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Marker assisted introgression of bacterial blight resistance gene into NR-9 (Sairam) rice variety

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Abstract

Xanthomonas oryzae pv. *oryzae* (Xoo) causes Bacterial Blight (BB) in rice result huge yield losses worldwide. Host plant resistance offers the best solution for economic management of bacterial blight. NR-9 (Sairam) is a widely cultivated variety of Nirmal Seeds Pvt. Ltd. Pachora, Jalgaon (M.S), India, adopted for its superior grain quality and yield potential. Despite it NR-9 exhibits high susceptibility for bacterial blight. The resistance gene Xa21 is widely utilized in rice breeding because it confers strong, wide range resistance to bacterial blight. In current study we introgressed Xa21, into the NR-9(Sairam) through marker-assisted backcross breeding to provide resistance against bacterial blight using MR-6 as a donor parent for Xa21 gene. Foreground and background selection was performed using allele specific PCR markers. BC₃F₁ plants homozygous for Xa21 and exhibiting more than 92% recurrent parent genome recovery were advanced to the BC₃F₄ generation. Twelve MAS derived lines demonstrated resistance to bacterial blight and showed other traits comparable to NR-9. An improved version of NR-9, designated MAS-14-283, was developed and made available to farmer as NR-1033 (Sairaj) for commercial cultivation during Kharif 2023.

Keywords: Bacterial blight, Xa21, NR-9, NR-1033

Introduction

Rice (*Oryza sativa* L.) is a major staple crop that supports the food security of more than half of the global population. It is especially crucial in Asia and Africa, where it forms the foundation of daily diets and contributes significantly to economic stability (Khatun *et al.*, 2024) [17]. Bacterial Blight (BB) caused by the *Xanthomonas oryzae* pv. *oryzae* (Xoo) is a widespread and devastating disease of rice affecting crops in both temperate and tropical regions and is particularly destructive result in heavy yield loss. (Yugander *et al.*, 2017) [35]. It spreads throughout the vascular system often resulting in reduced yield and quality under BB infection (Chukwu *et al.*, 2019; Mew *et al.*, 1993) [6, 22]. Infections during the tillering stage result in total crop failure. resistant varieties, natural cultural practices, biological control and chemical measures are way to tackle BB infection in rice. It has been shown that the bacterial blight gene Xa21 originally derived from *Oryza longistaminata* is on 11 number chromosome of rice with pTA248 as gene linked marker confers resistance against multiple bacterial blight races by activating defense signaling pathway (Ronald *et al.*, 1992; Peng *et al.*, 2015; Khan *et al.*, 2023) [29, 24, 15]. The most impactful method for tackling BB is use of resistance varieties (Khush *et al.*, 1989) [18]. Because of the masking effect of genes, linkage drag, and epistasis (i.e., interallelic gene interaction), it would be challenging to choose rice crops with many resistance genes using the traditional method alone. Gene from resistance varieties transferred to susceptible one using marker assisted breeding method and many BB resistant rice varieties and hybrid's parents have been developed using this method that typically reducing varietal development duration upto 4-5 years in compassion with conventional breeding (Chen *et al.*, 2000; Gopalakrishnan *et al.*, 2008; Sundaram *et al.*, 2008; Ellur *et al.*, 2016; Abhilash Kumar *et al.*, 2017; Balachiranjeevi *et al.*, 2017; Singh *et al.*, 2019; Pradhan *et al.*, 2019) [4, 9, 31, 7, 1, 2, 30, 25].

NR-9 is a long duration (140-145 days), mid-tall (110-120 cm), non-lodging, high yielding and premium short slender variety released during 2005 and is popular among farmers. Despite its popularity, NR-9 is highly susceptible to bacterial blight disease and sporadic occurrence of BB

results in considerable loss to farmers who cultivate NR-9. Therefore, introgression of wide range resistant gene against BB in NR-9 offers an valid solution. In current work, we have successfully introgressed gene (Xa21) into the background of NR-9 to develop near isogenic lines (MAS-14-283 named as NR-1033) that has combined resistance for BB disease with all other features similar to NR-9

Materials and methods

Plant material

NR-9 (Sairam) is a long duration (140-145 days), mid-tall (110-120 cm), non-shattering, high yielding, short slender and superfine grain quality variety released in 2005 by Nirmal Seeds Pvt. Ltd., Pachora used as recurrent parent. Grains have better cooking quality with intermediate amylose content (23.50%) and very good consumer preference. The donor parent MR-6, carrying the Xa21 resistance gene was used for introgression.

