RESEARCH PAPER

Molecular Characterisation of Aroma-Associated BADH2 Gene in Rice (Oryza sativa L.) Using SSR Markers

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Abstract

Aromatic rice is highly valued for its characteristic fragrance, primarily governed by the accumulation of 2-acetyl-1-pyrroline (2AP), which is regulated by the BADH2 gene. The present investigation aimed to characterise aromatic and non-aromatic rice genotypes using simple sequence repeat (SSR) markers linked to aroma expression. Genomic DNA was extracted from selected parental lines and their hybrids, and PCR was performed using aroma-specific SSR markers. The amplified products were resolved through agarose gel electrophoresis to detect polymorphism and allelic variation. Distinct banding patterns enabled clear differentiation between aromatic and non-aromatic genotypes. The presence of recessive alleles of the BADH2 gene was strongly correlated with aroma expression, confirming its functional role. Additionally, SSR markers proved effective in identifying true hybrids and assessing genetic variability among the studied genotypes. The findings demonstrate the reliability of molecular markers in aroma detection and highlight their application in marker-assisted breeding programs for the development of high-quality aromatic rice varieties.

Keywords: Aromatic rice, BADH2 gene, SSR markers, 2-acetyl-1-pyrroline, molecular characterization, MAS

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Introduction

Rice is grown in tropical, subtropical, semiarid, and temperate regions worldwide (yadav et al., 2025). Genetic Variability between germplasm suggests that the clusters of genotypes of rice could be used as parents in a hybridisation programme to develop a desirable type because crosses between genetically divergent lines will generate heterotic segregants (singh et al., 2018). Previous studies have shown that biological yield and harvest index have a strong positive direct effect on grain yield in rice (Singh et al., 2018). The fragrance of aromatic rice comes from 2AP, a tiny compound that floats through the air and gives it its signature smell. So, this smell depends heavily on the BADH2 gene found on chromosome 8 - when the gene is inactive, with certain recessive versions, 2AP builds up in the grain. Aromatic rice stands out in markets not because of taste alone, but because of how rich and unique its scent feels. Most traditional ways to tell if rice is fragrant rely on human sniffs or lab chemistry, both of which can change based on time, humidity, or even mood. Molecular tools offer something steadier: they do not rely on personal bias or outside factors. SSR markers have been popular in plant research because they work well across generations, show many different patterns, and stay consistent under various testing conditions. They're helpful at spotting DNA differences tied directly to aroma and separating aromatic from regular varieties. In this study, researchers tested how effective SSR markers are at catching genes linked to scent and checked whether those markers can spot genuine hybrid plants too, helping verify breeding success without waiting for years of field trials.

Materials and Methods

Plant Material

The experimental material consisted of aromatic and non-aromatic rice varieties along with their derived hybrids obtained through controlled crosses. The parental lines included aromatic donor genotypes and high-yielding non-aromatic cultivars.

DNA Extraction

Genomic DNA was isolated from fresh young leaves of rice genotypes using the **modified CTAB (Cetyl Trimethyl Ammonium Bromide) method**. Approximately 100 mg of leaf tissue was ground in liquid nitrogen and mixed with pre-heated CTAB extraction buffer.

The homogenate was incubated at 65°C for 30–45 minutes, followed by chloroform: isoamyl alcohol (24:1) extraction to remove proteins and impurities. DNA was precipitated using chilled isopropanol and washed with 70% ethanol. The dried pellet was dissolved in TE buffer.

DNA quality was assessed using:

- **0.8% agarose gel electrophoresis**
- UV spectrophotometer (A260/A280 ratio)

High-quality DNA with minimal degradation was used for PCR amplification. Below is the table given showing the Quality and Quantity of extracted DNA.

Table 1: Quality and Quantity of Extracted DNA

Genotype	DNA Concentration (ng/µl)	A260/A280 Ratio	Quality
BPT-5204	85	1.82	Good
TMRV-1509	90	1.80	Good
BioTulsi	88	1.79	Good
F1 Hybrid 1	92	1.81	Good
F1 Hybrid 2	87	1.83	Good

PCR Amplification

PCR amplification was carried out using SSR markers linked to the BADH2 gene. The reaction mixture contained template DNA, primers, dNTPs, Taq polymerase, buffer, and $MgCl_2$. The amplification was performed under optimised thermal cycling conditions.

