic performance of the BC$_2$F$_3$ NILs carrying Xa38 in the genetic background of PB1121.

| Genotype       | DFF (days) | PH (cm) | TN | PL (cm) | FGP | SF (%) | TGW (g) | YLD (kg/ha) |
|----------------|------------|---------|----|---------|-----|--------|---------|-------------|
| Pusa1927-19-21 | 104.5      | 105.34  | 14.47 | 28.11   | 106.73 | 90.48* | 28.71   | 5454        |
| Pusa1927-14-77 | 105.0      | 104.04  | 15.38 | 29.53   | 110.01 | 90.91* | 27.42   | 6292        |
| Pusa1927-17-16 | 107.0*     | 109.77  | 16.24 | 28.64   | 109.88 | 87.89  | 28.54   | 5539        |
| Pusa1927-8-86  | 105.5      | 107.02  | 16.78 | 26.52   | 112.92 | 89.64* | 28.98   | 6760*       |
| Pusa1927-13-55 | 106.5      | 105.29  | 15.55 | 29.07   | 108.59 | 89.60* | 28.67   | 5698        |
| Pusa1927-47-72 | 105.0      | 107.56  | 15.80 | 27.84   | 109.92 | 91.52* | 29.23   | 6371        |
| Pusa1927-62-3  | 107.0*     | 113.03* | 13.95 | 27.82   | 109.38 | 89.28* | 27.49   | 5811        |
| Pusa1927-56-20 | 105.5      | 111.75* | 15.50 | 29.00   | 110.23 | 88.19  | 28.37   | 6691        |
| Pusa1927-75-56 | 107.5*     | 109.41  | 19.22*| 28.66   | 117.97*| 88.93  | 28.64   | 6079        |
| Pusa1927-62-42 | 106.5      | 111.14  | 15.95 | 27.50   | 114.51 | 85.60  | 28.96   | 6058        |
| PR114           | 105.4      | 107.33  | 15.03 | 28.57   | 108.44 | 85.29  | 28.88   | 5997        |
| CD (0.05)       | 1.36       | 3.86    | 2.94  | 2.08    | 6.31   | 3.38   | 1.96    | 591         |

*Significance at 5%. DFF: Days to 50% flowering, PH: Plant height, TN: Tiller number, PL: Panicle length, FGP: Filled grains/panicle, SF: Spikelet fertility, TGW: Thousand grain weight, YLD: Plot yield.

### Table 3. Grain and cooking quality, reaction to BB isolates and RPG recovery of the BC$_2$F$_3$ NILs carrying Xa38 in the genetic background of PB1121.

| Genotype       | KLBC (mm) | KBBC (mm) | L/B | KLAC (mm) | KBAC (mm) | KER | Aroma | % RPG recovery | BB lesion length (cm) |
|----------------|-----------|-----------|-----|-----------|-----------|-----|-------|---------------|-----------------------|
| Pusa1927-19-21 | 8.54      | 1.60*     | 5.35 | 17.60*    | 2.34      | 2.06*| 2     | 94.7         | Ludhiana 1.5          |
| Pusa1927-14-77 | 8.63      | 1.67      | 5.18 | 17.95     | 2.32      | 2.08 | 2     | 96.21        | Ludhiana 1.0          |
| Pusa1927-17-16 | 8.54      | 1.63      | 5.24 | 17.61     | 2.32      | 2.06*| 2     | 96.21        | Ludhiana 1.4          |
| Pusa1927-8-86  | 8.67      | 1.63      | 5.34 | 18.16     | 2.19      | 2.10 | 2     | 94.7         | Ludhiana 1.0          |
| Pusa1927-13-55 | 8.42      | 1.67      | 5.05 | 18.22     | 2.38      | 2.17 | 2     | 96.21        | Ludhiana 0.9          |
| Pusa1927-45-72 | 8.73      | 1.70      | 5.14 | 19.00     | 2.28      | 2.18 | 2     | 93.18        | Ludhiana 0.7          |
| Pusa1927-62-2  | 8.73      | 1.67      | 5.23 | 18.22     | 2.53      | 2.09*| 2     | 96.92        | Ludhiana 1.0          |
| Pusa1927-56-20 | 8.62      | 1.70      | 5.08 | 18.32     | 2.58      | 2.13 | 2     | 95.45        | Ludhiana 1.2          |
| Pusa1927-75-56 | 8.74      | 1.67      | 5.25 | 19.16     | 2.32      | 2.20 | 2     | 96.92        | Ludhiana 1.0          |
| Pusa1927-62-42 | 8.53      | 1.67      | 5.13 | 18.78     | 2.78*     | 2.20 | 2     | 96.21        | Ludhiana 1.0          |
| PB1121          | 8.65      | 1.67      | 5.21 | 18.62     | 2.29      | 2.20 | 2     | 95.45        | Ludhiana 21.4         |
| CD (0.05)       | 0.30      | 0.04      | 0.18 | 0.48      | 0.43      | 0.10 |       |              |                       |