Crossing and marker assisted backcross breeding

In Rabi-2016 crosses taken between NR-9 and MR-6 (Xa21 donor line) at NSPL Paddy Research Station, Dhasai, Murbad, Dist-Thane, Maharashtra, India and the F₁s were grown during the

Kharif 2017 season. True F₁s were confirmed with the help of foreground marker for the gene Xa21. The true F₁s were then backcrossed with the recurrent parent NR-9 to generate BC₁F₁ population developed from cross F₁ with NR-9 were subjected to foreground selection using gene linked marker and background selection using polymorphic markers. A set of 101 SSR markers evenly distributed across 12 chromosomes was used for parental polymorphism analysis to identify informative markers for background selection. BC₂F₁ population obtained by backcrossing selected BC₁F₁ plants with Xa21 gene and maximum RPG recovery with NR-9 and the MABB process was continued up to the BC₃ generation (Fig.1). Selected plants from BC₃F₁ generation with Xa21 and highest RPG were selfed to generate BC₃F₂s and plants that possessed Xa21 gene in the homozygous condition were identified from BC₃F₂s further advanced to the BC₃F₄ generation. The BC₃F₄ families were then evaluated in Initial Varietal Trial (IVT) Rabi-2020 and Advanced Varietal Trial (AVT)-I & II during Kharif-2021 & Rabi-2021 respectively. In Advanced varietal trial-I & II, the entry MAS-14-283 (NR-1033) was shown at par performance with NR-9 in respect of days to 50% flowering, plant height, yield grain quality parameters in addition to resistance to BB.

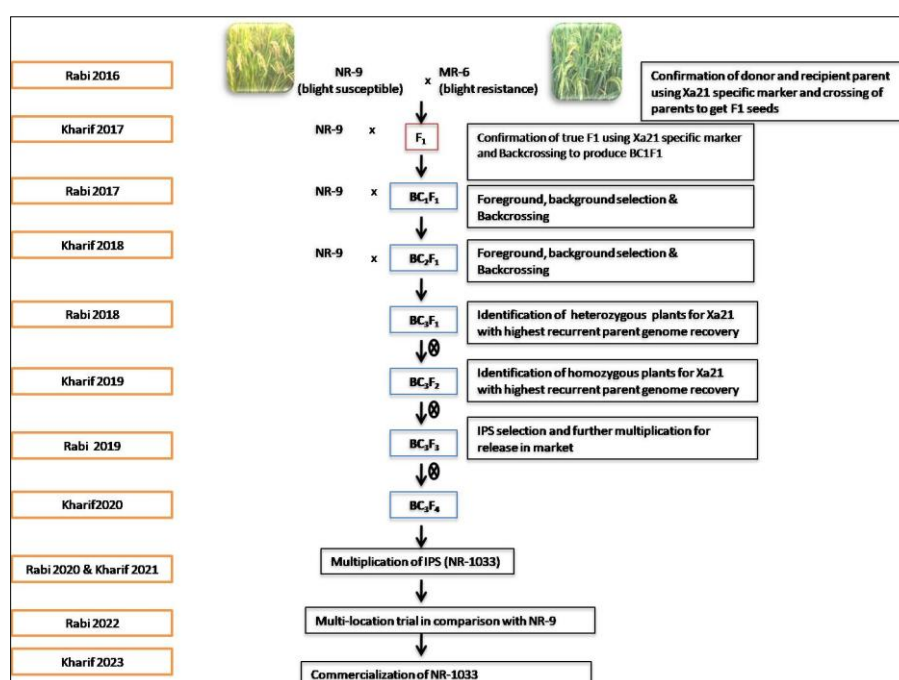


Fig 1: Crossing scheme for the marker-assisted introgression of Xa21 in the genetic background of Nirmal NR-9 (Sairam)

