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SSR-BASED MOLECULAR PROFILING OF ADVANCED RICE BREEDING LINES FOR YIELD, DISEASE RESISTANCE AND GRAIN QUALITY TRAITS

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ABSTRACT
Present study was aimed to evaluate advanced rice breeding lines for yield and its associated traits and to screen these lines for bacterial leaf blight (BLB) resistance and quality traits using molecular markers. Thirty-eight advanced breeding lines along with three checks were evaluated in Randomized Complete Block Design (RCBD) with three replications having a plot size of 10 m^2 and spacing of 20 x 15 cm. Analysis of variance revealed significant variation among the lines for all the traits recorded. Molecular screening using marker pTA248 targeting the Xa21 gene amplified a 925 bp product, marker xa-13 prom targeting the xa13 gene amplified a 450 bp product, while marker RM122 targeting the xa5 gene locus, amplified a product size of 240 bp. In addition, aroma marker BADEX7-5 was employed to target the badh2 gene which amplified a product size of 95 bp. Five advanced breeding lines along with one check exhibiting a moderately resistant response based on phenotypic performance revealed the presence of at least two resistance genes thereby, indicating that the observed phenotypic resistance was due to the presence of resistance genes.

KEYWORDS: Bacterial leaf blight, marker-assisted selection, molecular markers, aroma

INTRODUCTION

Rice (Oryza sativa L., 2n = 2x = 24) is the most important staple cereal that sustains more than half of the global population. In India, rice was cultivated over an area of 46.38 million hectares with an annual production of 135.80 million tonnes and productivity of 2.93 t ha^{-1} (Anonymous. 2024) while, in the Union Territory of Jammu and Kashmir, it occupies 258.84 thousand hectares, producing 6516 thousand quintals with a productivity of 25.17 quintals ha^{-1} (Anonymous. 2025). Enhanced productivity, resistance and grain quality of rice are the central goals of breeding programmes because of the steadily rising demand. Grain yield is the most critical trait which is highly complex, governed by multiple genes and strongly influenced by environmental conditions. Alongside, yield, quality traits such as grain appearance, cooking quality and aroma determine consumer preference and market value. Amylose content plays a key role in texture, while aroma, primarily contributed by 2-acetyl-1-pyrroline (2AP), regulated by the badh2 gene, is a defining feature of premium basmati varieties. In addition, biotic stresses, particularly bacterial leaf blight (BLB) caused by Xanthomonas oryzae pv. oryzae (Xoo), pose a severe challenge, leading to yield losses of 20-40 per cent during the tillering stage and 50-80 per cent under severe infection (Ullah et al, 2022). Although, more than forty bacterial leaf blight resistance genes have been identified (Pradhan et al, 2019) but among these Xa21, xa13 and xa5 are being widely used in breeding programmes as they confer durable wide spectrum resistance against many strains of pathogen in combination.

Despite having good potential, advanced breeding lines often remain under-utilized due to the lack of one or more desirable traits and critical evaluation of these lines is imperative. Conventional breeding based on phenotypic selection has been the backbone of rice improvement, but its efficiency is often constrained by environmental influences on trait expression. In contrast, molecular markers such as SSRs, STSs, SNPs and functional markers provide a precise, environment-independent means of identifying alleles linked to key traits, thereby, accelerating selection. The integration of morphological evaluation with molecular profiling enables a balanced assessment of advanced breeding lines for improvement and helps identifying superior ones with desirable combination of traits. Keeping these considerations in view, the aim of the present study was to profile advanced rice breeding lines by evaluating their yield and yield-attributing traits, assessing their resistance to bacterial leaf blight employing both phenotypic and molecular approaches and assessing key grain quality traits including aroma using functional markers.

MATERIALS AND METHODS

Experimental material comprised of thirty eight stable advanced generation (F_8) lines along with three checks viz., Basmati 370, Pusa Basmati 1847 and Jammu Basmati 118 (Table 1). Twenty five days old healthy seedlings of these lines were transplanted in a Randomized Complete Block Design (RCBD) with three replications. Each line was

transplanted with one seedling per hill in a plot size of 10 m^2 in each replication with row to row and plant to plant spacing of 20 x 15 cm. Standard rice cultivation practices were followed to ensure optimal growth of crop plants. For each line, yield and yield-related traits were recorded by randomly selecting five plants from each replication.

