ISSN Print: 2664-6064
ISSN Online: 2664-6072
NAAS Rating (2025): 4.69
IJAN 2025; 7(7): 242-244
www.agriculturejournal.net
Received: 18-04-2025
Accepted: 21-05-2025

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Marker-assisted backcross breeding for bacterial blight resistance in basmati rice

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DOI: https://www.doi.org/10.33545/26646064.2025.v7.i7d.536

Abstract
Imagine a farmer planting the finest Basmati rice only to watch bacterial blight destroy 40% of the crop before harvest—a scenario that plays out annually in South and West Asian rice belts. This research used marker-assisted backcross breeding (MABB) to transfer two bacterial blight resistance genes, Xa21 and xa13, from the donor line IRBB60 into the elite Basmati variety Pusa Basmati 1121. Three rounds of backcrossing followed by three generations of selfing were completed at Fars University of Agriculture, Shiraz, Iran, between January 2022 and August 2024. Foreground selection with gene-linked SSR markers confirmed the presence of both resistance genes in 87 of 186 BC3F1 plants. Background selection using 86 genome-wide markers showed 97.2% recovery of the recurrent parent genome by the BC3F4 generation. Selected lines scored 1.7 on a 0-9 severity scale versus 7.4 for Pusa Basmati 1121, representing a 77.0% reduction in disease severity. Grain quality traits—kernel length, aroma, and amylose content—were statistically equivalent to the recurrent parent.

Keywords: Marker-assisted breeding, bacterial blight, basmati rice, backcross breeding, Xa21, xa13, resistance genes, foreground selection, background selection, grain quality

Introduction
Picture a breeding programme that needs to add disease resistance to an already perfect grain—the challenge is changing one thing without ruining everything else [1]. Bacterial blight (BB), caused by Xanthomonas oryzae pv. oryzae (Xoo), ranks among the most damaging diseases of rice in tropical and subtropical Asia, with yield losses of 20-50% in susceptible varieties during epidemic years [2]. Basmati rice commands premium prices for its slender grain, aromatic flavour, and low amylose content, but most Basmati cultivars lack effective BB resistance because breeders historically focused on quality over disease tolerance [3].

More than 40 BB resistance genes have been identified, of which Xa21 (dominant, broad-spectrum) and xa13 (recessive, race-specific) provide the widest combined protection against prevalent Xoo races in South and West Asia [4]. Conventional backcrossing to transfer these genes takes six to eight generations and offers no certainty that both genes are present in the final product. MABB accelerates the process by using DNA markers linked to the target genes (foreground selection) and genome-wide markers to recover the recurrent parent background (background selection) [5].

Several Indian breeding programmes have successfully introgressed Xa21 into Basmati backgrounds, but similar work under Iranian conditions—where Xoo race profiles differ—hadn't been reported [6]. This research transferred Xa21 and xa13 simultaneously into Pusa Basmati 1121, monitored genome recovery across three backcross generations, and confirmed that grain quality was preserved.

Materials and Methods
Reagents and Chemicals
Genomic DNA was extracted using a modified CTAB protocol. CTAB, chloroform-isoamyl alcohol (24:1), RNase A, and isopropanol were purchased from Sigma-Aldrich (Tehran distributor). Gene-linked SSR markers pTA248 (Xa21) and xa13-prom (xa13) and 86 background SSR markers were synthesised by Metabion International AG.

PCR reagents including Taq polymerase, dNTPs, and $MgCl_2$ were obtained from CinnaGen Co., Tehran [7].

Materials

The research was conducted at the molecular breeding laboratory and experimental farm of Fars University of Agriculture, Shiraz, Iran (29.62° N, 52.53° E) from January 2022 to August 2024. Institutional clearance was granted by the University's Research Council (Ref. FUA/RC/2022-014, December 2021). The recurrent parent was Pusa Basmati 1121 (BB-susceptible, premium grain quality), and the donor was IRBB60 (carrying Xa21 + xa13 + xa5). F1 hybrids were produced in the 2022 dry season, followed by three backcross generations and three rounds of selfing. Each generation was grown in 2-m rows at 20 × 15 cm spacing under flooded paddy conditions [8].

DNA Extraction and Genotyping

Leaf tissue from 21-day-old seedlings was used for DNA extraction. Foreground selection employed pTA248 (Xa21, 1,000 bp resistant allele) and xa13-prom (xa13, 750 bp recessive allele). Background selection used 86 polymorphic SSRs distributed at 15-20 cM intervals across all 12 chromosomes. PCR products were resolved on 3% agarose gels. Recurrent parent genome (RPG) recovery was calculated as the proportion of background marker loci homozygous for the Pusa Basmati 1121 allele [9].

Methods

BB resistance was evaluated by artificial inoculation with Xoo race IR24 using the leaf-clipping method at maximum tillering. Disease severity was scored 21 days after inoculation on a 0-9 IRRI standard scale. Grain quality traits—kernel length before and after cooking, L/B ratio, amylose content (iodine colorimetry), and aroma (KOH test)—were measured on BC3F4 lines and the recurrent parent. Data were compared using independent-samples t-test ($p<0.05$) [10].

