

# Agronomic and genetic characteristics of f1 black rice × white glutinous rice crosses

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**Abstract.** Sholikhah U, Mujahidin I, Fanata WID, Ratnasari T, Tanzil AI. 2025. Agronomic and genetic characteristics of f1 black rice × white glutinous rice crosses. *Biodiversitas* 26: 528-535. Black rice cultivation faces obstacles such as long maturity periods, tall plant height, and low productivity. This study investigated the agronomic characteristics and genetic linkage of rice color traits (anthocyanin) in the F1 generation of crosses between black rice and white glutinous rice. The genetic study utilized DNA markers to detect the linkage of rice color traits, with RM 252 serving as the DNA marker. The plant materials used were Paketih white glutinous rice, which was used as the female parent, Purwokerto local black rice served as the male parent, and F1 plants derived from crosses between Paketih white glutinous rice and Purwokerto local black rice. The results revealed that genetic analysis using the RM 252 DNA marker produced two bands within a single genome of the F1 plants, indicating that the F1 plants exhibit heterozygous traits. Agronomic data, analyzed in the independent sample t-test, showed that F1 plants in the vegetative phase were classified as tall, with substantial number of tillers and productive tillers. In the generative phase, F1 plants displayed faster flowering, longer panicle length, a moderate number of grains per panicle, and heavier grain weight per clump than those of the parents, and a 1000-grain weight intermediate between the parents. The results of this study also confirm that the inheritance of rice color traits and their agronomic potential can be used to improve the efficiency of breeding programs to produce superior varieties. The genetic relationship detected with RM 252 can be used to accelerate the selection of anthocyanin-containing plants.

**Keywords:** Agronomic characters, black rice, F1 plants, rice color traits, white glutinous rice

## INTRODUCTION

Rice is a staple food for the majority of the world population, with varieties of white, red, and black rice (Prasad et al. 2019). Black rice is perceived highly beneficial for enhancing overall health and well-being, increasing antioxidant intake, and providing essential nutrients as well as contributing to diversifying agricultural production (Cañizares et al. 2024). In contrast to white or red rice, black rice contains higher levels of vitamins and minerals and richer bioactive components which include anthocyanins, phytic acid, polyphenols, flavonoids, and B-complex vitamins as antioxidant compound (Pang et al. 2018). The high antioxidant content makes black rice a more nutritious choice compared to white or red rice. Therefore, its use in various types of food can enhance nutritional quality (Rahim et al. 2022).

Anthocyanins, predominantly found in the aleurone and endosperm layers, are responsible for the distinctive purple-dark color of black rice, which tends to get darker with a higher level of anthocyanin (Sholikhah et al. 2021). Anthocyanins are perceived as the most effective dietary antioxidants which contribute to fighting both chronic and non-chronic diseases like atherosclerosis (Yamuangmorn et al. 2021), offer numerous health benefits, help reduce the risk of various chronic illness, improve digestive health, stabilize high blood pressure, reduce allergies, detoxify the

body, control diabetes, aid in weight management, and slow down cancer progression (Panda et al. 2022).

Black rice is one of the rare types of rice germplasm that offers a variety of health benefits (Xia et al. 2021). Black rice germplasm has spread and developed in various regions of Indonesia, often referred to as local black rice. The drawbacks of local black rice from Indonesia include long maturity periods, tall plant height, low productivity, and high amylose content which makes the rice texture hard (Nandariyah et al. 2023). In addition, currently available black rice varieties still have limitations in yield and adaptation to the environment compared to white rice varieties (Brunet-Loredo et al. 2023). The challenge in black rice breeding is how to improve superior agronomic traits while maintaining high anthocyanin content. Anthocyanin, the pigment that gives rice its black color, has an important role as a natural antioxidant with significant health benefits (Fatchiyah et al. 2020). However, this trait is controlled by several genes, so conventional breeding is often less efficient in obtaining the desired superior varieties (Ahmar et al. 2020).