Table 1: Description of advanced breeding lines of rice evaluated in the present study

Code Allocated	Breeding Line/Check	Cross combinations	Code Allocated	Breeding Line/Check	Cross combinations
GP-1	SJR 76-1-2	HKR-02-486/Saanwal basmati	GP-22	SJR 1506-33	Pusa basmati 1121/Pusa 1509
GP-2	SJR 80-1-1	Yamini/Basmati 370	GP-23	SJR 1506-34	Pusa basmati 1121/Pusa 1509
GP-3	SJR 82-2-3	RR 564/MAUB-13	GP-24	SJR 1506-35	Pusa basmati 1121/Pusa 1509
GP-4	SJR 92-2-2	IR 75483-385-2-2/PB 1	GP-25	SJR 1507-40	Basmati 370/Pusa basmati 1121
GP-5	SJR 82-4-1	RR 564/MAUB-13	GP-26	SJR 1507-45	Basmati 370/Pusa basmati 1121
GP-6	SJR 103-4-1	Pusa 2517-2-51-1/Vasumati	GP-27	SJR 1509-58	Basmati 370/Pusa basmati 1509
GP-7	SJR 103-2-2	Pusa 2517-2-51-1/Vasumati	GP-28	SJR 1401-61	Basmati 564/Pusa basmati 1509
GP-8	SJR 103-2-3	Pusa 2517-2-51-1/Vasumati	GP-29	SJR 1401-62	Basmati 564 / Pusa basmati 1509
GP-9	SJR 103-4-1	Pusa 2517-2-51-1/Vasumati	GP-30	SJR 1402-67	Sanwaal basmati/Pusa basmati 1509
GP-10	SJR 103-4-2	Pusa 2517-2-51-1/Vasumati	GP-31	SJR 1402-68	Sanwaal basmati/Pusa basmati 1509
GP-11	SJR 121-2-2	Pusa 2517-2-51-1/PB-1/PB-1	GP-32	SJR 1403-71	Type 3/Pusa 1460
GP-12	SJR 123-2-1	Ranbir basmati/Pusa 2517-2-51-1/P-2511	GP-33	SJR 1403-72	Type 3/Pusa 1460
GP-13	SJR 1501-2	Basmati 370/ Pusa 1460	GP-34	SJR 1404-81	Basmati 386/Pusa basmati 1460
GP-14	SJR 1501-4	Basmati 370/ Pusa 1460	GP-35	SJR 1406-84	VB 24/Pusa basmati 1121
GP-15	SJR 1501-7	Basmati 370/ Pusa 1460	GP-36	SJR 1407-89	Ranbir basmati/Pusa 1121
GP-16	SJR 1501-13	Basmati 370/ Pusa 1460	GP-37	SJR 1409-96	Pusa basmati 1121/Ranbir basmati
GP-17	SJR 1502-18	Basmati 564/ Pusa 1460	GP-38	SJR 1409-99	Pusa basmati 1121/Ranbir basmati
GP-18	SJR 1503-20	Sanwaal basmati/ Pusa 1460	GP-39	Basmati 370	Check 1
GP-19	SJR 1504-26	Pusa basmati 1121/ Pusa 1460	GP-40	Pusa Basmati 1847	Check 2
GP-20	SJR 1504-27	Pusa basmati 1121/ Pusa 1460	GP-41	Jammu Basmati 118	Check 3
GP-21	SJR 1504-28	Pusa basmati 1121/Pusa 1460			

Field pathotyping

Natural field screening for bacterial leaf blight was carried out using the 0 to 9 Standard Evaluation System (SES) scale, IRRI, 2014 (Table 2) and disease severity index was calculated. Further, the lines exhibiting less than 10 per cent disease severity were selected for screening to ascertain presence/absence of resistant genes using molecular markers.

Table 2: Standard Evaluation Scale (SES), IRRI, 2014 used for screening lines against bacterial leaf blight

Score	Diseased (affected lesion area)	Description
1	1-5 per cent	Resistant (R)
3	6-12 per cent	Moderately resistant (MR)

5	13-25 per cent	Moderately susceptible (MS)
7	26-50 per cent	Susceptible (S)
9	51-100 per cent	Highly susceptible (HS)

DNA extraction and purification

Genomic DNA was isolated from three-week-old seedlings using the cetyl trimethyl ammonium bromide (CTAB) method (Doyle, J.J. and Doyle, J.L., 1987) with slight modifications. Approximately 5 g of leaf tissue was ground in liquid nitrogen and DNA was extracted using preheated CTAB extraction buffer containing β-mercaptoethanol. DNA was precipitated with chilled isopropanol, washed twice with 75% ethanol, air-dried and dissolved in TE buffer. Purification was performed by RNase treatment followed by phenol-chloroform extraction. The DNA concentration and purity were assessed using a NanoDrop spectrophotometer (Thermo Scientific) and bands were confirmed on 0.8% agarose gel electrophoresis.