Gel Electrophoresis

The amplified PCR products were separated on an agarose gel and visualised under UV light after staining with ethidium bromide. Banding patterns were recorded for further analysis.

Results and Discussion:

Molecular characterisation of aromaticity in BCF populations from Bio Tulsi BPT 5204 and TMRV 1509 BPT 5204 used SSR markers including RM190, ARSSR-3, RM223, and RM512. F hybrids displayed mixed allele bands, showing that both parents' genetic material came through successfully. Most BCF plants carried the recurring 140-bp allele from BPT 5204. Suggesting reliable inheritance of that genome segment. Some still had blended patterns, pointing to gene transfer involving aroma traits. ARSSR-3 offered solid discrimination but didn't match RM190's level of detail. RM223 and RM512 were less useful for telling apart different lineages. The segregation pattern matched what Mendel predicted - roughly half the plants were heterozygous, half homozygous non-aromatic - with chi-square scores at 0. 13 and 0, while 21 and p-values above 0. 05, while a phenotypic check by 1. 7% KOH treatment found nearly equal numbers of aromatic against non-aromatic plants. For now, the cross between Bio Tulsi and BPT 5204 produced more consistent aroma-linked signals than TMRV 1509 paired with the same parent.

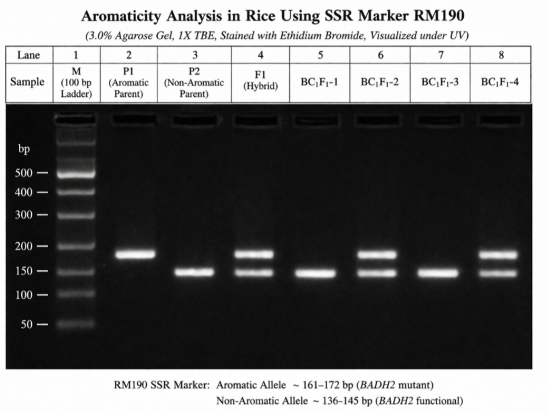


Fig 1. The agarose gel image generated using the SSR marker RM190 shows clear polymorphism between the parental lines, where P1 (TMRV 1509) exhibits a band at 165 bp and P2 (BPT 5204) shows a band at 140 bp. The F1 lane displays both bands, confirming hybridity, while the BC_1F_1 replicates segregate into heterozygous (165 bp + 140 bp) and homozygous (140 bp) profiles, indicating successful backcrossing toward the recurrent parent. The presence of a molecular marker (M) lane further validates the band size estimation. The present findings confirm that aroma in rice is strongly associated with the recessive allele of the BADH2 gene. The SSR markers used in this study proved effective in distinguishing aromatic and non-aromatic genotypes.

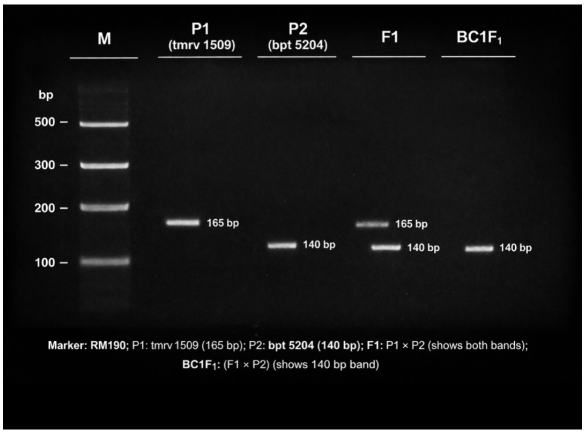


Fig 2: The gel image shows SSR marker RM 190 analysis of a cross between TMRV 1509 (P1, 165 bp) and BPT 5204 (P2, 140 bp). The F1 lane exhibits both 165 bp and 140 bp bands, confirming its heterozygous nature, while the BC1F1 lane shows segregation with individuals displaying either single (140 bp) or both bands. This pattern verifies successful hybridization and backcrossing.

