



# Agronomic Performance of Aromatic Rice (BC4F1) Derived from Inpari 33 × Merah Wangi Crosses

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## ABSTRACT

**Background:** Aromatic rice quality is primarily determined by loss-of-function mutations in the *BADH2* gene, which lead to the accumulation of 2-acetyl-1-pyrroline (2AP). This study aimed to introgress the *BADH2* aromatic allele from Merah Wangi into the high-yielding variety Inpari 33 and to evaluate the agronomic performance of the resulting BC4F1 population.

**Methods:** A breeding program consisting of four backcross cycles was conducted using Inpari 33 as the recurrent parent and Merah Wangi as the donor parent. Marker Marker-assisted breeding using Bradbury markers was applied to identify plants carrying the *BADH2* mutation. Agronomic traits, including heading date, plant height, panicle length, tillering capacity, yield components and grain quality were assessed to characterize phenotypic performance.

**Result:** Twenty heterozygous aromatic plants were identified, confirming successful introgression of the *BADH2* mutant allele. The BC4F1 population showed uniform heading date, intermediate plant height and increased panicle length relative to Inpari 33. The productive tillers, filled grains per panicle and grain yield per plant demonstrated improved performance, even though variability persisted in grain filling and 1000-grain weight. These findings indicate that the integration of the *BADH2* allele and the overall agronomic response validate the effectiveness of Marker Assisted Breeding combined with repeated backcrossing for developing improved aromatic rice lines, while further selection is still required to stabilize key traits.

**Key words:** Agronomic traits, Aromatic rice breeding, *BADH2* aromatic allele, BC4F1, Marker-assisted breeding.

## INTRODUCTION

Aromatic rice is highly valued in global markets due to its distinctive fragrance produced by the compound 2-acetyl-1-pyrroline (2AP), which drives strong consumer preference and premium pricing over non-aromatic varieties (Buttery, 1982; Calingacion *et al.*, 2014; Okpala *et al.*, 2019). Advances in breeding and genome editing have enabled the enhancement of fragrance-related genes and the development of aromatic cultivars with improved agronomic performance (Ashokkumar *et al.*, 2020; Imran *et al.*, 2023; Imran *et al.*, 2023). The integration of fragrance genes with desirable agronomic traits has further produced elite aromatic lines with improved yield potential and adaptability (Singh *et al.*, 2012; Vanavichit *et al.*, 2018; Wang *et al.*, 2023).

The *BADH2* gene on chromosome 8 is the major genetic determinant of aroma, encoding betaine aldehyde dehydrogenase, which converts gamma aminobutyraldehyde into GABA. Mutations in *BADH2* disrupt this pathway, causing the accumulation of 2AP. The most common aromatic allele is an 8-base pair deletion in exon 7 (Bradbury *et al.*, 2005; Hui *et al.*, 2022). Marker-assisted backcrossing is an effective approach for introducing fragrance alleles into agronomically superior varieties, as it enables the precise identification of plants carrying the aromatic genotype (Bradbury *et al.*, 2005; Ishak and Fedora, 2024). This method has also been used to combine aroma with other beneficial traits such as disease resistance (Kumar *et al.*, 2023; Sagar *et al.*, 2020).

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Despite its agronomic advantages, the rice variety Inpari 33 lacks aroma, whereas Merah Wangi possesses a strong aroma but inferior agronomic performance. Introgression of the *BADH2* aromatic allele from Merah Wangi into Inpari 33 through marker-assisted backcrossing offers a promising strategy to combine fragrance with high yield potential. Therefore, this study aimed to validate the introgression of the *BADH2* aromatic allele and to evaluate the agronomic performance of the resulting BC4F1 population compared with the parental varieties.

## MATERIALS AND METHODS

### Research site and experimental period

This study was conducted from September 2024 to March 2025 at Universitas Jember, East Java, Indonesia. Field experiments were carried out at UPA Taman Agroteknologi, Universitas Jember. Molecular and laboratory analyses were performed at the Integrated Laboratory and Waste Management Unit (UPA-PL2T), Universitas Jember.