PCR amplification

Four gene-specific molecular markers based on available literature viz., BADEX7-5, pTA248, xa13-prom and RM122 were used for molecular profiling (Table 3). PCR was performed in 15 µL reactions containing template DNA (~50 ng), primers, dNTPs, $MgCl_2$, PCR buffer and Taq DNA polymerase. Thermal cycling was conducted in an Eppendorf thermocycler using the following programme: initial denaturation at 94°C for 5 min, followed by 38 cycles of denaturation (94°C for 30 sec), annealing (54-58°C for 30 sec) and extension (72°C for 30 sec) with a final extension at 72°C for 7 min.

Table 3: Molecular markers used for screening advanced breeding lines against bacterial leaf blight resistance and aroma

S. No.	Primer	Targeted gene	Chr. No.	Sequence	T_m (°C)	Reference
1.	BADEX7-5	badh2	8	F- TGTTTTCTGTTAGGTTGCATT R- ATCCACAGAAATTTGGAAAC	54	Sakthivel et al., 2009
2.	pTA248	Xa21	11	F- AGACGCGGAAGGGTGGTTCCCGGA R- AGACGCGGTAATCGAAAGATGAAA	58	Ronald et al., 1992
3.	xa13-prom	xa13	8	F- GGCCATGGCTCAGTGTTTAT R- GAGCTCCAGCTCTCCAAATG	55	Hajira et al., 2016
4.	RM122	xa5	5	F- GAGTCGATGTAATGTCATCAGTGC R- GAAGGAGGTATCGCTTTGTTGGAC	55	Chen et al., 1997

Analyzing amplified PCR products

A 3% agarose gel was prepared by mixing 3 g of agarose with 100 ml of 1xTE buffer and heating it in the microwave for 2-3 minutes until fully dissolved. Once slightly cooled, 10 µL of ethidium bromide was added for DNA visualization under UV light. The gel solution was poured into a casting tray with combs to form wells and left to solidify at room temperature for 20-25 minutes. For sample preparation, 2 µL of loading dye was mixed with 4 µL of PCR product, gently mixed and loaded into the wells of the agarose gel. This process was repeated for all PCR products from different primer pairs. A 100 bp DNA ladder was loaded as a molecular marker to estimate the size of the PCR products generated by the specific primers. Electrophoresis was conducted at 100 V for 3 hours. Afterward, the gel was visualized under UV light using a Gel Documentation System. The size of each DNA band was compared to the marker bands to analyze the band profiles for each primer set.

Analysis of variance (ANOVA)

Analysis of variance (ANOVA) for a Randomized Complete Block Design (RCBD) was performed on replicated data to assess genetic variability among lines, following the method of Cochran and Cox (1957). The F-test was used to determine significant differences in treatment means by comparing each mean square against the error mean square (M_e). The analysis of variance was carried out using the fixed effect model given below:

Model:

$$Y_{ij} = \mu + g_i + r_j + e_{ij}$$

Where,

Y_{ij} = phenotypic effect of the i^{th} genotype in the j^{th} replication

μ = general mean

g_i = effect of the i^{th} genotype

r_j = effect of the j^{th} replication

e_{ij} = random error associated with the i^{th} genotype in the j^{th} replication.

The total variance was divided into different components using ANOVA for a Randomized Complete Block Design (RCBD).

RESULTS AND DISCUSSION

Analysis of variance revealed substantial genetic variation among the advanced breeding lines for all the traits recorded (Table 4) which indicates the genetically diverse nature of the lines for utilizing in future breeding programmes.

Table 4: Analysis of variance for yield, yield-attributing and quality traits (and * represents significance level at 1 and 5 per cent respectively)**

Source of variation	df	Number of days to 50 per cent flowering (no.)	Plant height (cm)	Number of effective tillers per plant (no.)	Panicle length (cm)	Days to maturity (no.)	1000 grain weight (g)	Grain yield per plant (g)	Kernel length (mm)	Kernel breadth (mm)	Kernel length/ breadth ratio	Amylose content (%)
Mean Sum of Squares (MSS)												
Replications	2	0.89	84.45	1.89	0.03	0.62	3.59	2.78	0.33	0.03	0.12	127.78
Treatments	40	223.62**	638.18**	12.50**	19.44**	17.86**	29.69**	85.49**	0.61**	0.05*	0.44**	36.04**
Error	80	0.77	43.92	1.62	0.91	0.14	1.32	3.57	0.16	0.03	0.06	0.78