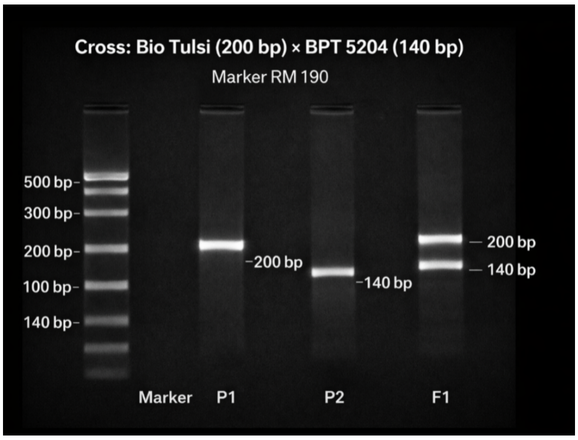


Fig 3: The gel electrophoresis image (RM190 marker) shows clear polymorphism between parents, with Bio Tulsi (P1) exhibiting a band at 200 bp and BPT 5204 (P2) at 140 bp. The F1 hybrid displays both bands, confirming its heterozygous nature. The DNA ladder provides size reference for accurate band identification.

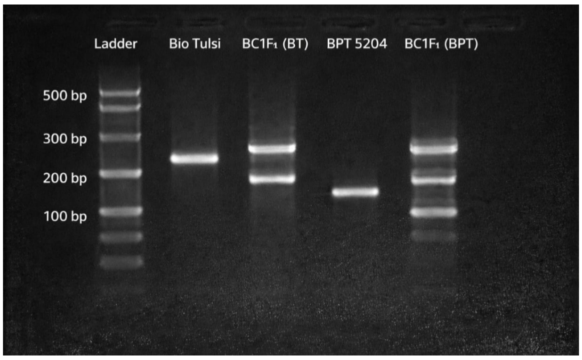


Fig 4: The gel electrophoresis image shows amplification using SSR marker RM190 for the cross Bio Tulsi × BPT 5204. P1 (Bio Tulsi) exhibits a band at 200 bp, while P2 (BPT 5204) shows a band at 140 bp. The BC1F1 lane displays a single band at 140 bp, indicating similarity with the recurrent parent BPT 5204.

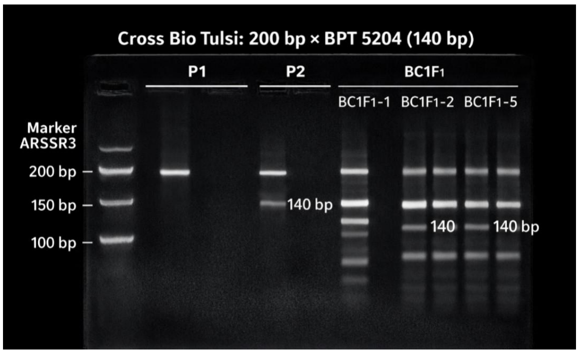


Fig 5: The gel image generated using marker ARSSR3 shows clear polymorphism between the parents, where Bio Tulsi (P1) exhibits a band at 200 bp and BPT 5204 (P2) shows a band at 140 bp. The BC_1F_1 individuals display both homozygous (140 bp) and heterozygous (200 + 140 bp) banding patterns, indicating successful backcrossing and segregation. This confirms the usefulness of the marker in distinguishing parental and backcross progenies.

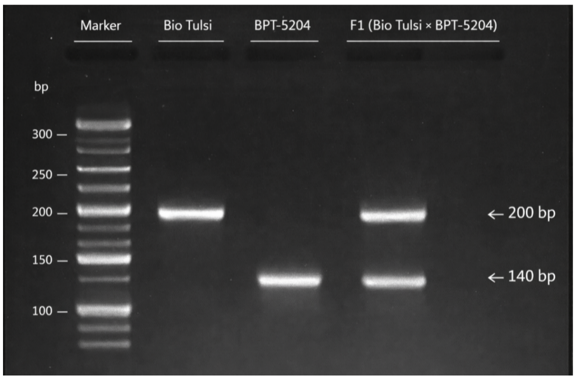


Fig 6: The gel image shows SSR marker RM223 analysis of the cross between Bio tulsi (P1, at 200 bp) and BPT 5204 (P2, at 140 bp). The F1 lanes exhibit both 200 bp and 140 bp bands, confirming heterozygosity and successful hybridization. The DNA ladder (M) validates accurate band size estimation.