Inoculation and evaluation of bacterial blight culture response:

Donor line, recurrent parent, susceptible check and backcross populations derived from parents were screened using *Xanthomonas oryzae* pv. *oryzae* (Xoo) culture which received from IIRR, Hyderabad, India (Supp.table1). The bacterium was grown on wakimotos medium at 27 °C. The growth was scrapped off the plates, resuspended in sterile water and adjusted OD₆₀₀ by using colorimeter (CL223, Make-Elico) and the suspension was

used as inoculum. At the maximum tillering stage (45 to 55 days after transplanting), the rice plants were clip-inoculated with a bacterial suspension of Xoo strains (Kauffman *et al.*, 1973) [14]. Scissor was dipped in the inoculum (bacterial suspension) and one-fourth of top leaves was clipped through it. Each plant received an inoculation of five to ten leaves, and fourteen days after the inoculation, the disease response was recorded (Fig.2). The disease incidence was evaluated using the IRRI Standard Evaluation System (SES) scale (table1).

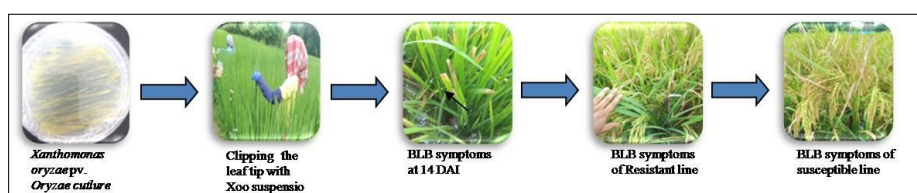


Fig 2: *Xanthomonas oryzae* culture inoculation by rice clipping method to plant for infection

Table 1: BB scoring as per IRRI Standard Evaluation System (SES)

Disease rating	Disease reaction	Description
1	Resistant	1- 5% leaf lesion area
3	Moderately resistant	6-12% leaf lesion area
5	Moderately susceptible	13-25% leaf lesion area
7	Susceptible	26-50% leaf lesion area
9	Highly susceptible	51-100% leaf lesion area

Screening for agro-morphological traits

The agronomic performance was conducted at NSPL Paddy Research Station, Dhasai, Murbad, and Dist-Thane, Maharashtra India (19.260N, 73.390E, and 83 m above MSL). Improved lines of NR-9 were field evaluated for the different agro-morphological traits by reordering data from 5 competitive plants chosen at random. Different traits evaluated as involving days to 1000 grain weight (g), grain number per panicle (no.), plant height (cm), spikelet fertility (%), number of productive tillers (no.), 50% flowering (days), panicle length (cm), grain type and yield (quintal/acre).

Evaluation of the grain quality characters

A laboratory dehuller was used to dehull 200 grams of raw rice kernels in order to assess head rice recovery (HRR) by taking ratio for the whole polished grains weight to raw grains weight (Lapis *et al.*, 2019) [20]. Ten grains from each entry was used to record observations for various grain quality parameter such as Kernel Length After Cooking (KLAC), Kernel Width Before Cooking (KWBC), Elongation Ratio (ER) and length-width ratio (Khanna *et al.*, 2015). The whole milled kernels were selected and soaked for 30 min in 10 ml of distilled water taken in test tubes. The lower portion of the tube containing the rice kernels was then fully submerged for eight to ten minutes after the tubes were dropped into a boiling water bath. Data was recorded after the fried kernels were transferred into a petri plate and allowed to cool at room temperature. Alkali Spreading Value (ASV) is an indicator of gelatinization temperature and cooking texture. Six ground entire kernels were spread out in a petri-plate and immersed in 1.7% KOH for 23 hours at 30°C in order to calculate ASV. The ASV per sample was recorded as per the 17 score scale (Little *et al.*, 1958) [21]. The amylose content of the improved lines was estimated as per standard protocol (Juliano, 1971). All measurements were replicated appropriately to ensure reliability.