### Experimental design

The experiment was conducted as a non-replicated observational progeny evaluation in an open-field condition. A total of 42 BC4F1 individuals were grown alongside five plants of both parental varieties (Inpari 33 and Merah Wangi) for comparative assessment. Plants were arranged in a completely randomized layout under uniform agronomic management. Each individual plant served as an experimental unit. Observations were recorded per plant and summarized using descriptive statistics to characterize phenotypic variation within the population.

### Plant materials and growth conditions

Seeds of Inpari 33, Merah Wangi and BC4F1 lines were dried at 50°C for one day to break dormancy (Wan *et al.*, 1997), germinated for three days on moist paper and transplanted after one week into soil-filled buckets placed in open field plots protected with netting to minimize bird disturbance. Plants received standard irrigation and fertilization using urea, monocalcium phosphate and potassium chloride at rates of 200, 50 and 50 kg/ha, respectively. Pest and disease management followed routine field practices to maintain crop health throughout growth.

### Genomic DNA extraction and genotyping

Leaf samples from all BC4F1 plants were collected for molecular analysis. Genomic DNA was extracted using a standard phenol-chloroform protocol and dissolved in TE buffer. PCR amplification was performed using GoTaq Green Master Mix (Promega Madison, WI, USA) and the Bradbury primer set (ESP, EAP, INSP and IFAP) to detect the *BADH2* aromatic allele (Bradbury *et al.*, 2005). PCR reactions were carried out in a T100™ Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA). The PCR reaction conditions followed Fitriyandi *et al.* (2025) consisting of an initial denaturation at 95°C for 5 min,

followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 1 min, with a final extension at 72°C for 5 min. Amplified products were electrophoresed on 1% agarose gel to distinguish non-aromatic (355 bp) and aromatic (257 bp) alleles (Bradbury *et al.*, 2005). Heterozygous individuals were identified by the presence of both fragments.

### Measurements of agronomic traits

Agronomic traits were evaluated at maturity. Plant height was measured from the soil level to the tip of the tallest panicle. The heading date was recorded as the number of days from sowing until the emergence of at least five panicles. Total and productive tillers were counted during the vegetative and reproductive phases. Panicle length was measured from base to tip. Filled and empty grains per panicle were quantified manually and the percentage of empty grains was calculated. Grain yield per plant was determined from all harvested filled grains. The weight of 1000 grains was determined after oven-drying at 37°C for 7 days to constant weight in a B-ONE IN-65-OL drying oven (B-ONE Corporation, Shanghai, China). The dried grains were weighed using an analytical balance with a precision of 0.01 g (Precisa ES 1200 C, Precisa Gravimetrics AG, Switzerland). Kernel length and width were measured using a digital caliper.

### Statistical analysis

Data from BC4F1 and parental plants were analyzed descriptively to obtain the mean, range, standard deviation and coefficient of variation (CV). Variability was categorized as broad or narrow following the criteria of Chaniago *et al.* (2022). All descriptive statistical analyses were performed using spreadsheet software (Microsoft Excel).

Segregation of the *BADH2* allele in the BC4F1 population was evaluated using a chi-square ( $\chi^2$ ) test of goodness-of-fit to the expected 1:1 segregation ratio for a backcross population. The chi-square value was calculated using the formula:

$$\chi^2 = \sum (O-E)^2/E$$

Where,

O= Observed frequency.

E= Expected frequency.

The calculated  $\chi^2$  value was compared with the critical value at the 5% significance level with 1 degree of freedom.

## RESULTS AND DISCUSSION

### Molecular analysis of BC4F1 plants

Molecular screening using the Bradbury primer set identified 20 heterozygous BC4F1 plants out of 42 individuals analysed (Fig 1A). These plants showed both the 355 bp fragment associated with the non-aromatic allele and the 257 bp fragment associated with the aromatic allele. Chi-square analysis indicated that the segregation pattern followed the expected 1:1 ratio for a backcross population ( $\chi^2 = 3.0 < 3.841$ ), confirming the predicted

distribution of genotypes. Additionally, ten representative BC4F1 plants carrying the aromatic allele were documented alongside both parental varieties for morphological comparison (Fig 1B).