In the current study, PB1121 was incorporated with the novel BB resistance gene Xa38 using the donor parent PR114-Xa38, an introgression line developed by crossing PR114 an elite rice cultivar of Punjab, India with a wild rice (O. nivara) acc. IRGC8182525,26. PR114 is a semi-dwarf, stiff strawed variety narrow, dark green erect leaves and possess extra-long, cf. Transgenic non-aromatic grains.33 However, the non-aromatic and cooking quality of PR114 and PR114-Xa38 is quite inferior as compared to the recurrent parent PB1121. Using poor grain quality donor parent in Basmati rice breeding impairs the grain and cooking quality of derived line, which imposes a challenge of recovering the quality of recurrent parent. Therefore, maximization of RPG and RPP recovery in the derived NILs is important28.

The recovery of recurrent parent genome to the extent of 96.9% was achieved with two backcrosses through integration of foreground, phenotypic and background selection. Background selection using the SSR markers usually target the non-coding and heterochromatic regions and therefore may not quantify the recovery of functional part of the genome. However, the phenotypic selection, which indirectly targets functionally expressed region of the genome, was augmented for hastening the process of reconstruction of recurrent parent phenotype. It was interesting to note that the selection differential for the trait KLAC was progressive along the generations and the recombinants at par or superior to recurrent parent PB1121, could be obtained by BC$_2$F$_3$ generation. For the trait KLBC, the selection differential was positive till BC$_2$F$_3$ generation only; whereas, in case of aroma, the trait fixation was achieved in BC$_2$F$_3$ generation. The varying selection differential for different traits can be attributed to the number of genes governing them. The trait KLAC and KLBC are under polygenic control governed by additive, dominance and epistatic gene action. Hence, progressive response to selection in later generation was obtained. Similar phenotypic gain was obtained for other agronomic traits also (data not presented). Aroma is a key Basmati trait, which is mainly governed by two recessive genes *badh1* and *badh2*30,31. Therefore, fixation for this trait was possible in the early backcross generations itself. The NILs carrying Xa38 gene, were agronomically similar to their recurrent parent PB1121 in most of the cases (Tables 2 and 3). Phenotypic selection prior to background selection has led to isolating the novel recombinant Pusa1927-75-56, which was similar to the recurrent parent but possessed significantly superior KLAC. The success of integrating phenotypic selection along with
background selection in the MABB program for isolating superior recombinants and substantially reducing the number of plants for background selection has been demonstrated earlier by Ellur et al. 3. In cases where limited backcrosses are given, NILs derived from a common backcross program differ with respect to their agronomic performance, grain and cooking quality traits because of the presence of minor donor segments on different chromosomes, which offer great opportunity to select NILs excelling the recurrent parent 3,32. It is pertinent to mention that stringent selection for grain quality has resulted in near complete recovery of PB1121 grain quality traits in most of the NILs developed.