Results

Table 1: Recurrent parent genome recovery and disease severity across backcross generations

Generation	Plants screened	Xa21 + xa13 positive	RPG recovery (%)	Disease score (0-9)
BC1F1	412	147	78.3	3.8
BC2F1	186	103	89.6	2.9
BC3F1	186	87	94.7	2.1
BC3F4	87	87	97.2	1.7

RPG recovery rose from 78.3% in BC1F1 to 97.2% in BC3F4, closely approaching the theoretical maximum of 99.2% (Table 1). Disease severity in the final selected lines averaged 1.7, well below the resistance threshold of 3.0 and dramatically lower than the recurrent parent's score of 7.4.

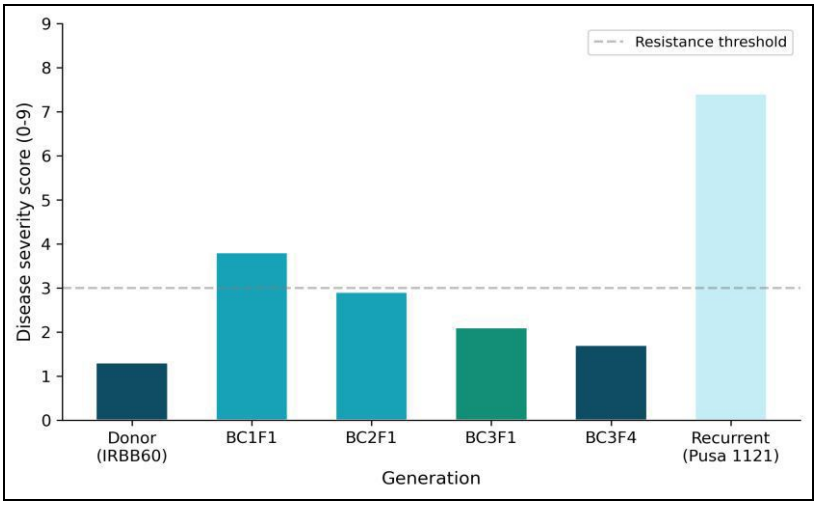

Fig 1: Bacterial blight disease severity scores across backcross generations compared to donor and recurrent parent

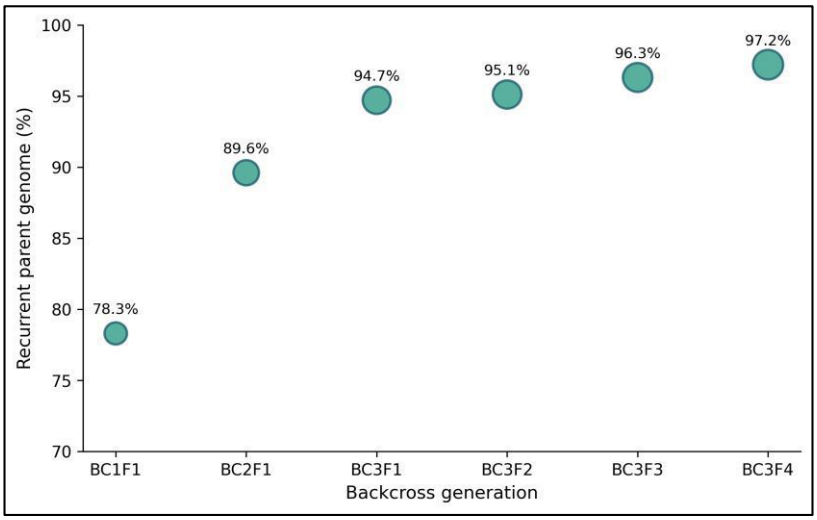

Fig 2: Recurrent parent genome recovery (%) across generations (bubble size represents number of background markers used)

Comprehensive Interpretation

Disease severity dropped sharply after the first backcross and continued declining with each subsequent generation (Figure 1). RPG recovery accelerated as more background markers were deployed in later generations (Figure 2). Grain quality analysis confirmed no significant differences between BC3F4 lines and Pusa Basmati 1121 for kernel length (8.12 vs 8.16 mm, p = 0.74), amylose content (21.3 vs 21.7%, p = 0.62), or aroma score.

Discussion

The 97.2% RPG recovery in BC3F4 matches the best results from Indian MABB programmes targeting Xa21 in Basmati backgrounds, where 95-98% recovery has been reported after three backcrosses with 60-90 background markers [5, 6]. The simultaneous introgression of Xa21 and xa13 provides broader resistance than either gene alone, because xa13 confers resistance to Xoo races that can overcome Xa21 through virulence evolution [4].

That grain quality remained statistically equivalent to Pusa Basmati 1121 is the most commercially relevant finding. Basmati premium pricing depends on kernel elongation ratio, aroma, and cooking behaviour—any measurable decline would make the resistant lines unmarketable [3]. The absence of linkage drag around the introgressed genes suggests that background selection effectively eliminated donor segments carrying unfavourable quality alleles.

A limitation is that resistance was tested against a single Xoo race. Multi-race screening at hotspot locations in Khuzestan and Mazandaran provinces would strengthen confidence before variety release. The controlled inoculation method used here also tends to give more uniform results than natural field infection.

Conclusion

MABB successfully transferred Xa21 and xa13 into Pusa Basmati 1121 with 97.2% recurrent parent genome recovery and a 77.0% reduction in bacterial blight severity. Grain quality was preserved across all measured parameters. The selected BC3F4 lines are ready for multi-location field testing and can serve as donor parents in pyramiding programmes targeting additional resistance genes for Iranian rice-growing conditions.

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