Statistical analysis

The experiment was laid out using a Randomized Complete Block Design (RCBD), and the resulting data were subjected to statistical analysis. Agro-morphological observations and phenotypic BB screening data were analyzed following standard statistical procedures, including the calculation of means, significant Standard Error of the Mean (SEM $\pm$), Analysis of Variance (ANOVA), and Least Significant Difference (LSD) at the 5% significance level. All computations related to the significant SEM ($\pm$) were performed using MS Excel.

Results and Discussion

BB is the main obstacle to rice cultivation and results in significant yield loss. Given the need for greater rice output in a rapidly expanding population, molecular breeding is important in the fight against BB disease in rice, even though it cannot be used in place of conventional breeding. Through MAS, a number of BB resistance genes have been found, described, integrated, and pyramided. (Fiyaz *et al.*, 2022; Chukwu *et al.*, 2019; Baliyann *et al.*, 2018; Yugander *et al.*, 2018; Laha *et al.*, 2016) [8, 6, 34, 19]. By

reducing the from donor parents, MABB offers an excellent chance for the exact transfer of a desired donor segment. MABB program and emphasize strict phenotypic selection (Yugander *et al.*, 2018; Sundaram *et al.*, 2008; Sundaram *et al.*, 2014) [34, 31, 32]. Although Xa21 confers broad-spectrum resistance, monitoring pathogen evolution and pyramiding with additional genes in future programs will be important for durability.

BB resistance gene Xa21 was added to the genetic background of NR-9 by combining MABB with phenotype selection. Total 35 F₁ seeds produced from cross between NR-9 and MR-6. The most successful breeding strategy was found to be combining marker-assisted foreground and background selection with phenotypic selection, which significantly improved the breeding program's efficiency. Nine true F₁s heterozygous for the Xa21 gene were found using foreground selection, and these were backcrossed with NR-9 to produce 62 BC₁F₁ plants. Foreground selection using the markers pTA248, for Xa21 gene on the BC₁F₁s found 30 plants with gene of interest (Fig.3). Out of total 101 marker used for parental polymorphic study identified total 36 polymorphic markers for background selection (Fig. 4; table 2). The selected phenotypically desirable plants from BC₁F₁ were genotyped for background selection with 36 polymorphic markers which showed RPG between 57% to 71%. the BC₁F₁ plants was backcrossed with NR-9 to produce 81 BC₂F₁s. A similar selection approach was adopted and 43 BC₂F₁ plants heterozygous for Xa21 gene with more than 82% RPG recovery were identified. Total 71 BC₃F₁ plants produced by crossing the BC₂F₁ plant with NR-9 to generate and out of 71 plants total 38 were positive for gene of interest Xa21, and the RPG recovery in these positive plants ranged between 92.7% and 95.1% (table 3). Positive BC₃F₁ plant with the maximum RPG recovery was selfed to generate total 343 plants. Foreground selection of BC₃F₂s found 91 plants positive for gene of interest Xa21 and out of 91 plants 12 plants based on phenotypic selection were advanced up to the BC₃F₄ generation using pedigree method as BC₁F₁ → BC₂F₁ → BC₃F₁ → BC₃F₄. Twelve improved lines of NR-9 with target genes (Xa21) in homozygous conditions had increased resistance screened with the artificial inoculation of *Xoo* isolates in the BC₃F₂ generation. Marker-assisted background analysis of trait introgressed lines identified lines with highest recurrent parent genome recovery (Sundaram *et al.*, 2008; Nandi *et al.*, 2023) [31, 23]. RPG levels above 92% indicate successful introgression with minimal donor genome retention, reducing linkage drag and ensuring recovery of NR-9 agronomic characteristics. In present study introgressed line MAS-14-283 showed the highest recurrent parent genome recovery.

