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Agronomic performance of BC3F2 aromatic rice lines derived from Inpari 32 × Merah Wangi crosses

MUHAMMAD RASSYA DHIO ANANTA[1], TRI AGUS SISWOYO[3,5], SHOLEH AVIVI[4,5], TRI HANDOYO[1,5], UMMI SHOLIKAH[1], TRI RATNASARI[2], AHMAD ILHAM TANZIL[2], WAHYU INDRA DUWI FANATA[4,5,♥]

[1]Graduate Program of Agronomy, Faculty of Agriculture, Universitas Jember. Jl. Kalimantan No. 37, Jember 68121, East Java, Indonesia
[2]Department of Agrotechnology, Faculty of Agriculture, Universitas Jember. Jl. Kalimantan No. 37, Jember 68121, East Java, Indonesia
[3]Graduate Program of Biotechnology, Universitas Jember. Jl. Kalimantan No. 37, Jember 68121, East Java, Indonesia
[4]Doctoral Program in Agricultural Sciences, Faculty of Agriculture, Universitas Jember. Jl. Kalimantan No. 37, Jember 68121, East Java, Indonesia
[5]Laboratory of Molecular Biology and Biotechnology, Center for Development of Advanced Science and Technology (CDAST), Universitas Jember. Jl. Kalimantan No. 37, Jember 68121, East Java, Indonesia. Tel.: +62-331-337877, ♥email: wahyuindra.faperta@mail.unej.ac.id

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Abstract. *Ananta MRD, Siswoyo TA, Avivi S, Handoyo T, Sholikah U, Ratnasari T, Tanzil AI, Fanata WID. 2025. Agronomic performance of BC3F2 aromatic rice lines derived from Inpari 32 × Merah Wangi crosses. Biodiversitas 26: 6417-6426.* Aroma is a key grain-quality trait that strongly influences consumer preference and market value in rice. Enhancing fragrance while maintaining high agronomic performance remains a key breeding objective. This study aimed to introgress the *BADH2* aromatic allele from Merah Wangi into the high-yielding Inpari 32 background using Marker-Assisted Backcrossing (MAB), and to evaluate the resulting BC_3F_2 population for agronomic traits and aroma expression. A total of 98 BC_3F_2 individuals and both parents were assessed under field conditions. Genotypic screening using the Bradbury marker identified fourteen homozygous *BADH2*$^{-/-}$ plants, confirming successful introgression of the recessive fragrance allele. Agronomic evaluation revealed that the BC_3F_2 population exhibited a stable heading date, intermediate plant architecture, and improved tillering capacity compared to its parents. Yield-related traits, including filled grain number, productive tillers, and 1000-grain weight, contributed to a higher grain yield per plant compared with both Inpari 32 and Merah Wangi. Grain-quality assessment further indicates a favorable kernel morphology, suggesting effective recombination of grain-size determinants. Organoleptic testing confirmed consistent aromatic expression across all homozygous lines, demonstrating strong agreement between molecular genotype and fragrance phenotype. Although segregation deviated from the expected 1:2:1 Mendelian ratio, the reliable identification of homozygous aromatic plants indicates that the fragrance trait was effectively fixed in this generation. Overall, the results highlight the efficiency of MAB for transferring recessive fragrance alleles into the elite rice backgrounds while maintaining desirable agronomic performance. The fourteen BC_3F_2 aromatic lines represent promising materials for further advancement, multilocation evaluation, and potential development of high-yielding aromatic derivatives of Inpari 32.

Keywords: *BADH2*, fragrant rice, homozygous lines, marker-assisted backcrossing, molecular breeding

INTRODUCTION

Aromatic rice plays a crucial role in both global and regional rice markets due to its distinctive sensory attributes, with fragrance serving as a key determinant of consumer preference and price differentiations. In many rice-producing countries, aromatic cultivars command significant price premiums and are often associated with premium market segments, specialty products, and export-oriented value chains. The characteristic pandan-like aroma of fragrant rice is primarily attributed to the volatile compound 2-acetyl-1-pyrroline (2AP), which strongly influences market acceptance in both domestic and export-oriented varieties (Calingacion et al. 2014; Okpala et al. 2019). As consumer awareness of grain quality continues to increase, demand for aromatic cultivars has expanded, driving breeding efforts aimed at combining fragrance with high-yield potential, improved grain quality, and broader adaptability. Modern breeding approaches have expanded opportunities for integrating aroma with agronomically important traits, such as stress tolerance and disease resistance (Singh et al. 2012; Vanavichit et al. 2018; Wang et al. 2023). Given its strategic economic value, aroma remains a high-priority target in rice breeding programmes, particularly in regions where fragrant rice commands strong consumer preference.

The molecular basis of fragrance in rice is primarily controlled by the betaine aldehyde dehydrogenase 2 (*BADH2*) gene located on chromosome 8. The functional *BADH2* enzyme catalyzes the conversion of γ-aminobutyraldehyde (GAB-ald) into γ-aminobutyric acid (GABA), a key metabolite in plant physiological pathways. A loss-of-function mutation in *BADH2*, most commonly an 8-bp deletion in exon 7, disrupts this pathway, leading to the accumulation of 2-AP and the expression of fragrance (Bradbury et al. 2005a; Chen et al. 2008). Because aroma is inherited as a recessive trait, aromatic expression occurs only when both alleles carry the inactivating mutation. This deletion has been widely conserved among aromatic genotypes and serves as a reliable molecular marker for distinguishing between fragrant and non-fragrant alleles.