#### Evaluation of heading date, plant height and panicle length

Heading date is a key phenological trait in rice. The BC4F1 plants had a mean heading date of 61 days after transplanting (DAT), which was later than Inpari 33 (57.2 DAT) and comparable to Merah Wangi (61.2 DAT) (Fig 2A, Table 1). The low coefficient of variation (2.49%) indicates that heading date was relatively uniform within the BC4F1 population.

The average plant height of the BC4F1 plants was 100.38 cm, slightly taller than Inpari 33 (98.4 cm) but shorter than Merah Wangi (128.6 cm) (Fig 2B, Table 1) and the narrow variability observed for this trait (6.13%) indicates limited phenotypic dispersion within the population. Panicle length averaged 25.27 cm, which was longer than Inpari 33 (23.66 cm) and shorter than Merah Wangi (28.17 cm) (Fig 2C, Table 1) and this trait exhibited broad variability (5.75%), reflecting considerable differences in panicle size among BC4F1 individuals.

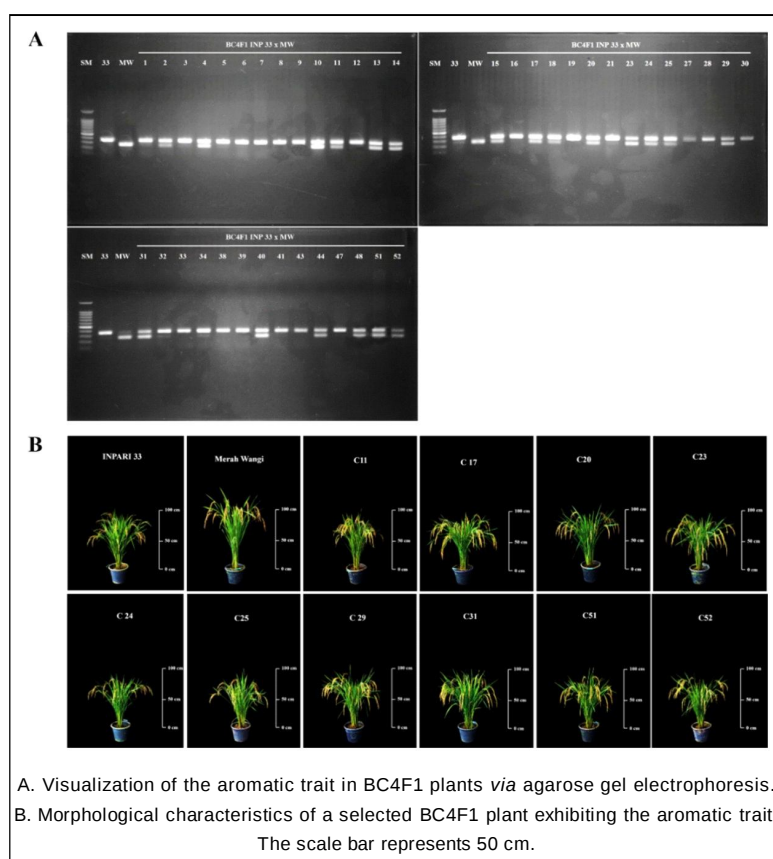
#### Evaluation of productive tillering and yield component

Tillering is an important trait in rice because each tiller has the potential to develop into a panicle and contribute directly

to yield. The BC4F1 plants produced an average of 44.7 tillers per plant, an intermediate value between Inpari 33 (51.6) and Merah Wangi (26.6), with broad variability (15.53%) (Fig 3A, Table 2). On average, BC4F1 plants formed 32.65 productive tillers per plant, fewer than Inpari 33 (44.2) but more than Merah Wangi (21) and this trait also displayed broad variability (24.63%) (Fig 3B, Table 2).

The number of filled grains per panicle is a vital yield component because it directly contributes to total grain production. The BC4F1 plants averaged 105.55 filled grains per panicle, a value higher than Inpari 33 (77.33) but lower than Merah Wangi (175.85) (Fig 3C, Table 2). The proportion of empty grains was 25.81%, which was slightly lower than Inpari 33 (28.72%) and higher than Merah Wangi (23.79%) and this trait exhibited broad variability (23.1%) (Fig 3D, Table 2). For grain yield per plant, the BC4F1 lines produced an average of 43.41 g, exceeding Inpari 33 (33.40 g) but remaining below Merah Wangi (50.31 g), with yield showing broad variability (9.15%) across the population (Fig 3E, Table 2).