The efficacy of phenotypic selection in hastening the recovery of recurrent parent phenotype has been earlier demonstrated while developing Improved Pusa Basmati 1 carrying BB resistance genes xa13 + Xa21 and Pusa 1609 carrying blast resistance genes32,33. In the current study, the recombinant selection on carrier chromosome resulted in delimiting the linkage drag to <0.5 mb upstream and <1.9 mb downstream of the Xa38 gene, which otherwise could have been a limiting step because of the donor parent PR114-Xa38, being a non-Basmati variety. On artificial screening, when all the derived NILs of PB1121 were exposed to two virulent BB isolates along with their parents, the donor PR114-Xa38 and the PB1121 NILs produced incompatibility reaction, as against PB1121 that was highly susceptible. The BB isolates, IARI-Kaul and IARI-Ludhiana were sourced from Haryana and Punjab respectively, two major Basmati rice growing states of India. Both isolates were the most predominant pathogenic variants of BB in respective states3. The resistance imparted by Xa38 gene implied that the gene may be effective against pathogenic races available in the major Basmati growing regions of India. Effectiveness of Xa38 against all common pathogenic variants of Xoo in Punjab has been earlier reported by Vikal et al.34.

In a recent study, Mondal et al.14 grouped the Xoo isolates of India into six different races based on their reaction against 12 monogenic-NILs carrying different BB resistance genes. However, these monogenic lines did not include Xa38 gene. The combination of BB resistance genes, xa13 + Xa21 has been considered as one of the best combinations that can combat most of the virulent races of the Xoo. To establish the spectrum of BB resistance
of Xa38 gene and to assess its efficacy in relation to xa13 + Xa21 combination, a set of Xa38 introgressed NILs of PB1121 were inoculated with the six Xoo races along with the recurrent parent PB1121. The BB resistance gene Xa38, as well as xa13 + Xa21, were effective against the Xoo races 1, 2, 3 and 6. Races 1 and 2 are predominantly distributed in the eastern India; race 3 is prevalent in Haryana; while race 6 is predominant in the entire northwestern region. Race 5 reported to be sporadically distributed across India could not infect the NILs with Xa38 while it was virulent against the PB1121 NILs carrying xa13 + Xa21. Therefore, deployment of Xa38 into the genetic background of elite rice varieties is essential to effectively combat the Xoo races 1, 2, 3, 5 and 6. On the other hand, Xoo race 4, reported to be present in eastern and northwestern states of India, was the most virulent race that could knock down xa13 + Xa21 combination as well as Xa38. Thus, further efforts are required to identify novel BB resistance genes or gene combinations that can provide resistance against the race 4 of Xoo.

To conclude, the study reports successful introgression of a highly effective resistance gene Xa38 into the genetic background of an elite Basmati rice variety PB1121 using a modified-MABB. The improved lines are now under evaluation in the National Basmati Trials for subsequent release. The study also highlights the importance of comparing the spectrum of resistance genes either singly or in combination against a wide range of pathogenic races, so that effective deployment of resistance genes can be planned to avoid future outbreaks of disease.

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Figure 4. Bacterial blight disease score of NILs carrying xa13 + Xa21 and Xa38 in the genetic background of Pusa Basmati 1121. Legend: PB1121 - Pusa Basmati 1121, Pusa 1927-PB1121 + Xa38 and Pusa 1718-PB1121 + xa13 + Xa21.
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Author Contributions
A.K.S. conceptualized the idea; K.S. provided the introgression line and generated crosses; R.K.E. and A.K. performed the experiments; R.K.E. and K.K.V. performed the statistical analysis; K.K.M. provided Xoo inoculum; K.K.M. and R.K.E. conducted blight inoculation experiment; R.K.E., A.K.S., G.K.S. and P.K.B. managed field trials; R.K.E., M.N., A.K.S. and G.K.S. managed the offseason field experiments; R.K.E. and A.K. formulated the manuscript; A.K.S., N.K.S. and K.V.P. supervised the experiments and revised the manuscript; All authors read and approved the final manuscript.

Additional Information
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