Field pathotyping under natural field conditions

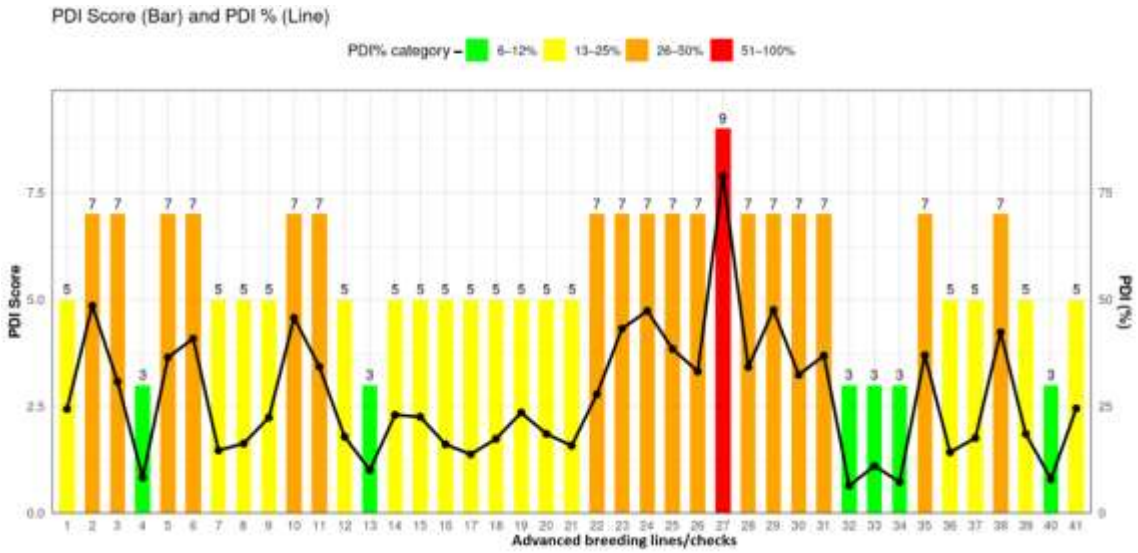
Phenotypic screening of advanced rice breeding lines was carried out under natural field conditions by utilizing the Standard Evaluation System (SES) for rice, developed by IRRI, 2014. A wide range of responses, ranging from moderately resistant to highly susceptible were observed in advanced breeding lines and notably, none of the lines exhibited complete resistance (Table 5 and Figure 1). However, six lines viz., GP-4, GP-13, GP-32, GP-33, GP-34 and GP-40 showed a moderately resistant reaction, seventeen lines viz., GP-1, GP-7, GP-8, GP-9, GP-12, GP-14, GP-15, GP-16, GP-17, GP-18, GP-19, GP-20, GP-21, GP-36, GP-37, GP-39 and GP-41, showed a moderately susceptible reaction. Another set of seventeen lines viz., GP-2, GP-3, GP-5, GP-6, GP-10, GP-11, GP-22, GP-23, GP-24, GP-25, GP-26, GP-28, GP-29, GP-30, GP-31, GP-35 and GP-38 showed a susceptible reaction while, only one line viz., GP-27 revealed a highly susceptible behaviour. These findings agree with previous reports by researchers, viz., Ashwini et al. 2021 and Verma and Tiwari, 2025 who have also documented variable resistance patterns in advanced breeding lines under natural field conditions in their respective studies.

Table 5: Phenotypic screening score and percent disease severity index for bacterial leaf blight under natural field conditions

Line/Check	Score	Description	PDI%	Line/Check	Score	Description	PDI%
GP-1	5	MS	24.38	GP-22	7	S	27.84
GP-2	7	S	48.57	GP-23	7	S	43.21
GP-3	7	S	30.82	GP-24	7	S	47.36
GP-4	3	MR	10.36	GP-25	7	S	38.45
GP-5	7	S	36.49	GP-26	7	S	33.17

GP-6	7	S	40.88	GP-27	9	HS	78.85
GP-7	5	MS	14.72	GP-28	7	S	34.23
GP-8	5	MS	16.32	GP-29	7	S	47.54
GP-9	5	MS	22.41	GP-30	7	S	32.41
GP-10	7	S	45.73	GP-31	7	S	36.89
GP-11	7	S	34.28	GP-32	3	MR	9.45
GP-12	5	MS	17.92	GP-33	3	MR	11.08
GP-13	3	MR	10.12	GP-34	3	MR	11.36
GP-14	5	MS	23.02	GP-35	7	S	36.96
GP-15	5	MS	22.57	GP-36	5	MS	14.28
GP-16	5	MS	16.14	GP-37	5	MS	17.62
GP-17	5	MS	13.78	GP-38	7	S	42.33
GP-18	5	MS	17.42	GP-39	5	MS	18.58
GP-19	5	MS	23.55	GP-40	3	MR	9.98
GP-20	5	MS	18.57	GP-41	5	MS	24.54
GP-21	5	MS	15.84				

Figure 1: Graphical representation of disease score and percent disease severity index