Although *BADH2* is linked to a pathway associated with the stress response, previous studies indicate that the loss-of-function mutation responsible for fragrance does not

cause severe impairment to plant growth or development. This characteristic makes the *BADH2* aromatic allele particularly suitable for introgression into high-yielding genetic backgrounds without compromising overall agronomic performance (Hui et al. 2022; Imran et al. 2023). The availability of tightly linked molecular markers has further facilitated the precise tracking of the aromatic alleles across the segregating populations, enabling efficient selection at early generations

Marker-Assisted Backcrossing (MAB) has become a highly effective strategy for introducing fragrance alleles into elite rice cultivars. Its precision enables the early selection of target alleles, accelerating the recovery of the recurrent parent genome. The Bradbury marker, designed to detect the 8-bp deletion in *BADH2,* exhibits strong cosegregation with the aromatic phenotype and has been widely adopted in breeding programs requiring the efficient identification of homozygous aromatic individuals (Bradbury et al. 2005b). MAB has also demonstrated its utility in integrating major genes for quality improvement and disease resistance without compromising yield potential (Sagar et al. 2020; Kumar et al. 2023), thereby reaffirming its value as a breeding tool for simultaneously improving multiple traits.

Despite these methodological advances, information on the agronomic stability, phenotypic variability, and aroma expression of early-generation backcross populations remains limited, particularly at the BC_3F_2 stage, where genetic segregation is still active. In Indonesia, Inpari 32 is a widely cultivated variety recognised for its high yield, adaptability, and production stability, yet it lacks the aromatic properties favoured by many consumers. In contrast, Merah Wangi exhibits a strong fragrance but possesses suboptimal agronomic performance. Strategic introgression of the *BADH2* aromatic allele from Merah Wangi into the Inpari 32 genetic background offers a promising approach to combining fragrance with desirable agronomic traits. However, the performance and stability of resulting BC_3F_2 individuals derived from this cross have not been comprehensively reported. Therefore, this study aimed to introgress the recessive aromatic allele from Merah Wangi into the Inpari 32 background using Marker-Assisted Backcrossing and to evaluate the resulting BC_3F_2 population for genotypic identity, agronomic performance, grain quality characteristics, and aroma expression. The findings are expected to support the development of elite aromatic derivatives of Inpari 32 and to strengthen the application of MAB for quality trait improvement in rice breeding programmes.

MATERIALS AND METHODS

Plant materials and growth conditions

The study was conducted at Agrotechnopark, Universitas Jember, Indonesia. Plant materials consisted of BC_3F_2 (Backcross 3 Filial 2) individuals derived from a backcross between Inpari 32 (non-aromatic, recurrent parent) and Merah Wangi (aromatic, donor parent). Seeds were dried under controlled conditions to break dormancy and then germinated according to standard procedures. Seedlings were raised for two weeks under nutrient-supported conditions before being transplanted into soil-filled buckets placed in open field plots protected by a mesh enclosure to prevent bird interference. A total of 98 BC_3F_2 plants were genotypically screened, and only the homozygous aromatic ($BADH2^{-/-}$) plants were selected for detailed agronomic evaluation, alongside five plants of each parental line used as controls. The experiment followed a non-replicated field design under uniform soil and environmental conditions, with no replication. Standard agronomic management practices were applied, including regular irrigation, pest control, and fertilization according to local recommendations. All plants were grown to maturity for the assessment of agronomic and grain-related traits.

Genomic DNA extraction and genotyping

Leaf samples were collected from each plant for molecular validation of the *BADH2* gene. Genomic DNA was extracted using a standard phenol-chloroform protocol, and the purified DNA was dissolved in TE buffer. The extraction buffer used for DNA extraction consisted of 200 mM Tris-Cl, 250 mM NaCl, 25 mM EDTA, and 0.5% SDS. A PCR-based assay employing the Bradbury marker system was used to distinguish homozygous aromatic ($BADH2^{-/-}$), heterozygous ($BADH2^{+/-}$), and non-aromatic ($BADH2^{+/+}$) genotypes. The nucleotide sequences of the Bradbury marker primer set were as follows: EAP (External Antisense Primer) 5'-AGT GCT TTA CAA AGT CCC GC-3', ESP (External Sense Primer) 5'-TTG TTT GGA GCT TGC TGA TG-3', INSP (Internal Non-Fragrant Sense Primer) 5'-CTG GTA AAA AGA TTA TGG CTT CA-3', IFAP (Internal Fragrant Antisense Primer) 5'-CAT AGG AGC AGC TGA AAT ATA TAC C-3'. The PCR was performed for 35 cycles under the following conditions: initial denaturation at 95°C for 5 min, denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, elongation at 72°C for 1 min, and a final elongation at 72°C for 5 min. PCR amplification followed by electrophoresis on a 1% agarose gel, and gel images were captured using a gel documentation imaging system to visualize the allele-specific fragments.

Phenotypic and agronomic observations

Phenotypic and agronomic observations were conducted at physiological maturity using standard rice evaluation guidelines. Recorded traits included heading date, plant height, total tillers, productive tillers, panicle length, grain number per panicle, percentage of unfilled grains, 1000-grain weight, grain yield per plant, and kernel dimensions. Measurements were performed on all selected homozygous aromatic BC_3F_2 plants and the parental controls to characterize morphological performance and yield components.

Organoleptic aroma evaluation

Aroma assessment was conducted using a sensory panel, following the method described by Sood and Siddiq (1978). The panel comprised ten assessors, five men and five women, all in good health and with no olfactory impairments. Leaf tissues from each plant were placed in

microtubes and treated with 1% KOH to facilitate the release of aroma. Assessors scored each sample independently in a single blind session using a binary scale: "+" (aromatic) or "-" (non-aromatic). No discussion was allowed during scoring. Participation was voluntary, and no personal data were collected; all procedures adhered to institutional ethical guidelines.