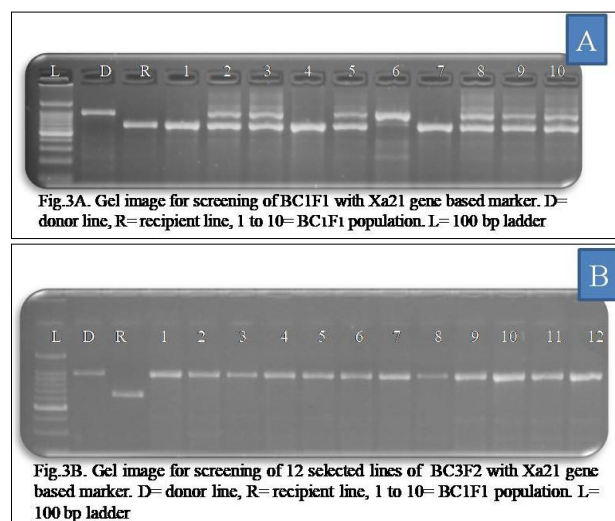


Fig 3: Foreground selection of F₁ and BC₃F₂ population using gene linked marker

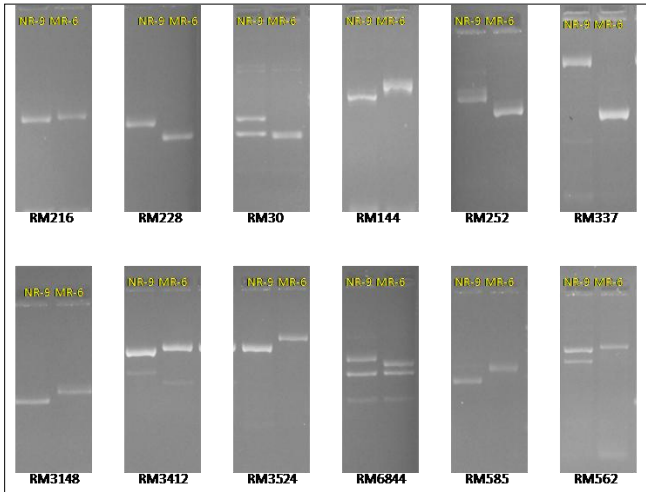


Fig 4: Polymorphic marker study for NR-9 and MR-6 parents

Table 2: Molecular markers used for polymorphic study between NR-9 and MR-6

Chromosome number	Total marker used for screening	Total polymorphic marker	Polymorphic markers
1.	21	8	RM 562, RM 493, RM 3412, RM3148, RM10033, RM11704, RM11012, RM12233
2.	11	5	RM 6844, RM 208, RM12398, RM324, RM424
3.	10	3	JGT03-26.8, RM15791, RM16030
4.	4	2	RM3524, RM 252
5.	3	2	RM18516, RM 164
6.	8	6	JGT06-6.81, JGT06-6.9, Chr 6-9.0, RM 585, RM 204, RM30
7.	11	3	RM21941, RM 70, RM6697
8.	8		RM22266, RM 337, RM6699
9.	2	0	
10.	10	3	RM 258, RM 228, RM216
11.	5	1	RM144
12.	8	3	RM5479, RM20, RM28157
Total	101	36	

Table 3: Summary of total plants screened using foreground and background marker in each backcross generation

Sr. No.	Generation	Number of plants screened	Number of plants positive for Xa21	Average recurrent parent genome recovery of selected backcross plant (%)
1.	F1	35	9	NA
2.	BC1F1	62	30	70
3.	BC2F1	81	42	85
4.	BC3F1	71	38	93
5.	BC3F2	343	91	NA

Twelve Xa21 gene introgressed lines and two parent’s agro-morphological data showed that the improved lines had superior recurring parent traits and were resistant to bacterial blight. In green house under artificial inoculation with BLB culture an average lesion length of 18.56 ± 3.30 cm for NR-9 as against MR-6 that showed average lesion length of 1.5 ± 0.24 cm. NR-9 improved lines showed an average lesion length of 1.5-2.8 cm that showed good level of BB resistance over NR-9 (Supplementary table1; Fig.5). The BB screening also carried out for Xa21 gene introgressed lines using bacterial blight culture from IIRR Hyderabad and symptoms clearly observed for susceptible and resistance lines (Abhilash Kumar *et al.*, 2017; Balachiranjeevi *et al.*, 2017) [1, 2].