These features can be improved to develop new superior black rice strains exhibiting characteristics of high productivity, early maturity, medium plant height, high anthocyanin content, and a soft rice texture. One promising approach to achieve this goal is to cross local black rice with high-yielding varieties that have better agronomic

quality. The utilization of Marker-Assisted Selection (MAS) can accelerate the breeding process by increasing efficiency in selecting individuals with superior traits. In addition to producing varieties with improved traits, this approach also contributes to food security by increasing the availability of high-quality black rice (Dadlani and Yadava 2023). The white glutinous rice variety Paketih is a superior variety in Indonesia known for its high productivity, lodging resistance, and low amylose content. Therefore, the results of this crosses are expected to inherit the superior traits or genes from both parents to the next generation (Patil 2021).

Selection in crossbreeding programs for local black rice can be enhanced by integrating molecular marker-based methods, specifically Marker-Assisted Selection (MAS). The use of DNA markers in MAS improves efficiency and precision in conventional plant breeding (Ishak and Fedora 2024). MAS becomes effective once appropriate marker information is identified and linked to the desired trait. The Quantitative Trait Loci (QTL) for rice color, particularly in the hull and pericarp is widely associated with SSR, such as RM 220 on chromosome 1 and RM 252 on chromosome 4 of the rice genome (Kristantini et al. 2018). Chromosome 4 and 1 plays a critical role in black pigmentation by identifying key genes involved in anthocyanin synthesis and purple pericarp traits. The genes Pp and Pb, located on chromosomes 4 and 1 respectively, collaborate to produce the characteristic color of black rice (Savitha et al. 2023). The genes purple pericarp B (Pb) and purple pericarp A (Pp) have been identified through conventional genetic research as key factors for black pigmentation (Oikawa et al. 2015). This study aimed to examine the agronomic characteristics and genetic linkage of rice color traits (black pigmentation) in F1 generation plants derived from white glutinous rice crosses × local black rice. It is important to understand the inheritance pattern of black color traits and the potential for improvement in agronomic traits such as plant height, maturity periods, and productivity. By understanding this, steps can be taken to improve more productive and adaptive black rice varieties.

## MATERIALS AND METHODS

### Plant materials

The Paketih white glutinous rice was used as the female parent because it has high productivity and resistance to lodging, which is expected to improve agronomic traits in the resulting F1 plants. Purwokerto local black rice was chosen as the male parent due to its high anthocyanin content, which provides a distinctive black color and health benefits. The crossbred F1 plants were analyzed with the Marker-Assisted Selection (MAS) through Polymerase Chain Reaction (PCR) to determine whether the crosses carried the genetic linkage for rice color traits. The crossing process was carried out in the greenhouse at Agrotechnopark, University of Jember, while the molecular analysis was conducted in the Molecular Biology and Biotechnology Laboratory of UPA Waste Management and Integrated Laboratory at the University of Jember.

### Procedure

#### *Crossing the two parents*

The crossing process was done in two steps: emasculation and pollination. Emasculation was performed on the female parent (Paketih white glutinous rice) by cutting one-third of the rice grain using scissors, removing all stamens with a vacuum pump or tweezers. The emasculated grains were then covered with paper temporarily to prevent external pollination. The pollination was carried out using the flowers from the male parent (Purwokerto local black rice) that reached the flowering stage. The cover paper on the female parent was carefully removed, the male flowers were brought close to the emasculated female panicle, then both flowers were gently shaken to ensure the pollen were attached evenly to the panicle.

#### *Planting F1 plants and both parents*

Seeding was done in a seedling tray prior for approximately 21 days, then transplanted in buckets filled with rice field soil as the planting medium. The F1 seeds were planted along with the male and female parents. The seeds were fertilized with urea, NPK, and ZA, and watered regularly to periodically inundate the planting medium until harvest. Pesticide was applied at appropriate dosage to control pest and disease. Approximately 3-4 weeks after the rice plants entered the grain-filling phase, the rice was harvested by cutting the yellow, fully-developed panicles.