The 1000-grain weight and kernel dimensions are important indicators of grain size and density, which influence both yield and market value. The BC4F1 plants had an average 1000-grain weight of 22.76 g, lower than both parents, Inpari 33 (25.51 g) and Merah Wangi (25.75 g) (Fig 4B, Table 3), with broad variability (5.27%).



**Fig 1:** Detection of the aromatic trait in BC4F1 rice plants using the bradbury marker.

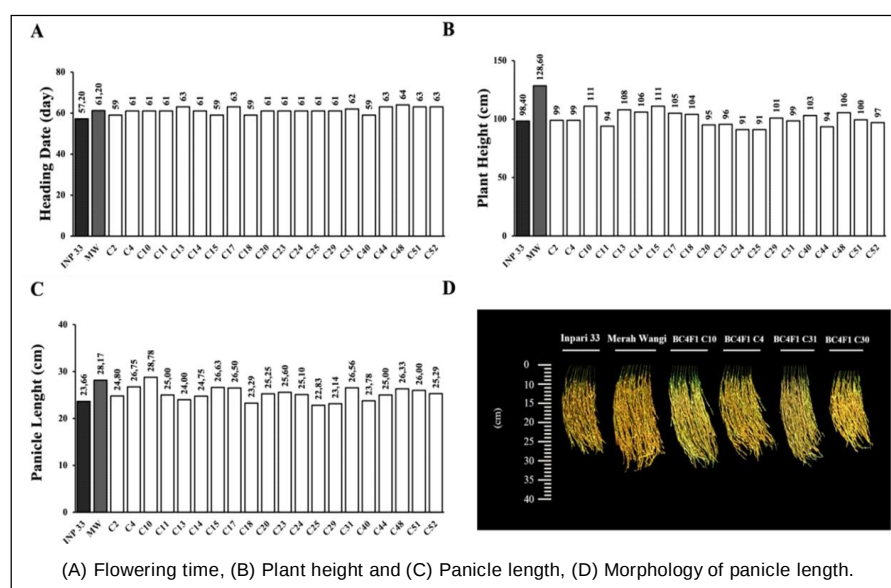
The average kernel length of the BC4F1 plants was 7.05 mm, an intermediate value between Merah Wangi (6.01 mm) and Inpari 33 (7.16 mm) (Fig 4C, Table 3). Kernel width averaged 2.22 mm, slightly smaller than Inpari 33 (2.23 mm) and markedly smaller than Merah Wangi (2.90 mm) (Fig 4D, Table 3). Kernel length and width exhibited broad variability (3.66% and 3.12%, respectively), reflecting considerable phenotypic diversity within the BC4F1 population.

This study demonstrates that Marker-Assisted Backcrossing (MAB) effectively introgressed the aromatic trait into Inpari 33, while maintaining desirable agronomic performance in the BC4F1 generation. Molecular screening using the Bradbury marker identified 20 heterozygous BC4F1 plants, confirming the transfer and stable inheritance of the *BADH2* aromatic allele. As a recessive loss-of-function mutation governs rice fragrance, aroma

develops only when both *BADH2* alleles are inactivated, leading to the accumulation of 2-acetyl-1-pyrroline (Behera and Panda, 2023; Bradbury *et al.*, 2005). The BC4F1 plants exhibited an intermediate heading date of 61 days after transplanting, with low variation indicating a uniform flowering response. Rice as a short-day crop typically requires 7 to 10 hours of light to induce flowering (Anggraeni *et al.*, 2021) and synchronized heading is beneficial for crop management (Khotasena *et al.*, 2022). A flowering time of around 60 days is advantageous in tropical environments such as Indonesia, where early- to medium-maturing varieties support higher cropping intensity (Adhi *et al.*, 2024; Liang *et al.*, 2024). Panicle length averaged 25.27 cm, longer than both parents, a pattern that may reflect transgressive segregation (Rieseberg *et al.*, 1999). Longer panicles are often associated with increased grain number per panicle (Fang *et al.*, 2024), although