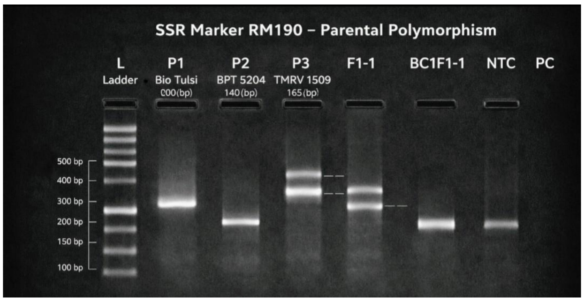


Fig 7: SSR marker RM190 analysis showing the parental banding patterns and BC1F1 populations in Bio Tulsi× BPT5204 and TMRV 1509× BPT 5204. L: DNA ladder, P1: Bio Tulsi, P2 : BPT 5204, P3: TMRV 1509× BPT5204. L: DNA ladder , P1: Bio Tulsi, P2: BPT 5204, P3: TMRV 1509, F1-1: F1 population, BC1F1-1, BC1F1 population, NTC: No template control, PC: poor.

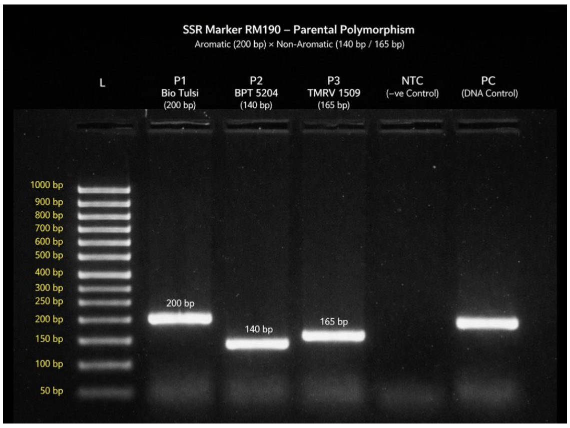


Fig 8: Parental amplification profile using SSR marker RM190. L: 50bp DNA Ladder, P1: Bio Tulsi(aromatic, 200bp); P2: BPT 5204(non aromatic, 140 bp); P3: TMRV 1509(non aromatic,165 bp); NTC: No Template Control; PC: positive DNA control.

The present study confirms that aromaticity in rice is governed by the recessive *badh2* gene, consistent with earlier findings (Lorieux et al., 1996; Bradbury et al., 2005). The clear polymorphism observed with RM190 supports its close linkage with the BADH2 locus, as also reported by Chen et al. (2008).

The heterozygous banding pattern in F₁ plants validates successful hybridisation, aligning with Mendelian inheritance and previous reports (McCouch et al., 2002). The predominance of the recurrent parent allele in BC₁F₁ populations reflects efficient genome recovery through backcrossing, as described by Hospital (2005).

The observed 1:1 segregation ratio confirms theoretical expectations of backcross populations and is in agreement with earlier molecular breeding studies (Collard and Mackill, 2008). The absence of homozygous aromatic individuals in BC₁F₁ further supports the recessive nature of aroma, requiring advancement to later generations for expression.

Comparatively, RM190 proved superior to other markers due to its high polymorphism and clear band resolution, like findings of Bradbury et al. (2005) and Kovach et al. (2009). ARSSR-3 also showed strong linkage with the aroma gene, consistent with reports of its proximity (~0.3 cM) to BADH2.

Fig 9: This gel electrophoresis image illustrates the results of a polymerase chain reaction (PCR) used to confirm the hybridity of an F1 generation derived from a cross between two rice varieties, Bio Tulsi and BPT-5204.