#### *Genomic DNA extraction of rice plants*

The extraction of genomic DNA from rice plants started by collecting a sample of rice plant leaf of  $\pm 1$  cm long and placing it in a microtube filled with with 500  $\mu$ L of extraction buffer. The leaf sample was crushed using a cell disruptor. Then, 500  $\mu$ L of the supernatant was drawn and mixed with 500  $\mu$ L of PCL, and the mixture was centrifuged at 12,000 rpm for 5 min. After centrifugation, 400  $\mu$ L of the top layer was transferred into a new tube, added with 100% ethanol 2.5 times the total volume, then recentrifuged at 12,000 rpm for 10 min to precipitate the DNA. The liquid was discarded into a collection container, then 700  $\mu$ L of 70% ethanol was incorporated into the tube and centrifuged again at 12,000 rpm for 5 min. Following this, 70% ethanol was discarded, the tube was spinned down, and any remaining ethanol was removed using a micropipette. Finally, the genomic DNA was dried at 37°C for 10 min, then added with 50  $\mu$ L of TE buffer to dissolve the genomic DNA (Fanata and Husna 2021).

#### *Polymerase Chain Reaction (PCR)*

PCR was performed by mixing several components that included 2.4  $\mu$ L of template DNA, 1.6  $\mu$ L of primers, 5  $\mu$ L of reaction mixture, and 1  $\mu$ L of RNase, resulting in a total volume of 10  $\mu$ L. DNA samples from F1 plants were subjected to PCR using specific primers related to rice color traits, namely RM 252. The RM 252 primers consist of a forward primer (TTCGCTGACGTGATAGGTTG) and a reverse primer (ATGACTTGATCCCGAGAACG) with a product size of 216 bp (Kristantini et al. 2018). The PCR cycle steps involved an initial denaturation at 95°C for 2

min, followed by denaturation at 95°C for 30s, annealing at 54°C for 30s, elongation at 72°C for 1 min, and a final elongation at 72°C for 5 min (Fanata and Husna 2021).

#### Electrophoresis

This process used 1.5% agarose gel (0.75 g) and incorporated it into 50 mL of TAE buffer. The solution was placed in a microwave for 1 min, then added with 4 µL of EtBr, and the solution was poured onto an agarose gel plate. Exactly 10 µL of PCR products were loaded into the agarose gel wells, which were submerged in TAE solution in the electrophoresis tank. The electrophoresis process was run at 100 volts for approximately 32 min. The visualization of the DNA bands was carried out in a gel documentation system.

#### Data analysis

The average data obtained were analyzed using a paired sample t-test with a significance level of  $\alpha = 0.05$ . Before conducting the t-test, a variance homogeneity test was conducted using the F-test. If the p-value  $>0.05$  the data is considered homogeneous so that the t-test analysis can be continued. The t-test results show whether there is a significant difference between pairs of samples. If the p-value  $<0.05$  the difference is statistically significant. Conversely, if the p-value  $>0.05$  there is not statistically significant difference. Next, a correlation analysis was conducted to see the relationship between the variables. Pearson correlation analysis was used with correlation values ranging from -1 to 1. The closer to 1, the stronger the relationship between the two variables. The results of the Pearson correlation analysis were visualized in the form of a heatmap to facilitate interpretation of the relationship between variables. The software used in this analysis is Microsoft Excel 2019 for the F-test and t-test, and Python with Google Colaboratory for Pearson correlation analysis and heatmap visualization.

