



# Agronomic Characteristics of F1 Rice from Crossing Aromatic Rice with Local Rice Varieties

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**Abstract.** The aroma of pandanus is the hallmark of aromatic rice, which is typically more expensive than regular non-aromatic rice. However, low-productivity cultivars yield the majority of fragrant rice. Thus, breeding aromatic rice is vital for developing new high-yield aromatic rice varieties. In this work, we used non-aromatic IR64 and Sigupai varieties as recipient parents, and aromatic trait donors, Merah Wangi and line 29. Using Bradbury markers, the aromatic genotype was identified based on the presence of the *badh2* allele caused by the *OsBADH2* gene mutation. Our findings demonstrated that the *badh2* allele is heterozygously present in the F1 progenies of the IR64/Merah Wangi, Sigupai/Merah Wangi, and Sigupai/line 29 cross-pollination. The results of the phenotypic study indicated that the F1 Sigupai/Strain 29 plant had the greatest number of tillers and the highest number of productive tillers, while the plant heights of F1 IR64/Merah Wangi and Sigupai/Strain 29 were classified as medium. Furthermore, three F1 lines were classified as medium in terms of both panicle length and number.

**Keywords:** Aromatic rice breeding, *OsBADH2* gene mutation, bradbury markers

## INTRODUCTION

Over half of the global population relies on rice (*Oryza sativa* L.) as a staple diet. Local rice is a naturally occurring type that comes from a region where people have long-farmed rice [1]. It is utilized as germplasm, or a collection of genes with specific features, to create new, improved rice varieties [2]. Aromatic rice is a favorite food of most people in Asia due to its unique ability to release pandanus aroma from 2-acetyl-1-pyrroline (2AP) compounds [3]. Most fragrant rice in Indonesia is produced by low-yielding local varieties that are prone to disease and pests [4]. In aromatic rice breedings, codominant markers are favored over dominant markers to identify aromatic traits because aroma is a recessive trait [5].

Merah Wangi is a type of aromatic brown rice that originated from Pacitan Regency East Java Indonesia [6]. One drawback of Merah Wangi is that the seed breaks easily during milling [7]. Line 29 is the result of crossing Merah Wangi and Pendok rice [8]. The characteristics of line 29 include a high number of tillers, stable aromatic trait, and an average height of 107.93 cm [9]. However, line 29 drawbacks include a poor head rice recovery level and a limited capacity for grain filling [10]. The Sigupai variety, renowned for having a large number of grains, originated in the Southwest Aceh district of Nangroe Aceh Darussalam Province [11]. IR64 variety is one of the rice varieties still widely planted by farmers in Indonesia due to its high productivity, shorter lifespan, and its fluffy taste [12].

In our experiment, we conducted three combinations of crossed breeding; IR64/Merah Wangi, Sigupai/Merah Wangi, and Sigupai/Line 29 [13]. This crossing is aimed to produce a new aromatic rice variety with superior agronomic traits [14]. The agronomic and molecular analysis was conducted on the F1 plants that emerged from the three cross combinations [15].

## METHOD

### Research Implementation

Research activities were carried out from March 2021 to December 2021 at the Center for Development of Advance Sciences and Technology (CDAST). The materials used in this study were seeds of Merah Wangi and line 29 as aromatic trait donors to non-aromatic IR64 and Sigupai varieties as recipient parents.

### Molecular Analysis

The presence of *badh2* allele was analyzed using PCR using genomic DNA from rice leaves [16, 17]. PCR analysis used Bradbury markers with primers ESP, EAP, INSP, and IFAP [18]. PCR analysis begins with making a solution with a total volume of 10  $\mu$ l with the following components; 2.5  $\mu$ l DNA template, 2  $\mu$ l Bradbury primers, 5  $\mu$ l GoTaq® DNA Polymerase (Promega, Madison, Wisconsin, USA), and 0.5  $\mu$ l RNase (Sigma Aldrich, St. Louis, MO, USA) [19]. The primer sequences used for the Bradbury markers were ESP 5'-TTGTTGGAGCTTGCTGATG-3', EAP 5'-AGTGCTTTACAAAGTCCCGC-3', INSP 5'TGGTAAAAAGATTATGGCTTCA-3', and IFAP 5'-CATAGGAGCAGCTGAAATATACC3' [20]. PCR was performed for 30 cycles with the following temperatures; 95°C for 5 minutes (initial denaturation), 90°C for 30 seconds (denaturation), 55°C for 30 seconds (annealing), 72°C for 1 minute (elongation), and 72°C for 5 minutes (final elongation) [21]. PCR products were then visualized using DNA electrophoresis with agarose gel concentration of 1.5% agarose gel (Vivantis, Shah Alam, Malaysia) in 1  $\times$  TAE buffer and 100 bp DNA ladder (Tiangen Biotech, Beijing, China). DNA band observation was carried out using a gel documentation system (FluorChem HD2 system, USA) [22, 23]. The phenotype analysis conducted included plant height, number of productive tillers, panicle length, number of grains in panicle, and weight of 1000 grains [24].