**Table 1:** Comparison of phenological and morphological traits of BC4F1 rice plants with the recurrent parent inpari 33 and donor parent merah wangi.

| Parameter           | Plant line | Sample number | Range       | Average | Standard deviation | Coefficient of variation | Variability |
|---------------------|------------|---------------|-------------|---------|--------------------|--------------------------|-------------|
| Heading date (day)  | Inpari 33  | 5             | 57-58       | 57.20   | 0.45               | 0.78                     | Narrow      |
|                     | MW         | 5             | 61-62       | 61.20   | 0.45               | 0.73                     | Narrow      |
|                     | BC4F1      | 22            | 59-64       | 61.30   | 1.53               | 2.49                     | Narrow      |
| Plant height (cm)   | Inpari 33  | 5             | 94-102      | 98.40   | 3.78               | 3.84                     | Narrow      |
|                     | MW         | 5             | 112-139     | 128.60  | 10.09              | 7.85                     | Narrow      |
|                     | BC4F1      | 22            | 91-111      | 100.38  | 6.15               | 6.13                     | Narrow      |
| Panicle length (cm) | Inpari 33  | 5             | 22.59-26.15 | 23.66   | 1.42               | 5.99                     | Broad       |
|                     | MW         | 5             | 25.9-29.2   | 28.17   | 1.39               | 4.95                     | Broad       |
|                     | BC4F1      | 22            | 22.83-28.78 | 25.27   | 1.45               | 5.75                     | Broad       |



**Fig 2:** Phenological and morphological traits of BC4F1 rice plants compared to parental lines.

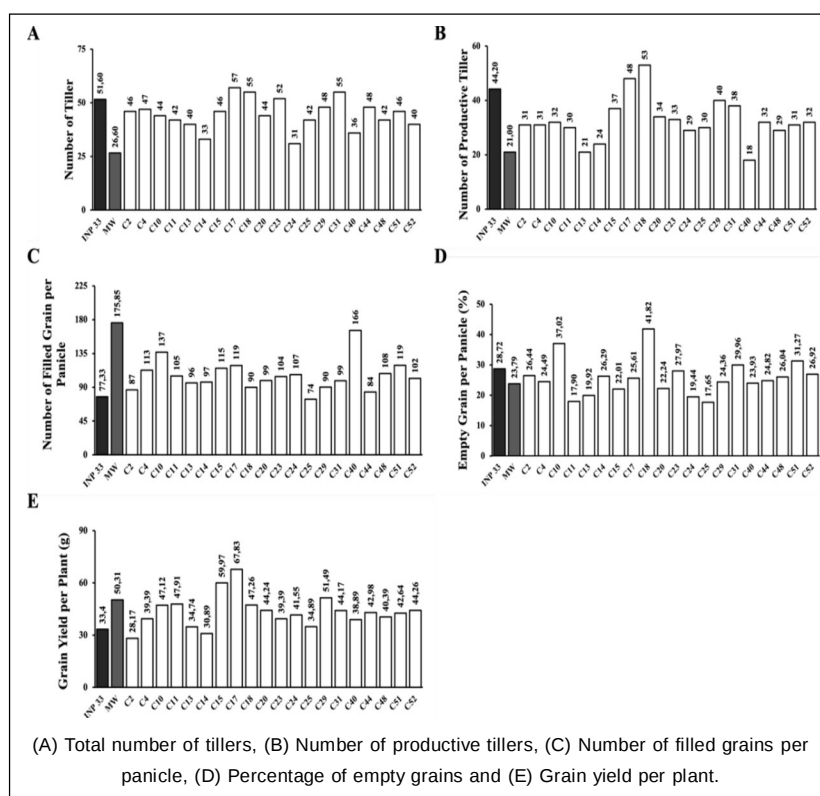
genetic and environmental factors jointly influence this trait (Yadav *et al.*, 2023) and optimal assimilate distribution is required to avoid increases in poorly filled grains (Li *et al.*, 2023; Won *et al.*, 2022).