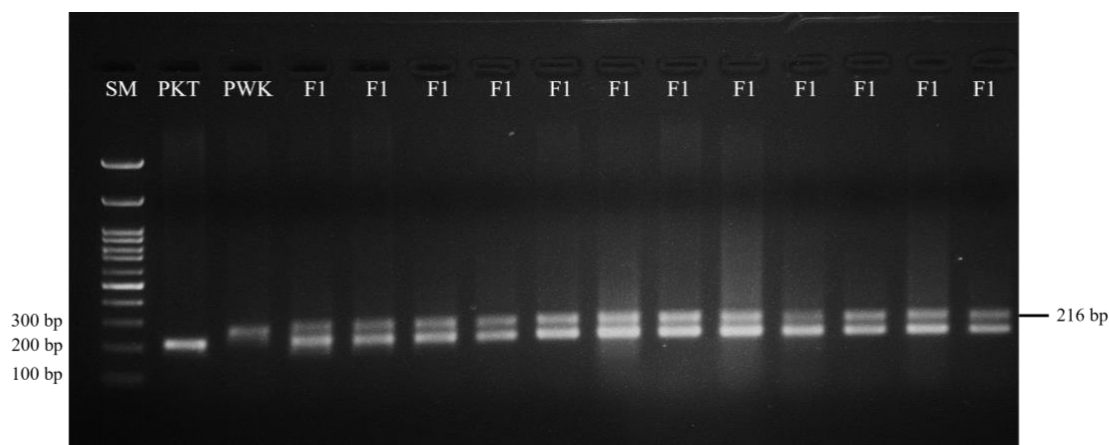
## RESULTS AND DISCUSSION

### Molecular identification of rice color traits in F1 plants

PCR (Polymerase Chain Reaction) analysis was conducted to select F1 plants based on the linkage of rice color traits (anthocyanins). The PCR samples were genomic DNA from the Paketih white glutinous rice variety as the female parent, Purwokerto local black rice as the male parent, and F1 plants of Paketih x Purwokerto crossbreed. The PCR analysis was performed to trace the linkage of rice color traits in F1 plants carrying the purple color trait in seeds from the local black rice. The primer RM 252 was the SSR marker related to rice color traits used in the PCR analysis.

The results of the PCR analysis showed clear DNA bands (Figure 1), indicating the presence of polymorphism marked by bands in both parental strains in different positions, and two bands were present in the F1 plant genome. The F1 plants exhibited a heterozygous trait because they had DNA bands resembling both parental strains of white glutinous rice (female) and local black rice (male). It is in line with Kristantini et al. (2018) that the RM 252 marker can identify heterozygosity in the cross of local black rice and Situbagendit white rice, producing DNA bands similar to those of both parents. Anthocyanins are mostly found in the pericarp of black rice. RM 252 is found in a prospective region on chromosome 4 (Utami et al. 2009). Chromosome 4 is responsible for encoding the basic helix-loop-helix (bHLH) transcription factor. This transcription factor is important for regulating anthocyanin synthesis (Savitha et al. 2023).

F1 plants show that the inheritance of black color in rice has a genetic link that can be observed through molecular markers. Inheritance of this trait occurs because a cross between two homozygous elders with genetic differences produces heterozygous offspring that carry one allele from each parent (Adi et al. 2021). These results reinforce the concept that alleles that determine black color in rice are dominant or semi-dominant (Fathima et al. 2022), so they can appear in the F1 population although with varying expression.



**Figure 1.** Visualization of PCR results using marker RM 252. PKT: Paketih white glutinous rice; PWK: Purwokerto local black rice; and F1 Paketih white glutinous rice × Purwokerto local black rice

The nature of heterozygosity in the F1 population can affect the expression of other agronomic traits such as plant height, flowering age, and yield (Ahmad et al. 2023). This phenomenon can be explained by the interaction between color genes and other genes involved in plant growth regulation. In addition, heterozygous F1 populations tend to show phenotypic uniformity compared to segregating populations in the next generation (Bradshaw 2022). Thus, these findings not only confirm the existence of genetic linkages in rice color traits but also provide insights into inheritance patterns and their implications in breeding programs to develop high-yielding varieties with desirable trait combinations.

### Agronomic characterization

#### Plant height

Plant height is used as a reference indicator for plant growth. The average height of the F1 plants was 151.57 cm, which was significantly taller than the Paketih white glutinous rice (113.53 cm) but shorter than the Purwokerto local black rice (165.57 cm) (Figure 2). The International Rice Research Institute (IRRI) (2013) classified plant height into three categories: short (<110 cm), medium (110-130 cm), and tall (>130 cm). Therefore, the F1 plants is categorized as a tall plant. While plant height is a growth parameter in rice plants, it is not directly proportional with the harvest yield, so a taller plant does not necessarily produce more yield. This result is consistent with Sholikhah et al. (2019) that shorter plants tend to have more tillers and photosynthesis than taller plants, thus increasing rice grain production. It is because the metabolic process for growth is reduced, directing more resources for panicle formation than height increase.