Molecular profiling

Molecular profiling was carried out to determine the presence or absence of bacterial leaf blight (BLB) resistance genes viz., Xa21, xa13 and xa5 as well as the aroma gene badh2, using gene-specific molecular markers (Table 6). To ascertain the presence or absence of Xa21 gene, the functional marker pTA248 was used. This marker amplified a 925 bp fragment specific to the resistant allele. Eleven breeding lines viz., GP-7, GP-8, GP-9, GP-13, GP-14, GP-17, GP-19, GP-32, GP-33, GP-34 and GP-40 exhibited this fragment, indicating the presence of Xa21 gene (Plate 1). Similar results, using the same amplicon size for Xa21 detection, have been reported in prior studies by Ronald et al, 1992 and Meesa et al, 2024. For xa13 gene, the marker xa13-prom was employed, which yielded a 450 bp amplicon specific to its resistant allele. This fragment was detected in thirteen breeding lines viz., GP-1, GP-4, GP-12, GP-13,

GP-15, GP-16, GP-18, GP-20, GP-21, GP-32, GP-33, GP-34, and GP-40, confirming the presence of the xa13 gene (Plate 2). Similar findings were observed by Hajira et al, 2016 and Gautam et al, 2023 in their respective studies. Marker RM122 was utilized to detect the presence of xa5 gene, which amplified a 240 bp fragment linked to the xa5 resistant allele and three lines viz., GP-4, GP-36 and GP-37 showed the presence of resistant bands (Plate 3). These results are in conformity with the results of Chen et al, 1997 and Kone et al, 2025. The functional marker BADEX7-5 was employed for the detection of the presence or absence of aroma gene viz., badh2. In thirty-eight lines viz., GP-1, GP-2, GP-3, GP-4, GP-5, GP-6, GP-7, GP-8, GP-10, GP-11, GP-12, GP-13, GP-14, GP-15, GP-16, GP-17, GP-18, GP-19, GP-20, GP-21, GP-22, GP-23, GP-24, GP-25, GP-26, GP-27, GP-28, GP-29, GP-30, GP-31, GP-32, GP-33, GP-36, GP-37, GP-38, GP-39, GP-40 and GP-41, the expected 95 bp fragment was observed (Plate 4) confirming the presence of the aroma allele. This marker has been successfully validated in earlier studies conducted by Sakthivel et al, 2009 and Islam et al, 2020. Overall, molecular profiling confirmed the presence of Xa21 in eleven lines, xa13 in thirteen lines, xa5 in three lines and the badh2 aroma allele in thirty eight lines, offering valuable insights for formulating breeding strategies that combine disease resistance and grain aroma.

Table 6: Presence/absence of bacterial leaf blight resistance genes (Xa21, xa13 and xa5) along with aroma (badh2) gene

Line/ Check	Xa21	xa13	xa5	badh2	Line/ Check	Xa21	xa13	xa5	badh2
	pTA248	xa13-prom	RM122	BADEX7-5		pTA248	xa13-prom	RM122	BADEX7-5
GP-1	-	+	-	+	GP-22	-	-	-	+
GP-2	-	-	-	+	GP-23	-	-	-	+
GP-3	-	-	-	+	GP-24	-	*	-	+
GP-4	*	+	+	+	GP-25	-	-	-	+
GP-5	-	-	-	+	GP-26	-	-	-	+
GP-6	-	-	-	+	GP-27	*	-	-	+
GP-7	+	-	-	+	GP-28	-	-	-	+
GP-8	+	*	-	+	GP-29	-	-	-	+
GP-9	+	-	-	-	GP-30	-	-	-	+
GP-10	-	-	-	+	GP-31	-	-	*	+
GP-11	-	-	*	+	GP-32	+	+	-	+
GP-12	-	+	-	+	GP-33	+	+	-	+
GP-13	+	+	-	+	GP-34	+	+	-	-
GP-14	+	-	-	+	GP-35	-	*	-	-
GP-15	-	+	-	+	GP-36	-	-	+	+
GP-16	-	+	-	+	GP-37	-	-	+	+
GP-17	+	-	-	+	GP-38	-	-	-	+
GP-18	-	+	-	+	GP-39	-	-	-	+
GP-19	+	-	-	+	GP-40	+	+	-	+
GP-20	-	+	-	+	GP-41	-	-	-	+
GP-21	-	+	-	+					

[Resistant (+); Susceptible (-); No amplification (*)]