Fig 5: Bacterial blight disease reaction of the selected pyramided lines and recurrent parent NR-9

Evaluating DUS features lastly in the improved lines is not preferable as there is no way to get the desirable RP characters back. Therefore, foreground-positive individuals should be chosen first, followed by those with a higher yield index and those chosen for higher recovery of RP traits (Nandi *et al.*, 2023) [23]. MABB programs also use careful visual selection to make sure the new lines that improved keep the plant traits and yield compared to the original parent variety (Joesph *et al.*, 2004; Hari *et al.*, 2013) [12, 11]. Various studies mentioned that some gene introgressed lines had higher yields than the recurrent parent; this could be because the recurrent parent inherited certain yield features or QTLs from the donor parent (Pradhan *et al.*, 2015; Pradhan *et al.*, 2016; Yugander *et al.*, 2018; Wang *et al.*, 2021) [26, 27, 34, 33]. All of the introgressed lines were on at par with the recurrent parent, according to an analysis of variance for several morphological and grain quality characteristics. *viz.* days to 50% flowering, plant height, 1000 grain weight (g), grains per panicle (No.), number of productive tillers (No.), spikelet fertility (%), panicle length (cm) & yield per acre (q). In our study, two improved lines were performed well over the NR-9 in terms of number of productive tillers per plant, spikelet fertility (%) and grain yield per acre (quintal/acre). During the *Kharif*- season, the

chosen improved lines of NR-9 at the BC₃F₄ generation along with parents were assessed for specific agro-morphological traits. The findings showed that, in terms of yield components, most selections were similar to recurrent parents. Various traits as panicle length (cm), grains per panicle (No.), days to 50% flowering, spikelet fertility, plant height (cm), 1000 grain weight, number of productive tillers (No.) & yield per acre (q) found non significant. Days to 50% flowering of the improved lines ranged from 115 to 119 days compared with 118 days of NR-9. Pant height of the improved lines ranged from 109 cm to 118 cm compared with 116 cm of NR-9. The mean number of productive tillers per plant of the improved lines ranged from 12 to 14 compared with 13 of the recurrent parent NR-9. Panicle length for improved lines were in the range of 24.8 cm to 25.4cm. The mean grains per panicle (No.) of the improved lines ranged from 338 to 345 (No.) compared with 341 (No.) of the recurrent parent NR-9. The entries such as MAS-14-283 and MAS-14-305 were promising with number of productive tillers/ plant (14 per plant), spikelet fertility (88.12% & 88.11% respectively) & yield (23.86 & 23.84 quintal/acre respectively) over the recurrent parent NR-9 i.e. 22.54 quintal/acre. All the improved lines had grain type, similar to NR-9 (table 4; Fig. 6).



Fig 6: Panicles of the selected pyramided lines and recurrent parent NR-9

Table 4: Agro-morphological characters of pyramided lines along with parents under field evaluation

Entry	Days to 50% flowering	Plant height (cm)	No. of productive tillers/plant	Panicle length (cm)	No. of grains/panicle	Spikelet fertility %	1000 grain wt. (g)	Grain type	Yield/acre (Q)
MAS-14-158	118	113	12	24.8	344	87.39	11.3	SS	23.04
MAS-14-260	118	116	13	25.4	344	87.78	11.4	SS	22.91
MAS-14-283	118	109	14	24.9	342	88.12	11.5	SS	23.86
MAS-14-305	115	117	14	25.3	340	88.11	11.2	SS	23.84
MAS-14-330	118	117	13	25.4	338	87.37	11.4	SS	23.21
MAS-14-362	117	115	13	25.2	345	86.82	11.5	SS	23.28
MAS-14-428	119	117	13	25.1	338	87.01	11.3	SS	22.91
MAS-14-469	117	110	13	25.2	342	86.38	11.4	SS	23.04
MAS-14-525	118	112	12	25.1	343	87.39	11.5	SS	22.54
MAS-14-575	116	113	13	25.2	338	87.32	11.4	SS	23.29
MAS-14-600	118	116	12	25.1	343	87.37	11.5	SS	22.74
MAS-14-619	115	118	13	25.4	340	87.05	11.3	SS	23.21
NR-9 (Recipient)	118	116	13	25.1	341	87.37	11.7	SS	22.54
MR-6 (Donor)	105	92	12	23.6	241	83.96	14.3	MS	18.22
C.D. @ 5%	1.683	4.95	0.552	0.452	7.052	1.972	0.287		2.472
SE(m)	0.576	1.694	0.155	0.155	2.412	0.675	0.098		0.846