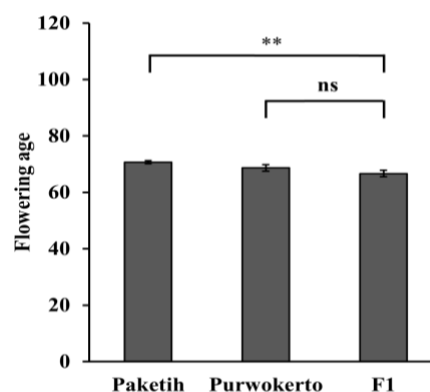
The International Rice Research Institute (IRRI) (2013), plant height can be classified into three categories: short (<110 cm), medium (110-130 cm), and tall (>130 cm). Based on this classification, the F1 plants (151.57 cm) fall into the tall category. Plant height is one of the growth parameters in rice plants, but a taller plant does not necessarily guarantee a larger yield. This result is consistent with the study by Sholikhah et al. (2019), which found that shorter plants tend to have more tillers than taller plants, potentially leading to higher rice grain production due to increased photosynthesis. This is because the metabolic

process for growth is reduced, allowing for more resources to be used for panicle formation rather than height increase.

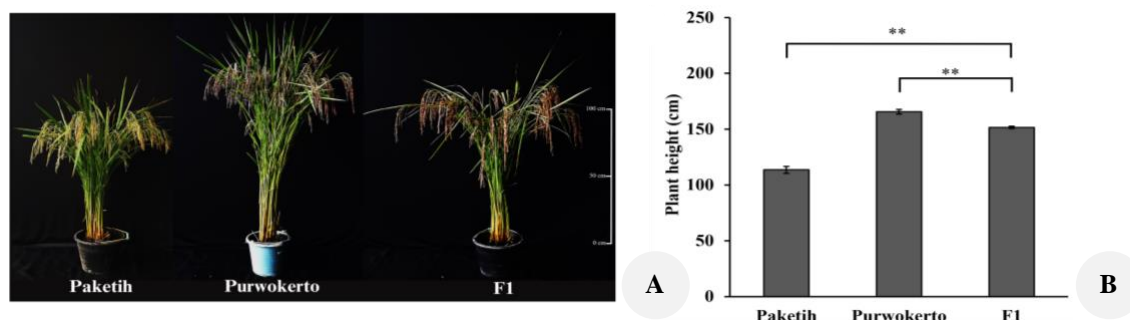
#### Flowering age

The early generative phase is characterized by the appearance of panicles, followed by flowering, which is a feasible characteristic for measurement parameter when 50% of the rice plants in a cluster have blossomed. The F1 plants flowered on 67 days after planting, which was significantly earlier than the white glutinous rice variety Paketih (71 days) but not significantly differ from the Purwokerto local black rice (69 days) (Figure 3).

The early-maturing rice can be determined by both vegetative and generative phases because rice plants that flower earlier generally have a shorter harvest period than their counterparts. It has been reported that the longer the flowering, the more delayed the harvest time (Kasim et al. 2020). Therefore, the age at which 50% of the plants have flowered is positively correlated with the plant's age or harvest time, meaning that plants with an earlier 50% flowering time will also have a shorter harvest period. The F1 plants flowered earlier than both parent plants, indicating that the F1 plants will have a shorter harvest period.



**Figure 3.** Comparison of flowering age of F1 plants with Paketih white glutinous rice and Purwokerto local black rice. Data were analyzed using t-test with a significance level of 0.05, and considering the homogeneity of variance using the F-test. Note: ns: Not significant; \*: Significant; \*\*: Highly significant



**Figure 2.** Comparison of F1 plant height with Paketih white glutinous rice and Purwokerto local black rice. A. Plants of Paketih rice, Purwokerto local black rice, and F1 at 87 days after planting; B. Plant height of Paketih rice, Purwokerto local black rice, and F1. Data were analyzed using the t-test with a significance level of 0.05, and considering the homogeneity of variance using the F-test. Note: ns: Not significant; \*: Significant; \*\*: Highly significant