Data analysis

Data from the selected homozygous aromatic BC_3F_2 plants and parental controls were summarized using descriptive statistics, including mean values, Standard Deviation (SD), and Coefficient of Variation (CV). As the experiment followed a non-replicated field layout, inferential statistics such as ANOVA were not applied. Phenotypic variability was interpreted using the CV threshold, where CV values <10% indicate narrow variability, values between 10% and 20% indicate moderate variability, and values >20% indicate broad variability (Ferreira et al. 2016). All figures and graphs were produced using Microsoft Excel 2019 in vector format for clarity.

RESULTS AND DISCUSSION

Genotypic validation of the *BADH2* aromatic allele

A total of 98 BC_3F_2 individuals were genotypically screened using the Bradbury marker to detect the 8-bp deletion in the *BADH2* gene associated with fragrance. The parental lines showed the expected allelic patterns with Inpari 32 producing a 355 bp amplicon and Merah Wangi producing a 257 bp fragment. Fourteen plants from the 98 genotyped individuals carried the 257 bp band corresponding to the homozygous aromatic genotype (*BADH2*$^{-/-}$), and these plants were subsequently selected for detailed phenotypic evaluation (Figure 1.A). The segregation of *BADH2* alleles in the BC_3F_2 population deviated from the expected 1:2:1 Mendelian ratio. A chi-square test (df = 2) yielded a value of 6.00, indicating a significant deviation from segregation expectations. Ten representative BC_3F_2 aromatic plants were photographed alongside parental varieties for morphological comparison (Figure 1.B).

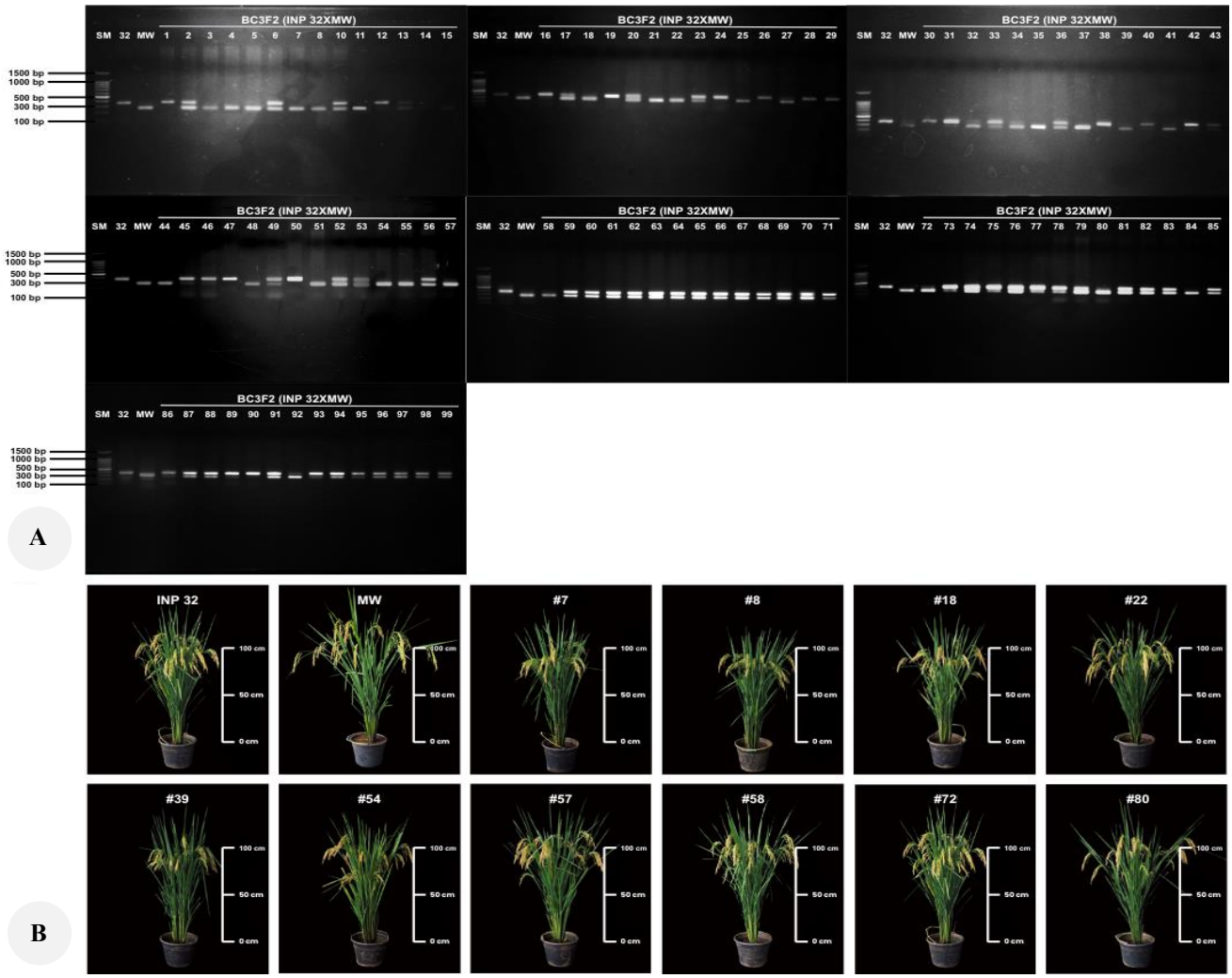

Figure 1. Detection of the *BADH2* aromatic allele in BC_3F_2 rice plants using the Bradbury marker: A. Agarose gel electrophoresis showing allele-specific bands for Inpari 32, Merah Wangi, and BC_3F_2 individuals, B. Morphology of representative BC_3F_2 plants carrying the homozygous aromatic allele. Scale bar: 100 cm

Phenological traits and plant architecture

The BC_3F_2 lines reached heading at an average of 60 DAT, slightly later than Inpari 32 with 57 DAT and comparable to Merah Wangi with 59 DAT (Figure 2.A; Table 2). The low coefficient of variation indicates uniform flowering across the selected plants. Plant height of the BC_3F_2 population averaged 126.21 cm, which exceeds the height of Inpari 32 (111 cm) and approaches the height of Merah Wangi (133 cm), with narrow variability (Figure 2.B; Table 1). The panicle length averaged 24.33 cm, falling between the two parents, and likewise showed narrow variability (Figure 2.C; Table 2). Overall, the BC_3F_2 population exhibited uniform phenology and an intermediate plant structure, consistent with expectation for a segregating backcross population.