C.V.	0.856	2.599	1.168	1.068	1.25	1.341	1.466		6.436
Mean	117	113	13	25.1	334	87.1	11.6		22.76
Minimum	105	92	12	23.6	241	83.96	11.2		18.22
Maximum	119	118	14	25.4	345	88.12	14.3		23.86

Rice Grain Quality (RGQ) and yield represent the most critical and economically significant traits in rice cultivation, as they directly influence consumer preferences and contribute substantially to global food security (Cheng *et al.*, 2022) [5]. Appearance, milling quality, cooking behavior, sensory traits, palatability and nutritional value are key factors that influence its market price. Rice physical quality includes traits such as grain shape, head rice yield, grain size, chalkiness, the amount of broken grains, grain breakage, color, and overall appearance (Handoka *et al.*, 2022) [10]. Measuring different parameters such as texture traits, cooking time, grain expansion, amylose content, gelatinization temperature, gel consistency, the levels of protein, lipids, and micronutrients helps in product establishment in market (Prodhan *et al.*, 2024) [28].

The selected BC₃F₄ improved lines were subjected to rigorous

phenotypic evaluation for grain quality and the results indicated that their quality attributes closely matched those of the recurrent parent. The improved lines have good level in of inbuilt resistance to BB and close features for agro morphological and grain related traits and yield at par with NR-9. All the improved versions possessed short slender grain type & showed similarity to recurrent parent in all the grain quality parameters analyzed, viz., kernel breadth (1.36 mm - 1.43 mm), hulling (76.5-81.5%), milling (67.3-71.4%), HRR (57.9-63.2%), kernel length (5.34 mm - 5.68 mm), L/B ratio (3.79 - 3.97), kernel length after cooking (9.0-10.20 mm), elongation ratio (1.66 - 1.87), alkali spreading value (5.0 - 6.0) and intermediate amylose content (21.33 - 24.88%) (table.5). MAS-14-283 (NR-1033) and MAS-14-305 (NR-1047) were shown very good cooking quality of rice as comparable with the recurrent parent NR-9 (Fig.7).