#### Total number of tillers and number of productive tillers

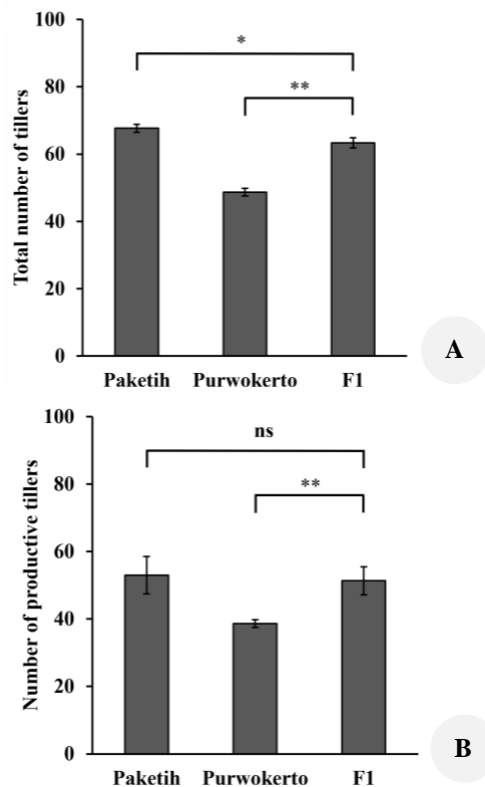
Plant growth influences the total number of tillers and the number of productive tillers during the vegetative and generative phases. This study showed an average of 63 tillers per clump recorded in the final vegetative phase. It was significantly different from 68 tillers of the Paketih white glutinous rice and 49 tillers of the Purwokerto local black rice. The F1 plants had an average of 51 productive tillers, which was not significantly different from 53 tillers of Paketih variety but significantly different from 39 tillers of Purwokerto local black rice (Figure 4).

IRRI (International Rice Research Institute) (2013) classified the number of productive tillers per clump into five categories: very few (<5 tillers), few (5-9 tillers), medium (10-19 tillers), ideal (20-25 tillers), and very many (>25 tillers). Therefore, the F1 plants were in the very many categories with its exceptionally high number of productive tillers. A positive correlation was identified between the total number of tillers and the number of productive tillers, indicating that the number of tillers is positively correlated with the number of productive tillers (Shrestha et al. 2021). Both total number and productive tillers have a positive and direct influence on grain yield, in which the total yield increased with the number of productive tillers (Renuprasath et al. 2023).

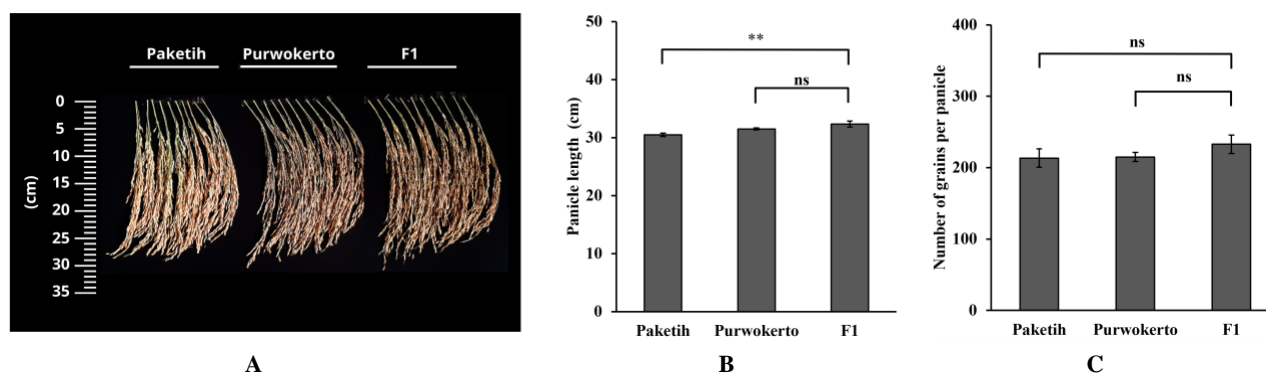
#### Panicle length and number of grains per panicle

As rice plants progressed into the final generative phase, key parameters such as panicle length and the number of grains per panicle were measured. The panicle is the part of the rice plant that contains the grains or seeds, making panicle length an important indicator of rice plant productivity. Panicle length has a positive relationship with the number of grains per panicle, especially in terms of grain formation on the panicle. The average panicle length of F1 plants was 32.35 cm, indicating a significant difference from the average of 30.5 cm of Paketih white glutinous rice, but not significantly different from 31.5 cm of Purwokerto local black rice. The average number of grains per panicle in F1 plants was 233, which did not