Tillering capacity and yield components

The BC_3F_2 plants produced an average of 27 tillers per plant, exceeding both Inpari 32 and Merah Wangi (Figure 3.A; Table 2). Productive tillers also showed higher values in the BC_3F_2 population, with a mean of 24 per plant (Figure 3.B). The BC_3F_2 plants had an average of 205 filled grains per panicle and a lower proportion of empty grains compared to the parental lines (Figures 3.C and 3.D; Table 3). Grain yield reached 96.17 g per plant, surpassing both parents (Figure 3.E). Most yield-related traits, including total tillers, productive tillers, filled grain number, and grain yield, exhibited moderate variability, indicating residual segregation within BC_3F_2 population. The empty grain percentage showed broad variability, reflecting greater dispersion for these traits. These observations indicate higher values than those of the parents, but remain preliminary, as the yield per plant under bucket-grown conditions does not directly represent field-scale yield per hectare.

Grain quality characteristics

The 1000-grain weight of BC_3F_2 plants averaged 27.57 g, which was higher than the values recorded for both parental lines (Figure 4.B; Table 4). Kernel dimensions also showed intermediate expression, with an average length of 7.06 mm and a width of 2.59 mm, falling between the parental values (Figures 4.C and 4.D; Table 3). Based on IRRI grain-length classification (IRRI 2013), the BC_3F_2 kernels fall within the long-grain category (6.61-7.50 mm). Inpari 32, with an average kernel length of 7.01, also belongs to the long-grain class, whereas Merah Wangi, with an average kernel length of 5.94, falls into the medium-grain category (5.51-6.60). Kernel measurement was obtained using standard grain-evaluation protocols widely applied in rice quality assessment (Bintoro and Zahra 2022). These intermediate kernel dimensions indicate close similarity between the BC_3F_2 lines and Inpari32, a variety widely preferred by farmers and consumers in Indonesia. All grain quality traits in the BC_3F_2 population exhibited narrow variability, reflecting consistent grain characteristics across the selected lines.

Aroma expression

All fourteen homozygous aromatic BC_3F_2 lines exhibited detectable aroma in the KOH leaf assay and were consistently recognised by the evaluation panel (Table 5). All BC_3F_2 lines received positive aroma responses from the majority of assessors, indicating uniform expression of the aromatic trait within the selected group. Merah Wangi was rated aromatic by all assessors, while Inpari 32 was consistently rated as non-aromatic across all evaluations, providing a clear parental contrast for assessing aroma in the BC_3F_2 individuals. Because the aroma scoring used a binary scale, variation in the aroma intensity could not be captured, which represents a limitation of the present sensory evaluation.

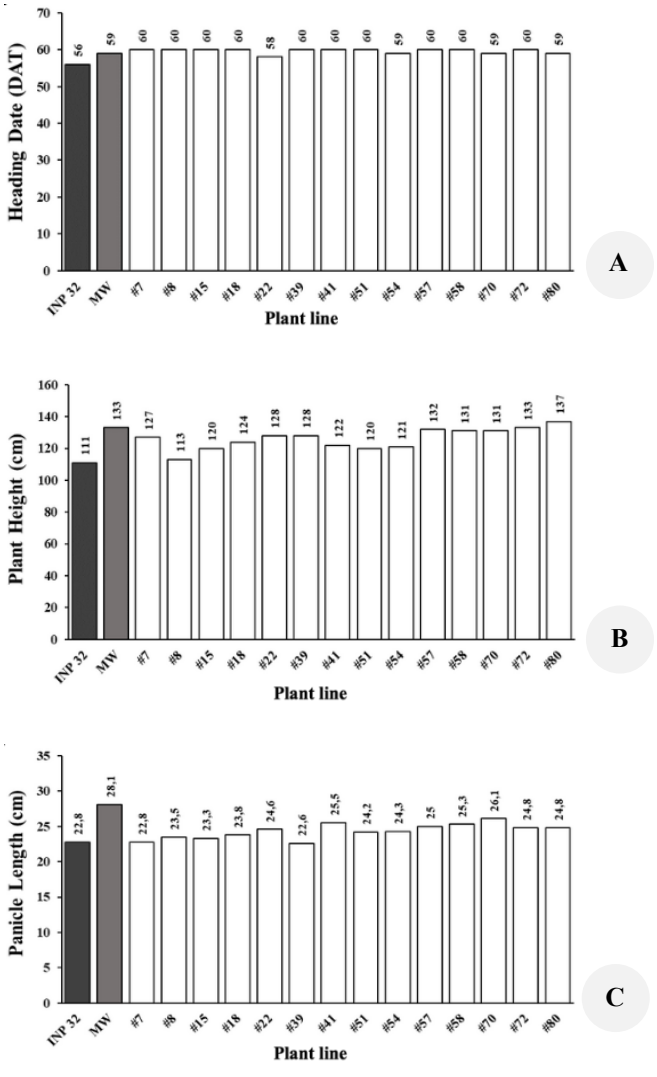

Figure 2. Phenological and morphological traits of BC_3F_2 plants compared with Inpari 32 and Merah Wangi: A. Heading date, B. Plant height, and C. Panicle length presented as mean values for each plant group