The BC4F1 plants also exhibited enhanced tillering, producing an average of 45 tillers per plant, representing

an intermediate expression level between Inpari 33 and Merah Wangi. Tillering is governed by genetic, hormonal and environmental regulations, involving the initiation of axillary buds and their subsequent elongation (Yuan *et al.*, 2024). Although the total number of tillers is one component of yield potential, productive tillers are

**Table 2:** Tillering capacity and grain yield components of BC4F1 rice plants compared to Inpari 33 and Merah Wangi.

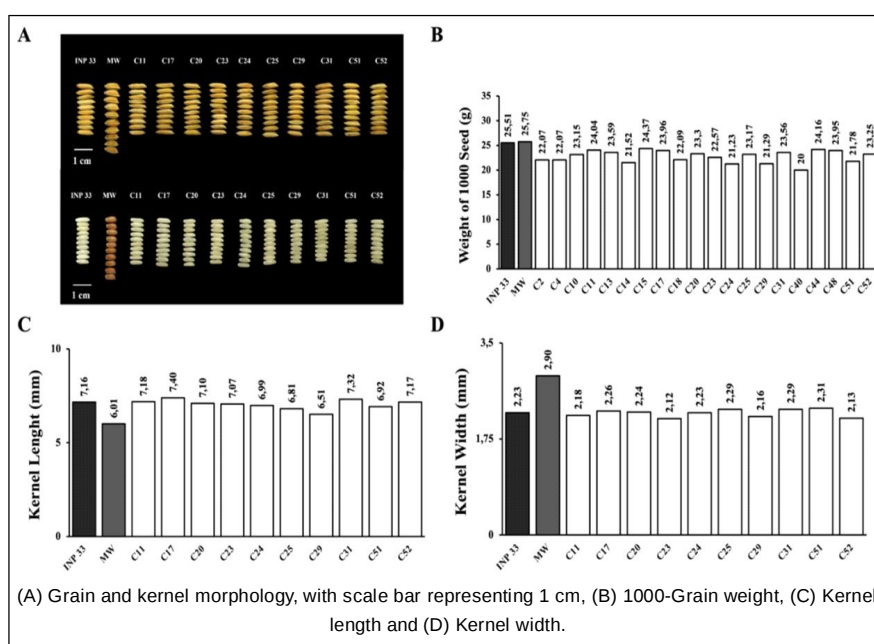
| Parameter                          | Plant line | Sample number | Range       | Average | Standard deviation | Coefficient of variation | Variability |
|------------------------------------|------------|---------------|-------------|---------|--------------------|--------------------------|-------------|
| Tiller number                      | Inpari 33  | 5             | 41-65       | 51.60   | 11.06              | 21.43                    | Narrow      |
|                                    | MW         | 5             | 17-32       | 26.60   | 6.15               | 23.11                    | Broad       |
|                                    | BC4F1      | 22            | 25-57       | 44.70   | 6.94               | 15.53                    | Broad       |
| Productive tiller number           | Inpari 33  | 5             | 33-56       | 44.20   | 12.74              | 28.81                    | Broad       |
|                                    | MW         | 5             | 15-25       | 21      | 3.81               | 18.13                    | Broad       |
|                                    | BC4F1      | 22            | 13-53       | 32.65   | 8.04               | 24.63                    | Broad       |
| Number of filled grain per panicle | Inpari 33  | 5             | 69-91       | 77.33   | 9.36               | 12.11                    | Narrow      |
|                                    | MW         | 5             | 167-204     | 175.85  | 23.85              | 13.56                    | Narrow      |
|                                    | BC4F1      | 22            | 66-166      | 105.55  | 20.14              | 19.08                    | Narrow      |
| Empty grain (%)                    | Inpari 33  | 5             | 25.15-32.93 | 28.72   | 2.91               | 10.12                    | Broad       |
|                                    | MW         | 5             | 18.62-29.37 | 23.79   | 4.17               | 17.52                    | Broad       |
|                                    | BC4F1      | 22            | 17.65-41.82 | 25.81   | 5.96               | 23.10                    | Broad       |
| Grain yield per plant (g)          | Inpari 33  | 5             | 26.1-39.51  | 33.40   | 6.54               | 19.58                    | Broad       |
|                                    | MW         | 5             | 32.65-66.25 | 50.31   | 14.57              | 28.97                    | Narrow      |
|                                    | BC4F1      | 22            | 28.17-67.83 | 43.41   | 9.15               | 21.07                    | Broad       |