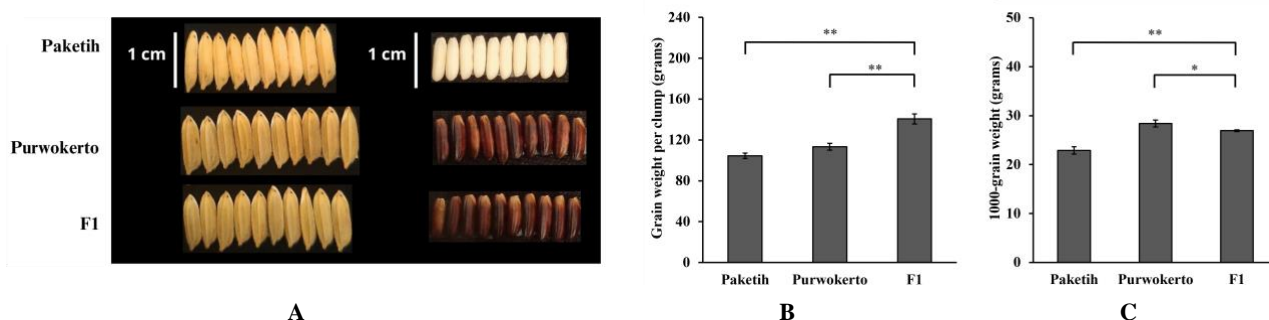
differ significantly from the 213 grains of Paketih white glutinous rice or the 215 grains average of Purwokerto local black rice (Figure 5).



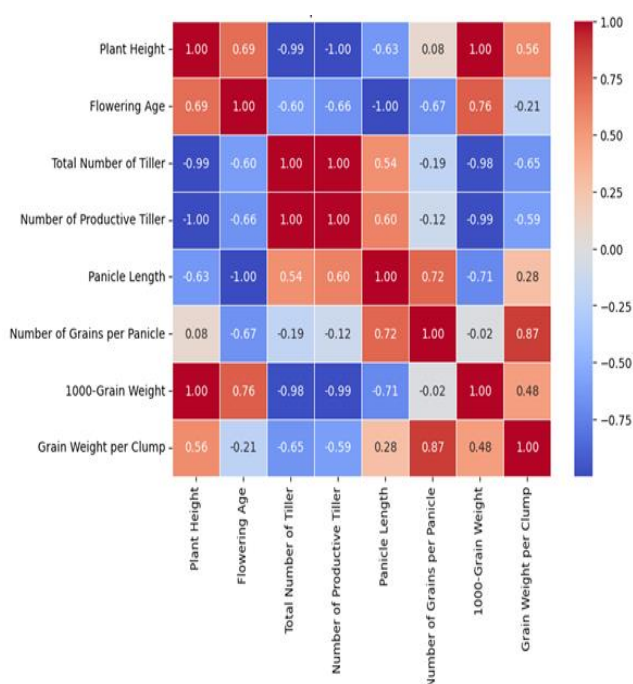
**Figure 4.** Comparison of the number of tillers of F1 plants with Paketih white glutinous rice and Purwokerto local black rice: A. Total number of tillers per clump of Paketih, Purwokerto local black rice, and F1; B. Number of productive tillers per clump of Paketih, Purwokerto local black rice, and F1. Data were analyzed using the t-test with a significance level of 0.05, and considering the homogeneity of variance using the F-test. Note: ns: Not significant; \*: Significant; \*\*: Highly significant



**Figure 5.** Comparison of panicle length and number of grains per panicle of F1 plants with Paketih white glutinous rice and Purwokerto local black rice. A. Samples of the 10 longest panicles of Paketih, Purwokerto local black rice and F1; B. Panicle length of Paketih, Purwokerto local black rice and F1; C. Number of grains per panicle of Paketih, Purwokerto local black rice and F1. Data were analyzed using t-test with a significance level of 0.05, and considering the homogeneity of variance using the F-test. Note: ns: not significant; \*: Significant; \*\*: Highly significant