Table 1. Genotype classification of BC_3F_2 plants based on PCR banding patterns using the Bradbury marker

Genotype	Description	Band size (bp)	Plant IDs	Count
BADH2$^{+/+}$	Non-aromatic, wild type alle	355	1, 12, 16, 19, 24, 26, 28, 29, 30, 31, 33, 38, 40, 42, 45, 46, 47, 50, 61, 71, 73, 75, 77, 78, 86, 89, 90, 93	28
BADH2$^{+/-}$	Heterozygous, segregating	355 and 257	2, 3, 4, 5, 6, 10, 11, 13, 14, 17, 20, 21, 23, 25, 27, 32, 34, 35, 36, 37, 43, 44, 48, 49, 52, 53, 55, 56, 59, 60, 62, 63, 64, 65, 66, 67, 68, 69, 74, 76, 79, 81, 82, 83, 84, 85, 87, 88, 91, 92, 94, 95, 96, 97, 98, 99	56
BADH2$^{-/-}$	Homozygous aromatic allele	257	7, 8, 15, 18, 22, 39, 41, 51, 54, 57, 58, 70, 72, 80	14

Table 2. Comparison of phenological and morphological traits of BC_3F_2 rice plants with recurrent parent Inpari 32 and donor Merah Wangi

Parameters	Plant line	Sample number	Average	Range	Standard deviation	Coefficient of variation	Variability
Heading date	Inpari 32	5	57	56-58	1.10	1.93	Narrow
	MW	5	59	58-62	1.67	2.82	Narrow
	BC_3F_2	14	60	58-60	0.63	1.06	Narrow
Plant height (cm)	Inpari 32	5	111	107-117	3.74	3.37	Narrow
	MW	5	133	130-137	2.86	2.15	Narrow
	BC_3F_2	14	126.21	113-137	6.51	5.16	Narrow
Panicle length (cm)	Inpari 32	5	22.88	21.8-24.4	1.01	4.40	Narrow
	MW	5	28.18	27.5-28.8	0.53	1.87	Narrow
	BC_3F_2	14	24.33	22.6-26.1	1.03	4.23	Narrow

Table 3. Tillering capacity and grain yield components of BC_3F_2 rice plants compared to Inpari 32 and Merah Wangi

Parameters	Plant line	Sample number	Average	Range	Standard deviation	Coefficient of variation	Variability
Tiller number	Inpari 32	5	21	20-25	2.07	9.69	Narrow
	MW	5	17	15-19	1.52	8.72	Narrow
	BC_3F_2	14	27	20-33	3.53	12.99	Moderate
Productive tiller number	Inpari 32	5	18	17-19	0.84	4.70	Narrow
	MW	5	16	15-17	0.84	4.74	Narrow
	BC_3F_2	14	24	18-30	3.53	14.71	Moderate
Number of filled grain per panicle	Inpari 32	5	156	114-172	24.33	15.57	Moderate
	MW	5	244	217-262	16.42	6.73	Narrow
	BC_3F_2	14	205	171-236	22.55	11.02	Moderate
Empty grain (%)	Inpari 32	5	7.67	4.9-10.7	2.59	33.78	Broad
	MW	5	6.94	2.4-9.9	3.05	44.03	Broad
	BC_3F_2	14	5.42	2.9-10.9	2.14	39.41	Broad
Grain yield per plant (g)	Inpari 32	5	49.05	39.65-55.1	6.24	12.72	Moderate
	MW	5	85.42	75.04-91.26	6.22	7.28	Narrow
	BC_3F_2	14	96.17	76.5-116.24	11.91	12.38	Moderate

Table 4. Grain quality characteristics: 1000-grain weight and kernel dimensions in BC_3F_2 rice plants compared to Inpari 32 and Merah Wangi

Parameters	Plant line	Sample number	Average	Range	Standard deviation	Coefficient of variation	Variability
Weight of 1000 seeds (g)	Inpari 32	5	25.78	25.25-26.38	0.49	1.91	Narrow
	MW	5	26.08	25-27.31	0.93	3.58	Narrow
	BC_3F_2	14	27.57	24.2-29.32	1.50	5.45	Narrow
Kernel width (mm)	Inpari 32	5	2.50	2.3-2.7	0.12	4.62	Narrow
	MW	14	2.78	2.5-3	0.16	5.82	Narrow
	BC_3F_2	14	2.59	2.48-2.73	0.07	2.90	Narrow
Kernel length (mm)	Inpari 32	5	7.01	6.4-8.9	0.73	10.41	Moderate
	MW	5	5.94	5.1-6.3	0.35	5.90	Narrow
	BC_3F_2	14	7.06	6.77-7.45	0.22	3.13	Narrow

Discussion

The results clearly demonstrate that Marker-Assisted Backcrossing (MAB) was effective in transferring the *BADH2* fragrance allele from Merah Wangi into the Inpari 32 background. The Bradbury marker successfully distinguished the parental genotypes and identified fourteen homozygous *BADH2*$^{-/-}$ BC_3F_2 plants, confirming the presence of the characteristic 8-bp deletion associated with fragrance (Bradbury et al. 2005a; Chen et al. 2008). Although the segregation ratio deviated from the expected 1:2:1 Mendelian ratio, such a distortion is frequently reported in segregating rice populations and can arise from several biological mechanisms. Segregation distortion may be caused by gametophytic selection, such as preferential transmission of male and female gametes, as well as zygotic selection, cytoplasmic effects, or hybrid weakness, all of which have been documented in rice (Xu et al. 1997; Matsushita et al. 2003; Wang et al. 2009). Despite this deviation, the consistent identification of homozygous aromatic plants demonstrates that the target allele was successfully preserved through backcrossing and marker-assisted selection effectively enriches for the recessive *BADH2*$^{-/-}$ genotype required for 2-acetyl-1-pyrroline accumulation (Bradbury et al. 2005b; Behera and Panda 2023). These findings illustrate that MAB remains a powerful and efficient strategy for integrating fragrance alleles into elite rice cultivars.