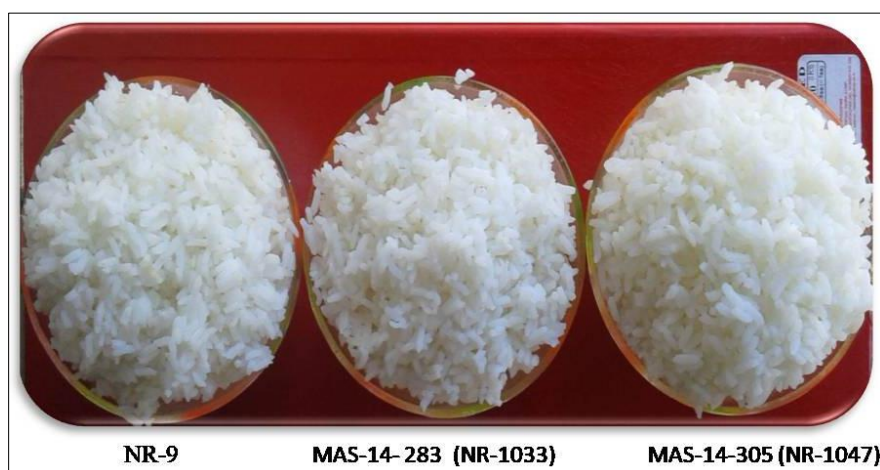


Fig 7: Cooking quality of the selected pyramided lines and recurrent parent NR-9

Table 5: Grain quality parameters of pyramided lines along with parents

Entry	Hulling (%)	Milling (%)	HRR (%)	KL (mm)	KB (mm)	L/B Ratio	KLAC (mm)	ER (mm)	ASV	A C (%)
MAS-14-158	79.5	69.4	59.7	5.37	1.39	3.87	9.50	1.77	5.0	22.76
MAS-14-260	80.0	70.4	60.3	5.45	1.37	3.97	9.70	1.78	6.0	21.43
MAS-14-283	78.5	69.8	62.6	5.46	1.39	3.92	9.40	1.72	5.5	23.50
MAS-14-305	79.8	70.4	63.2	5.35	1.36	3.94	9.80	1.83	5.0	23.88
MAS-14-330	81.5	68.3	58.4	5.68	1.43	3.98	9.40	1.66	5.5	23.25
MAS-14-362	80.4	71.4	60.4	5.34	1.41	3.79	9.00	1.69	6.0	24.39
MAS-14-428	78.4	68.3	58.4	5.40	1.39	3.88	9.40	1.74	5.0	23.80
MAS-14-469	81.5	69.4	57.9	5.47	1.40	3.90	9.70	1.77	6.0	24.88
MAS-14-525	79.4	70.3	60.4	5.46	1.38	3.97	10.20	1.87	5.5	23.23
MAS-14-575	76.5	67.3	58.7	5.42	1.39	3.90	9.80	1.81	5.0	24.50
MAS-14-600	78.6	68.3	62.4	5.40	1.40	3.85	9.40	1.74	6.0	24.50
MAS-14-619	79.4	67.9	61.4	5.45	1.39	3.92	9.70	1.78	5.0	21.33
NR-9 (Recipient)	79.8	69.4	60.4	5.36	1.42	3.79	9.40	1.75	5.0	23.50
MR-6 (Donor)	78.9	68.7	59.4	4.84	1.72	2.81	8.50	1.76	5.5	22.76
C.D. @ 5%	0.563	0.562	0.62	0.056	0.046	0.129	0.081	0.045	NA	0.316
SE (m)	0.193	0.193	0.213	0.019	0.016	0.044	0.028	0.016	NA	0.109
C.V.	0.422	0.483	0.61	0.618	1.927	2.01	0.508	1.527	NA	0.804

Note: HL/B ratio- >3 and seed length <6mm (short slender); L/B ratio- <2.5 and seed length <6mm (short bold); L/B ratio- 2.5-3 and seed length <6mm (medium slender), L/B ratio- >3 and seed length >6mm (long slender), L/B ratio- <3 and seed length >6mm (long bold), L/B ratio- >3 and seed length >7.5mm (Extra-long slender), L/B ratio- >3 and seed length >6.1mm (basmati)

After being assessed in multi-location trials, the introgressed lines made available to farmers as improved cultivars MAS-14-283 [NR-1033 (Sairaj)]. In the states of MH, CG, MP, GJ, TN, UP,

KA, AP, BR, and TS performance of NR-1033 in a multi-location farmers field trial carried out in Rabi-2022 demonstrates good farmer acceptance (Fig. 8).



Fig 8: Field acceptance of NR-1033 during multi-location trial

To enhance yield stability and sustainability in BB prone regions where NR-9 is cultivated, advanced breeding lines developed through MAS and phenotypic selection offer effective and durable resistance. In this study, MAS enabled the successful introgression of the broad spectrum BB resistance gene Xa21, resulting in twelve promising improved lines with high RPG recovery and strong agronomic performance. The introgressed line MAS-14-283 [NR-1033 (Sairaj)] exhibited grain yield and quality comparable to NR-9 and demonstrated excellent disease resistance. These improved lines not only serve as superior cultivars for farmers but can also be effectively used as BB resistant donors in breeding programs aimed at enhancing resistance in other elite short slender or fine grain varieties. Overall, MAS enabled backcross breeding facilitated the efficient transfer of Xa21 gene into NR-9 while maintaining grain quality and agronomic performance. Several improved lines exhibited strong BB resistance and yields comparable or superior to NR-9, with MAS-14-283 emerging as the most promising line. This demonstrates that MAS in conjunction with phenotypic selection may be a dependable approach in backcross breeding initiatives.

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