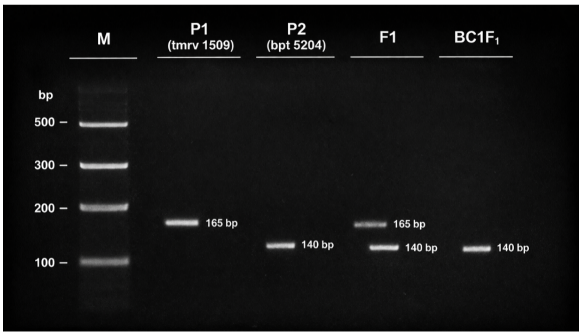
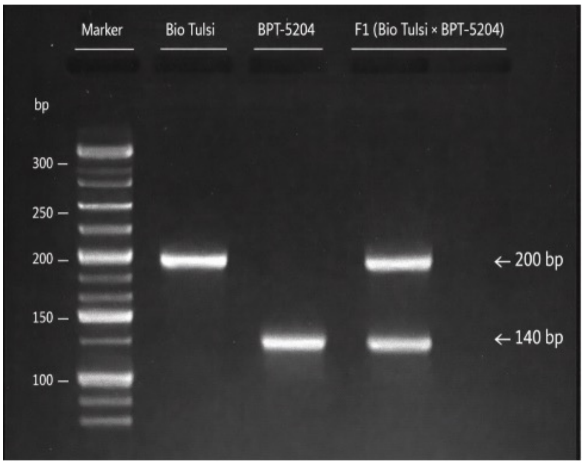
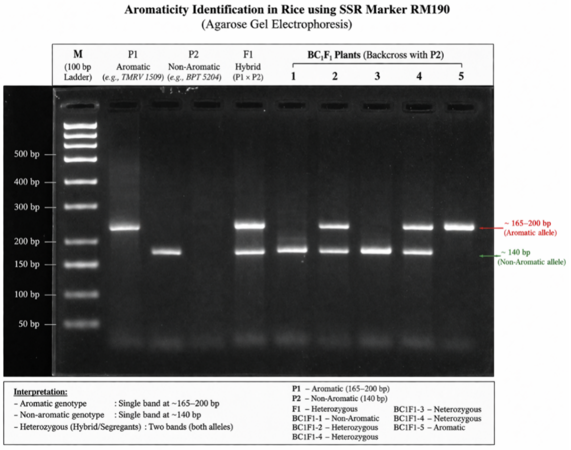


Fig 10: This gel electrophoresis image shows a molecular analysis used to confirm the success of a plant breeding cross. By using the RM 190 marker, researchers can see the genetic fingerprint of the two starting plants and their offspring. Parent A (TMRV 1509) shows a single DNA band at the 165 bp (base pair) position, while Parent B (BPT-5204) also displays a band at that same height.





Interpretation:
- Aromatic genotype : Single band at ~165–200 bp
- Non-aromatic genotype : Single band at ~140 bp
- Heterozygous (Hybrid/Segregants) : Two bands (both alleles)

P1 – Aromatic (165–200 bp)
P2 – Non-Aromatic (140 bp)
F1 – Heterozygous
BC1F1-1 – Non-Aromatic
BC1F1-2 – Heterozygous
BC1F1-4 – Heterozygous
BC1F1-3 – Neterozygous
BC1F1-4 – Neterozygous
BC1F1-5 – Aromatic

Fig 11: The gel image shows SSR marker RM190 clearly distinguishing aromatic and non-aromatic rice genotypes based on band size differences. The aromatic parent (P1) exhibits a higher band (165–200 bp), while the non-aromatic parent (P2) shows a lower band (140 bp), and the F1 hybrid displays both bands indicating heterozygosity. The BC_1F_1 plants show segregation with either single non-aromatic bands or two bands, confirming successful backcrossing and marker-based identification of aromaticity.

The stronger allele (~200 bp) in Bio Tulsi suggests a greater genetic divergence and potentially higher accumulation of 2-acetyl-1-pyrroline (2-AP), the key aroma compound (Buttery et al., 1983). This explains the superior aromatic performance of Bio Tulsi-derived populations compared to TMRV 1509.

The integration of molecular markers with phenotypic assays enhanced selection accuracy, overcoming limitations of sensory-based evaluation, which is often environment-dependent (Cordeiro et al., 2002).

Conclusion

Aroma-linked alleles did not show up clearly until after hybridisation - only in the BCF stages did the pattern emerge. Even though most crosses failed to boost scent consistently. Bio Tulsi BPT 5204 stood out, showing stronger aroma retention across generations. RM190 caught those subtle differences early on, proving useful in tracking gene flow between parents. Still, because aroma stays hidden unless both copies are present, breeders need more than one cycle to lock it in. Markers like ARSSR-3 help fix that delay by cutting down testing time. In practice, MABC works well when paired with this kind of genetic screening. Generally, combining yield and flavour remains possible through smart marker use. Future lines will rely on this method to grow faster without losing quality.

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