The BC_3F_2 population exhibits uniform heading dates and intermediate plant heights relative to both parents. These outcomes indicate the stable inheritance of phenological and architectural traits, which is desirable for ensuring synchronized maturity and reliable field performance (Adhi et al. 2024; Liang et al. 2024). The intermediate height is consistent with reports that backcross-derived lines often combine parental vigour and growth habit in segregating generations (Liu et al. 2017). Panicle length also displayed intermediate expression between the parents, indicating partial inheritance of spike-related morphology. A longer panicle structure supports a greater number of spikelets and is positively associated with yield potential (Bai et al. 2016; Shang et al. 2016). Such morphological patterns are typical of segregating populations and provide scope for further refinement in later generations. The variability observed for some traits suggests that continued selection will be needed to achieve more uniform phenotypes.

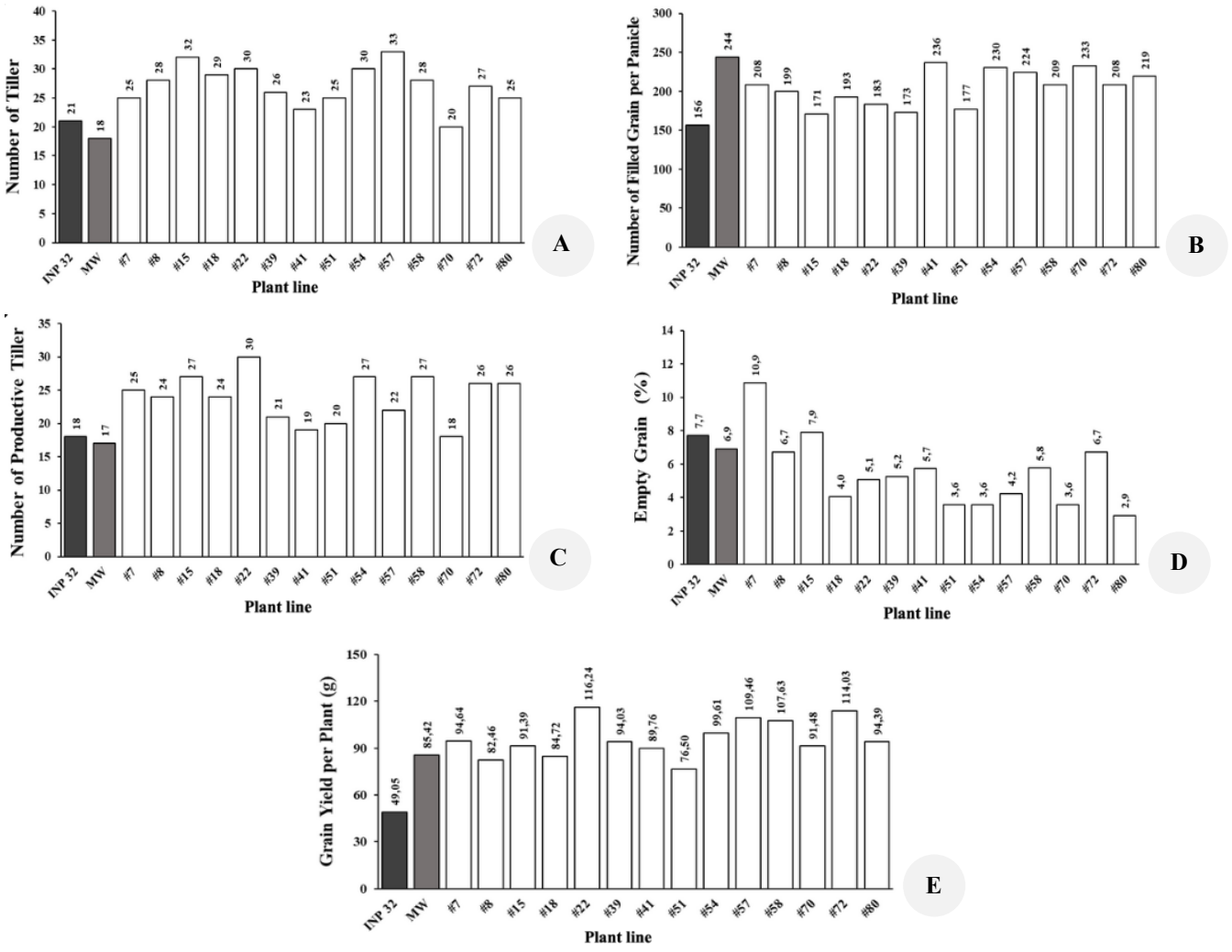

Figure 3. Tillering capacity and yield components of BC_3F_2 plants in comparison with Inpari 32 and Merah Wangi. A. Total tiller number, B. Productive tiller number, C. Filled grains per panicle, D. Percentage of empty grains, and E. Grain yield per plant