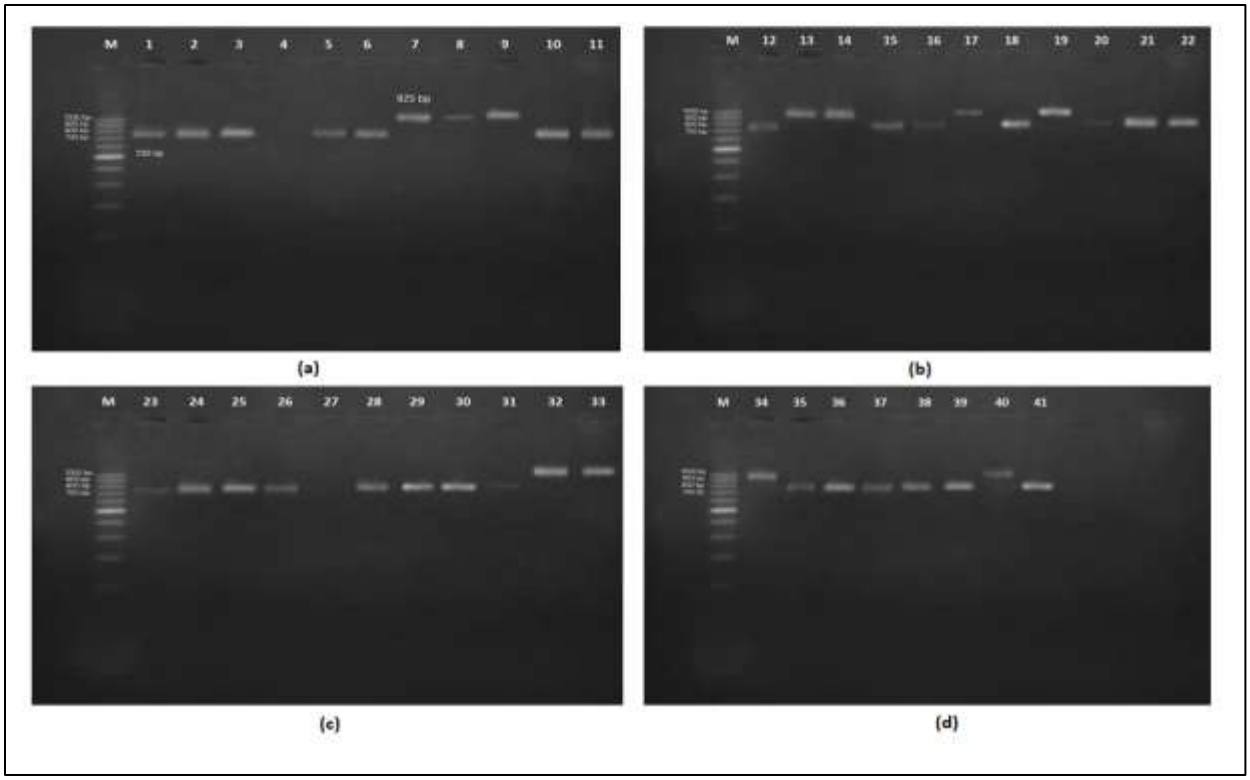


Plate 1: Banding profile of advanced breeding lines/checks with marker pTA248

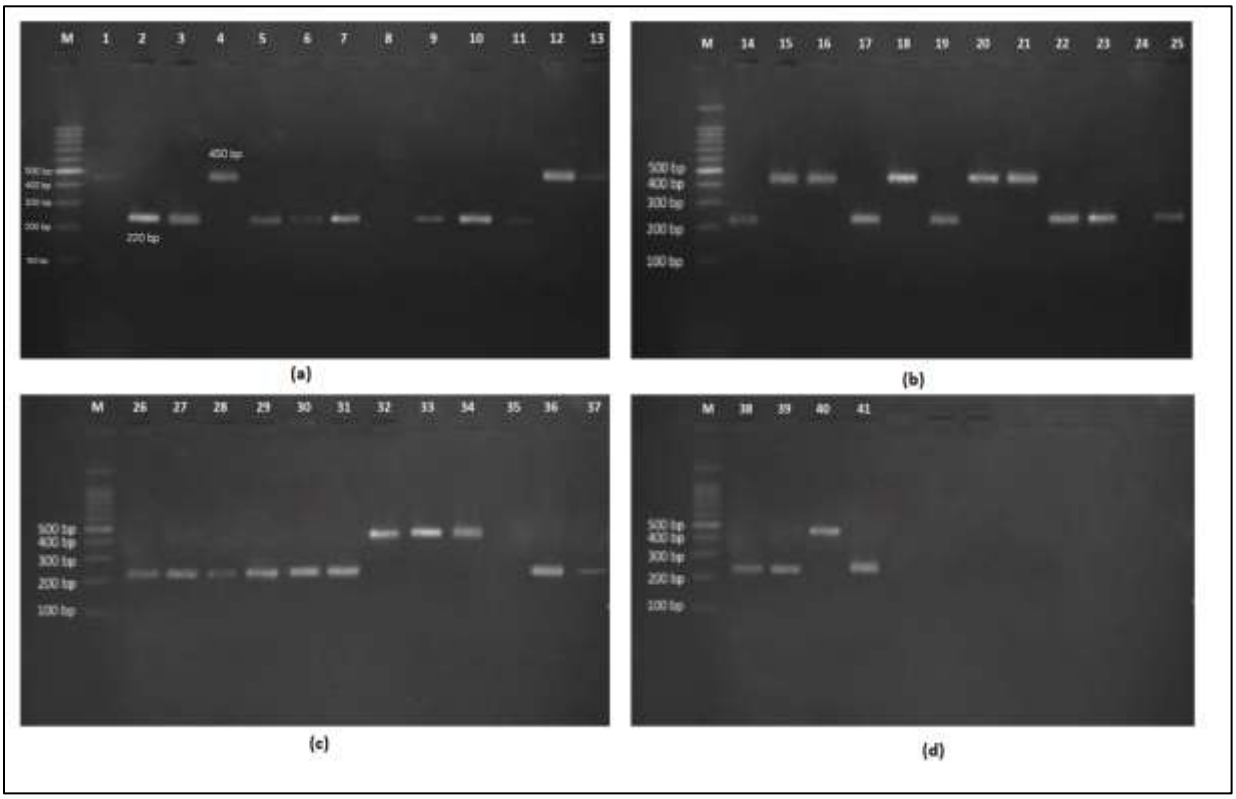


Plate 2: Banding profile of advanced breeding lines/checks with marker xa13-prom

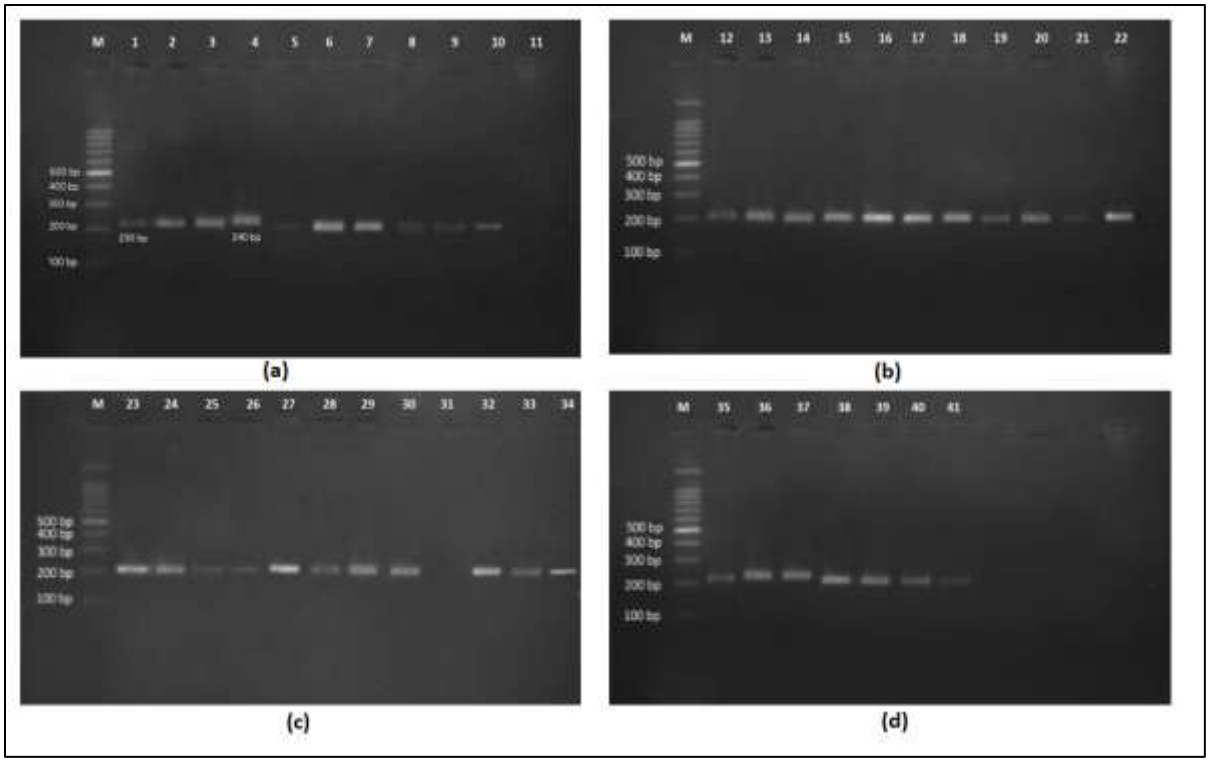


Plate 3: Banding profile of advanced breeding lines/checks with marker RM122

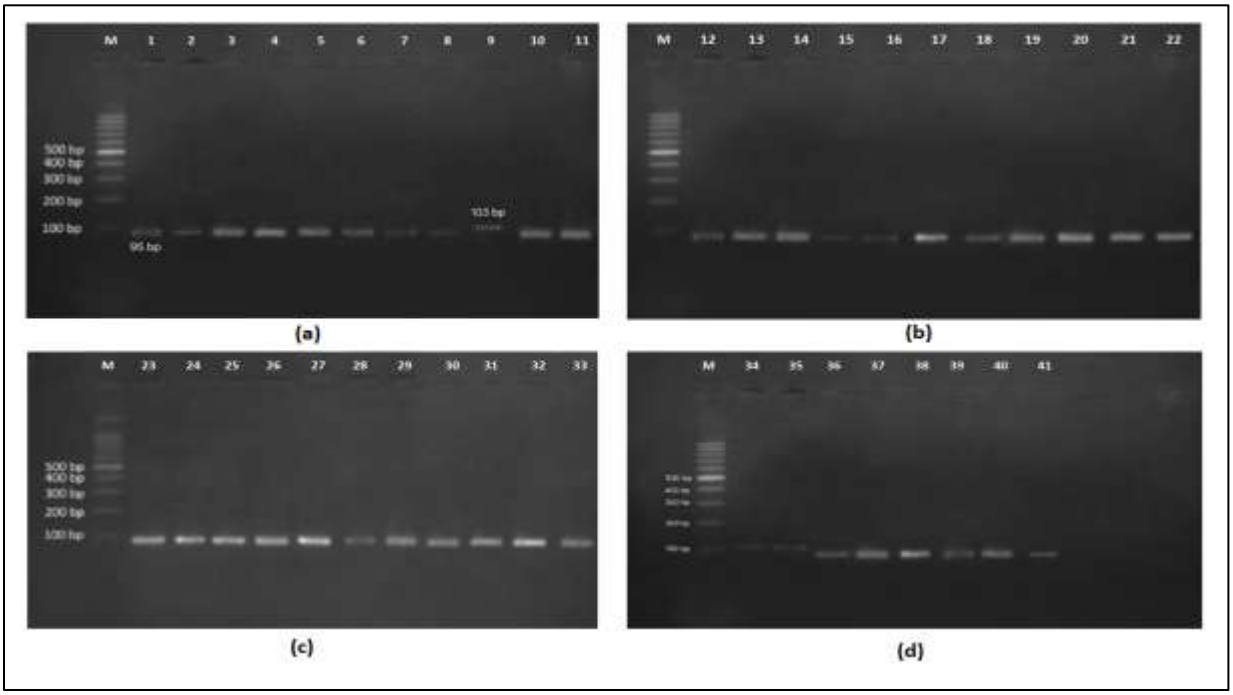


Plate 4: Banding profile of advanced breeding lines/checks with aroma marker BADEX7-5

Field pathotyping vs marker-based profiling

A comparative study of phenotypic screening under natural field conditions with molecular profiling, revealed a strong correspondence between field performance and genetic profiles. Notably, six lines viz., GP-4, GP-13, GP-32, GP-33, GP-34 and GP-40 which were classified as moderately resistant in the field were found to carry two of the three targeted BLB resistance genes. In contrast, GP-27, the only line identified as highly susceptible under field conditions

lacked all of the tested resistance genes. These observations underscore the complementary value of combining field evaluation and molecular analysis for selecting promising breeding lines. However, to confidently utilize these lines in breeding programmes, further validation through multi-location phenotyping and expanded molecular testing is necessary. These results are in conformity with the results of Nirala et al, 2025 and Khare et al, 2021.

CONCLUSIONS

Study revealed substantial genetic variation among breeding lines for yield, yield-attributing, disease resistance and grain quality traits, indicating their potential for use in future rice improvement programmes. Phenotypic screening under natural field conditions identified six breeding lines with a moderately resistant reaction to bacterial leaf blight. Molecular profiling further confirmed the presence of important resistance genes (Xa21, xa13 and xa5) and the aroma gene (badh2) in several breeding lines, demonstrating the usefulness of gene-specific molecular markers in validating field observations. The integration of phenotypic evaluation with molecular profiling enabled the identification of promising breeding lines possessing desirable combinations of disease resistance and grain quality traits. These lines can serve as valuable genetic resources in marker-assisted breeding programmes aimed at developing high-yielding, bacterial leaf blight-resistant and superior-quality rice cultivars. However, further validation through multi-location evaluation and additional molecular characterization is recommended before their utilization in varietal development programmes.

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