### Molecular Analysis

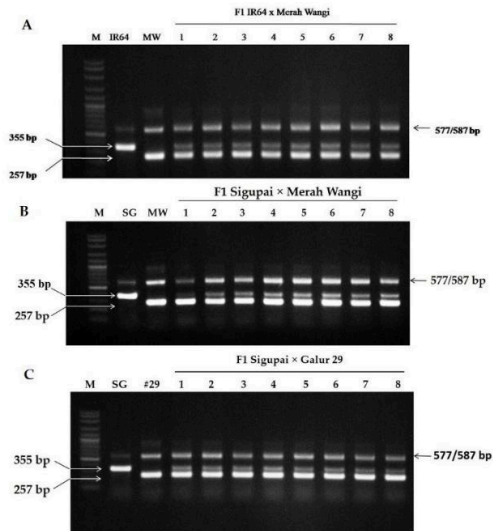
The data are presented as mean values from experiments with a minimum three replications. Statistical significance among multiple samples was examined using two-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) at 95% confidence level [25].

## RESULTS

### Analysis of the Presence of *badh2* Allele

The aromatic trait in rice is caused by a mutation of *OsBADH2* gene producing *badh2* allele. The presence of *badh2* can be detected using PCR analysis with Bradbury makers. The success of cross pollination is shown by the presence of *badh2* in F1 plants.

The visualization of PCR products showed that IR64 and Sigupai produced DNA fragments with 355 bp length whereas Merah Wangi and line 29 had 257 bp of DNA fragments. Moreover, eight F1 plants from IR64/Merah Wangi, Sigupai/Merah Wangi, and Sigupai/line 29 cross breeding combinations produced two DNA fragments of 355 bp and 257 bp (FIGURE 1). These results showed that the *badh2* allele is present in the heterozygous state in all examined F1 plants. In this PCR analysis, INSP/EAP primers amplified the 355 bp DNA fragment from non-aromatic and heterozygous plants, while ESP/IFAP primers amplified the 257 bp band from non-aromatic and homozygous plants. Internal positive controls, or DNA fragments with 577/585 bp, are amplified using ESP/EAP primers and are present in all PCR samples. The heterozygous non-aromatic plant produced DNA fragments of 355 bp and 257 bp as a result of chromosome combinations from non-aromatic and aromatic parental lines. According to Pratiwi et al., PCR analysis employing Bradbury markers revealed that the F1 rice plant resulting from the crossing of Fatmawati and Mentik Wangi similarly produced 355 and 257 bp DNA pieces.



**FIGURE 1.** Visualization of PCR results using Bradbury markers (A) visualization results of IR64, MW: Merah Wangi, and F1 cross of IR64 with Merah Wangi. (B) visualization results of SG: Sigupai, MW: Merah Wangi, and F1 cross of Sigupai with Merah Wangi. (C) SG: Sigupai, #29: Line 29, and F1 cross of Sigupai with Line 29.

### Vegetative Stage

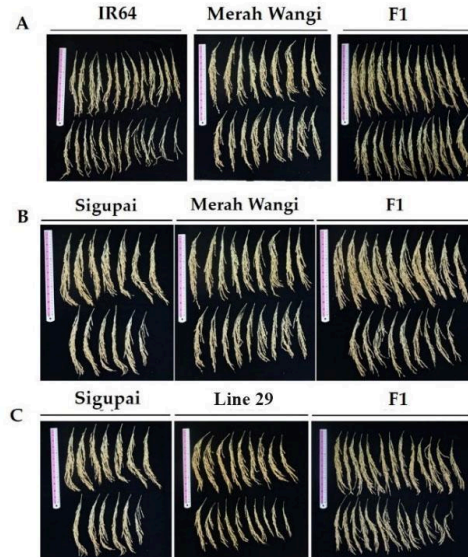
For every rice genotype, the average number of productive tillers per plant ranged from 13.25 to 23.25. The F1-IR64/Merah Wangi plants had a significantly higher number of productive tillers than the Merah Wangi (male parent) variety, but were not statistically different from the IR64 variety. The productive tiller numbers of F1-Sigupai/Merah Wangi were comparable to those of Sigupai, the female parent, and Merah Wangi, the male parent. Additionally, compared to Line 29 (the male parent) and Sigupai (the female parent), F1-Sigupai/Line 29 had a much higher number of productive tillers. This suggests that the cross between Sigupai and Line 29 resulted in a significant increase in productive tiller numbers. The findings indicate that the combination of these two parent varieties may have a positive impact on tiller production in the F1 generation.

**TABLE 1.** Comparative Value of Phenotype Characters of Sigupai, Merah Wangi, Line 29, dan F1 in Vegetative Phase.

Variety	Phenotype Characters			
	Number of Productive Tillers	Panicle Lengths	Grains per Panicle	1000 grains Weight
IR64	21cde	23.07b	104.58a	26.36a
Sigupai	13.25a	25.17cd	190.86cd	29.06d
Merah Wangi	16.25ab	24.48c	193.47cd	27.84bc
Line 29	17.75bc	21.52a	129.99ab	28.96cd
F1- IR64/Merah Wangi	21.5de	26.05d	156.73bc	26.25a
F1- Sigupai/Merah Wangi	17abc	25.5cd	206.8d	28.37cd
F1- Sigupai/ Galur 29	23.25e	23.0b	164.41bed	27.16ab

Numbers followed by the same letter are not statistically significantly different as tested by DMRT test at 95% confidence level.