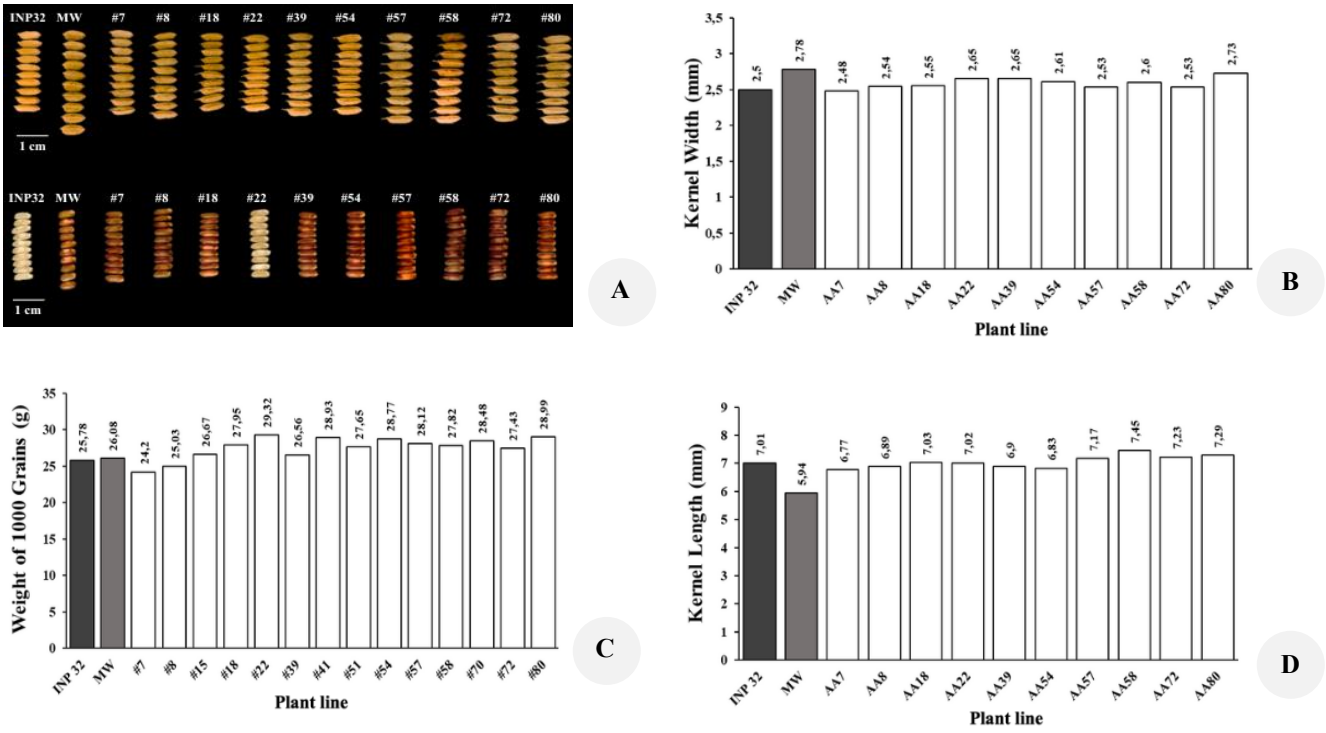

Figure 4. Grain quality characteristics of BC_3F_2 plants compared with parental lines. A. Grain and kernel morphology (scale bar: 1 cm), B. Weight of 1000-grain, C. Kernel width, and D. Kernel length based on average measurements

Table 5. Result of organoleptic aroma evaluation for BC_3F_2 rice plants compared to Inpari 32 and Merah Wangi

Plant line	Organoleptic aroma evaluation by panelist										Status
	A	B	C	D	E	F	G	H	I	J	
Inpari 32	-	-	-	-	-	-	-	-	-	-	Non aromatic
Merah Wangi	+	+	+	+	+	+	+	+	+	+	Aromatic
AA7	+	+	+	+	+	+	+	+	+	+	Aromatic
AA8	+	+	-	+	-	+	+	+	+	+	Aromatic
AA15	+	+	+	+	+	+	+	+	+	+	Aromatic
AA18	-	+	-	+	+	+	+	+	+	+	Aromatic
AA22	+	+	+	+	-	+	-	+	+	+	Aromatic
AA39	+	+	+	+	+	+	+	+	+	+	Aromatic
AA41	+	+	+	+	+	+	-	+	-	+	Aromatic
AA51	+	+	+	+	+	+	+	+	+	+	Aromatic
AA54	+	+	+	+	+	+	+	-	-	+	Aromatic
AA57	+	+	+	-	+	-	+	+	+	+	Aromatic
AA58	+	+	+	+	+	+	+	+	+	+	Aromatic
AA70	+	+	+	+	+	+	+	+	+	+	Aromatic
AA72	+	-	+	-	+	+	+	+	+	+	Aromatic
AA80	+	+	+	+	+	+	+	+	+	+	Aromatic

Yield-related traits showed a favorable combination of parental attributes. The BC_3F_2 plants produced more total and productive tillers, displayed improved grain filling, and recorded higher grain yield per plant than both parents. These results indicate that introgression of the fragrance allele did not negatively influence yield potential. Similar outcomes have been reported in breeding programs where single major genes were introgressed into elite backgrounds without compromising agronomic performance (Sagar et al. 2020; Kumar et al. 2023). Grain-quality characteristics also improved with higher 1000-grain weight and favorable kernel morphology. These outcomes reflect successful recombination of grain-size determinants, many of which are known to segregate widely in backcross-derived populations (Li et al. 2018; Xuedan et al. 2023). The intermediate grain shape, which aligns the BC_3F_2 lines with the long grain type of Inpari 32, a variety widely preferred by farmers and consumers in Indonesia due to its desirable eating quality. This similarity in grain type, together with the presence of an aroma trait, may enhance the potential

acceptability of BC3F2 lines in the consumer market. Although broad variability remains, such diversity is typical in early backcross generations and offers an opportunity for further selection toward higher uniformity and desirable profiles.