**Fig 3:** Assessment of tillering capacity and grain yield components in BC4F1 rice plants compared to parental lines.

**Table 3:** Grain quality characteristics: 1000-Grain weight and kernel dimensions in BC4F1 rice plants compared to inpari 33 and merah wangi.

| Parameter               | Plant line | Sample number | Range       | Average | Standar deviation | Coefficient of variation | Variability |
|-------------------------|------------|---------------|-------------|---------|-------------------|--------------------------|-------------|
| Weight of 1000 seed (g) | Inpari 33  | 5             | 24.61-26.5  | 25.51   | 0.70              | 2.76                     | Broad       |
|                         | MW         | 5             | 23.23-27.29 | 25.75   | 1.52              | 5.91                     | Broad       |
|                         | BC4F1      | 22            | 20-24.37    | 22.76   | 1.20              | 5.27                     | Broad       |
| Kernel lenght (mm)      | Inpari 33  | 5             | 6.9-7.9     | 7.16    | 0.30              | 4.12                     | Broad       |
|                         | MW         | 5             | 5.8-6.3     | 6.01    | 0.23              | 3.88                     | Broad       |
|                         | BC4F1      | 22            | 6.51-7.32   | 7.05    | 0.26              | 3.66                     | Broad       |
| Kernel width (mm)       | Inpari 33  | 5             | 2.2-2.3     | 2.23    | 0.05              | 2.17                     | Broad       |
|                         | MW         | 5             | 2.8-3       | 2.90    | 0.08              | 2.82                     | Broad       |
|                         | BC4F1      | 22            | 2.12-2.31   | 2.22    | 0.07              | 3.12                     | Broad       |

**Fig 4:** Grain quality assessment of BC4F1 rice plants compared to parental lines.

more critical. The BC4F1 plants produced an average of 33 productive tillers, indicating promising yield attributes, while the observed variability suggests the need for further selection to improve trait stability. Improved grain filling was also observed, as the BC4F1 plants produced more filled grains per panicle than both parents. Grain filling is strongly influenced by environmental conditions, including temperature, water supply and light availability (Hu *et al.*, 2021). A relatively high proportion of empty grains in the BC4F1 plants indicates heterogeneity in grain development, which may be associated with stress conditions such as heat, drought, or pest incidence during flowering (Shrestha *et al.*, 2022).

A comprehensive analysis of grain quality traits showed that the BC4F1 plants performed intermediately between Inpari 33 and Merah Wangi. The lower 1000 grain weight in the BC4F1 population suggests partial inheritance of grain

mass from both parents. The broad variation observed indicates considerable genetic diversity, which can be utilized to select lines with improved grain size and market value (Li *et al.*, 2018; Xuedan *et al.*, 2023). Kernel morphology also showed intermediate expression, with average kernel length of 7.05 mm and width of 2.22 mm. The coefficients of variation for these traits highlight meaningful phenotypic diversity, providing breeders with opportunities to refine grain shape and size characteristics that influence consumer preferences and market acceptance (Saidon *et al.*, 2020).

## CONCLUSION

The BC4F1 plants successfully incorporated the *BADH2* aromatic allele from Merah Wangi and showed improved agronomic performance compared with Inpari 33. Molecular and phenotypic assessments confirmed the

stable inheritance of key traits, including heading date and plant height, as well as increases in panicle length and grain yield. Although variation persisted in characteristics such as grain filling and 1,000-grain weight, this diversity offers valuable opportunities for further selection. The next phase of the breeding program will involve additional backcrossing with Inpari 33 to develop a stable, aromatic version of this high-yielding variety, supporting the eventual release of a commercially viable aromatic rice line adaptable to diverse production environments.

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## Conflict of interest

The authors declare that there is no conflict of interest associated with this manuscript.

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