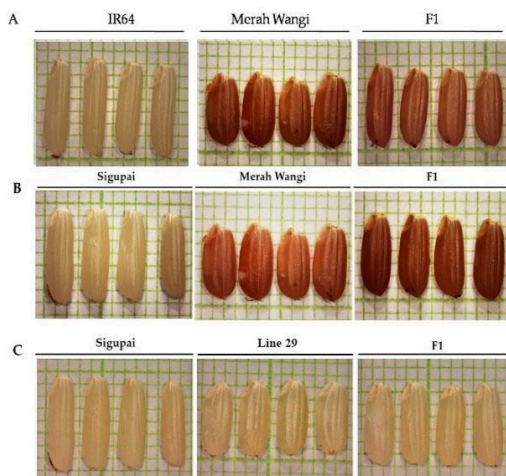
There are four classifications for the quantity of productive tillers on rice plants: extremely low (>5), few (5–9), medium (10–19), high (20–25), and very high (>25). According to this classification, F1-IR64/Merah Wangi and F1-Sigupai/Line 29 have a high number of productive tillers whilst F1- Sigupai/Merah Wangi is classified as having a medium type. Yield and the number of productive rice tillers are closely correlated. High yield rice cultivars have a large proportion of productive tillers.



**FIGURE 2.** Comparison of panicle length of Sigupai, Merah Wangi, Line 29, dan F1. (A) Panicle length of IR64, Merah Wangi, and F1. (B) Panicle length of Sigupai, Merah Wangi, and F1. (C) Panicle length of Sigupai, Line 29, F1.

F1-IR64/Merah Wangi demonstrated a significantly longer panicle than IR 64 and Merah Wangi in terms of the panicle length parameter; however, F1-Sigupai/Merah Wangi's panicle length did not differ significantly from Sigupai and Merah Wangi. Furthermore, F1-Sigupai/Line 29 had a panicle length that was noticeably longer than Line 29 but shorter than Sigupai. There are three classifications for panicle length: short (less than 20 cm), medium (20–30 cm), and long (more than 30 cm). The panicle length of F1-IR64/Merah Wangi, F1-Sigupai/Merah Wangi, and F1-Sigupai/Line 29 is included in the medium category. The length of the rice panicle is a major determinant of the number of grains per panicle that determines rice yield.

The F1-IR64/Merah Wangi grain number per panicle was substantially higher than IR 64, but it did not differ considerably from Merah Wangi. The grain number per panicle of F1-Sigupai/Merah Wangi was substantially higher than that of Sigupai and Merah Wangi. In addition, F1-Sigupai/Line 29 grain number per panicle did not differ significantly from Sigupai and Line 29. Three groups exist for the number of grains per panicle: low (<150 grains), medium (150-300 grains), and many (>300 grains). For all F1 plants, the number of grains per panicle is considered medium type. The total number of grains produced by a rice plant is positively correlated with productivity. Panicle length and nutrient availability are important factors in grain formation per panicle.



**FIGURE 3.** Comparison of seed shape of Sigupai, Merah Wangi, Line 29, dan F1. (A) Seed shape of IR64, Merah Wangi, and F1. (B) Seed shape of Sigupai, Merah Wangi, and F1. (C) Seed shape of Sigupai, Line 29, F1.

Together with the number of grains per panicle, the weight of 1000 grains also plays a role in determining the yield of rice plants. The weight of 1000 grains of F1-IR64/Merah Wangi was substantially lower than that of Merah Wangi but not significantly different from IR64; in contrast, the weight of 1000 grains of F1-Sigupai/Merah Wangi did not differ significantly from Sigupai and Merah Wangi. Furthermore, F1-Sigupai/Line 29 plants' 1000 grain weight did not change appreciably from that of Sigupai and Line 29. The amount of dry matter in the seeds has an impact on their weight. Photosynthetic material is used to provide dry matter for seed filling. Furthermore, the 1000-grain weight is determined by the genetic characteristics of each variety.

## CONCLUSION

Using PCR analysis with a Bradbury marker, the F1 plants from the IR64/Merah Wangi, Sigupai/Merah Wangi, and Sigupai/Line 29 cross combinations were successfully selected. F1-Sigupai/Merah Wangi has demonstrated a high level of plant height, a medium level of panicle length, a medium level of number of grains per panicle, and a medium level of productive tiller count. Medium-level plant height, a high level of productive tiller number, medium-level panicle length, and medium-level grain number per panicle were all displayed by F1-Sigupai/Line 29. Additionally, F1-IR64/Merah Wangi had a medium level of panicle length, a medium level of plant height, a high level of productive tiller number, and a medium level of grains per panicle.

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