A strong correspondence between molecular classification and organoleptic evaluation by KOH assays confirms the stable expression of fragrance in all homozygous *BADH2*$^{-/-}$ BC_3F_2 plants. The alignment between genotype and phenotype is consistent with reports that the *BADH2* mutation reliably determines aroma expression across diverse rice populations (Hui et al. 2022; Imran et al. 2023). While chemical and sensory traits, such as aroma, are often influenced by environmental factors or post-harvest handling (Yang et al. 2008; Varatharajan et al. 2021; Dutta et al. 2022), no such variability was evident among the BC_3F_2 lines evaluated in this study. The strong genotypic and phenotypic concordance also highlights the value of integrating molecular markers with organoleptic validation to ensure accurate selection in early-generation breeding populations.

Several limitations should be acknowledged. The non-replicated field layout limited the ability to conduct inferential statistical analyses and prevented evaluation of environmental influences on trait expression. Reliance on a single functional marker limits insight into background genome recovery or possible linkage drag. Furthermore, evaluation at a single site did not allow assessment of genotype and environment interactions. Future work should involve multilocation trials, advanced-generation testing, and broader genomic assessments to stabilise traits and support the development of elite aromatic lines. Overall, this study demonstrates that Marker-Assisted Backcrossing (MAB) can efficiently integrate the *BADH2* aromatic allele into a high-yielding background while maintaining desirable agronomic and grain-quality characteristics. The combined improvements in yield components, kernel properties, and fragrance expression indicate that the selected BC_3F_2 lines hold strong potential for advancement in aromatic rice breeding programmes.

In conclusion, this study successfully identified fourteen homozygous aromatic BC_3F_2 lines carrying the fragrance allele introgressed from Merah Wangi through Marker-Assisted Backcrossing. The selected plants exhibited consistent aroma expression and maintained desirable agronomic traits, including uniform heading data, intermediate plant stature, enhanced tillering capacity, improved grain filling, and higher yield performance. Grain-quality characteristics, particularly 1000-grain weight and kernel morphology, also showed favourable improvement. Although segregation for the *BADH2* aromatic allele did not follow the expected Mendelian ratio, the strong alignment between genotype and fragrance phenotype confirms effective transfer and expression of the target allele. Continued advancement, multilocation evaluation, and extended genomic assessment are recommended to stabilise segregating traits and support the development of an elite aromatic line suitable for wider cultivation.

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SUPPLEMENTARY DATA

Table S1. Chi-square calculation result

Genotype	Expected	Observe	d = O - E	X^2
BADH$^{+/+}$	24.5	28	3.5	0.50
BADH$^{+/-}$	49	56	7.0	1.00
BADH$^{-/-}$	24.5	14	-10.5	4.50
Total	98	98	-	6.00

Note: The critical value (df = 2) = 5.991. The chi-square analysis showed a total χ^2 value of 6.00, which exceeds the critical value of 5.991 at df = 2 and the 0.05 significance level for a 1:2:1 Mendelian segregation model. This indicates that the observed genotypic frequencies of the *BADH2* locus in the BC_3F_2 population significantly deviate from the expected 1:2:1 ratio (*BADH2*$^{+/+}$: *BADH2*$^{+/-}$: *BADH2*$^{-/-}$)

Table S2. Raw agronomic data of BC_3F_2 lines and parental controls

Parameters	Lines	Total sample	Range	Average	STDEV	CV (%)	Variability
Plant height (cm)	INP 32	5	107-117	111	3.74	3.37	Narrow
	MW	5	130-137	133	2.86	2.15	Narrow
	BC_3F_2	14	113-137	126.21	6.51	5.16	Narrow
Tiller number	INP 32	5	20-25	21	2.07	9.69	Narrow
	MW	5	15-19	17	1.52	8.72	Narrow
	BC_3F_2	14	20-33	27	3.53	12.99	Moderate
Productive tiller number	INP 32	5	17-19	18	0.84	4.70	Narrow
	MW	5	15-17	16	0.84	4.74	Narrow
	BC_3F_2	14	18-30	24	3.53	14.71	Moderate
Heading date	INP 32	5	56-58	57	1.10	1.93	Narrow
	MW	5	58-62	59	1.67	2.82	Narrow
	BC_3F_2	14	58-60	60	0.63	1.06	Narrow
Panicle length (cm)	INP 32	5	21.8-24.4	22.88	1.01	4.40	Narrow
	MW	5	27.5-28.8	28.18	0.53	1.87	Narrow
	BC_3F_2	14	22.6-26.1	24.33	1.03	4.23	Narrow
Number of filled grain per panicle	INP 32	5	114-172	156	24.33	15.57	Moderate
	MW	5	217-262	244	16.42	6.73	Narrow
	BC_3F_2	14	171-236	205	22.55	11.02	Moderate
Empty grain (%)	INP 32	5	4.9-10.7	7.67	2.59	33.78	Broad
	MW	5	2.4-9.9	6.94	3.05	44.03	Broad
	BC_3F_2	14	2.9-10.9	5.42	2.14	39.41	Broad
Weight of 1000 seeds (g)	INP 32	5	25.25-26.38	25.78	0.49	1.91	Narrow
	MW	5	25-27.31	26.08	0.93	3.58	Narrow
	BC_3F_2	14	24.2-29.32	27.57	1.50	5.45	Narrow
Grain yield per plant (g)	INP 32	5	39.65-55.1	49.05	6.24	12.72	Moderate
	MW	5	75.04-91.26	85.42	6.22	7.28	Narrow
	BC_3F_2	14	76.5-116.24	96.17	11.91	12.38	Moderate