**Figure 6.** Comparison of grain weight of F1 plants with Paketih white glutinous rice and Purwokerto local black rice. A. Comparison of seeds of the elders Paketih, Purwokerto local black rice and F1; B. Grain weight per clump of Paketih, Purwokerto local black rice and F1; C. 1000-grain weight of Paketih, Purwokerto local black rice and F1. Data were analyzed using t-test with a significance level of 0.05, and considering the homogeneity of variance using the F-test. Note: ns: not significant; \*: significant; \*\*: highly significant



**Figure 7.** Correlation between agronomic character variables, especially those that affect crop yield potential

The panicle length of rice is categorized into three groups: short (<20 cm), medium (20-30 cm), and long (>30 cm), while the number of grains per panicle is also categorized into three levels: low (<150 grains), medium (150-300 grains), and high (>300 grains) (Fanata et al. 2024). Therefore, the F1 plants were categorized into the medium category of panicle length and number of grains per panicle. The number of grains per panicle is positively correlated with yield, the more grains per panicle, the higher the potential for greater yield (Singh et al. 2018). Both panicle length and the number of grains per panicle directly affect the rice yield (Huang et al. 2021).

*Grain weight per clump and 1000-grain weight*

Rice plants, upon entering the generative phase, will continue to the yellowing phase until harvesting. The

harvested grain was oven dried then the grain content weight per clump and the 1000-grain weight were measured using analytical scales. The results indicated that F1 plants had an average grain weight of 140.64 g, which was significantly different from the Paketih white glutinous rice variety (104.51 g) and the Purwokerto local black (113.36 g). The average weight of 1000 grains of F1 plants was 26.93 g, which was significantly different from the 22.89 g of the Paketih variety of white glutinous rice and the 28.39 g of Purwokerto local black rice (Figure 6).

Grain weight and grain shape are important determinants of rice grain yield and quality (Zhang et al. 2024). Rice grain yield is influenced by three main morphological traits: grain weight, number of grains per panicle, and number of productive tillers, while rice grain weight is determined by seed filling and volume which are influenced by spikelet hull growth (Li et al. 2021). One of the criteria to measure the filling ability of rice grains is the 1000-grain weight, which is significantly affected by grain size and shape. Grain size and shape are controlled by genetics and it potentially increases the grain length and width, and 1000-grain weight (Bai et al. 2023). This study showed that the average 1000-grain weight, calculated after the grains were dried, of Purwokerto local black rice (28.39 g) was higher than that of the Paketih white glutinous rice (22.89 g) and F1 plants (26.93 g). It is because Purwokerto local black rice has round and oval-shaped grains which translates to heavier grain.

Agronomic characters have a significant positive correlation coefficient with grain weight per clump, except for the total number of tillers, number of productive tillers, and flowering age (Figure 7). Characters that have a positive correlation with grain weight per clump are the number of grains per panicle, plant height, 1000-grain weight, and panicle length. This correlation indicates that increasing grain weight per clump can be influenced by factors such as grain size and weight, as well as plant physical characteristics such as panicle height and length. The higher the number of grains per panicle and the 1000-grain weight, the greater the grain weight per clump produced. These results are in line with the research of Al-Daej (2022) which states that the increase in rice grain

yield is caused by a high number of grains per panicle and a larger grain weight.

The 1000-grain weight had a moderate positive correlation (0.48) with grain weight per clump, indicating that an increase in grain weight per clump contributes to an increase in yield. This indicates that selection of genotypes with greater grain weight can be an effective strategy in increasing yield. Akbar et al. (2021) large and fully filled grains will result in a higher harvest index and optimal grain weight per clump, thus contributing to an increase in total rice production. In addition, the strong positive correlation between the number of grains per panicle and grain weight per clump (0.87) indicates that the more grains that develop in a panicle, the greater the yield obtained. This factor is also supported by panicle length which has a moderate positive correlation (0.28), indicating that longer panicles tend to hold more grains, thus increasing total yield.

In conclusion, F1 agronomic characters on grain weight per clump, 1000-grain weight, number of filled grains per panicle, panicle length, number of productive tillers per clump, plant height and flowering age, as well as genotypes from crossing glutinous rice Paketih and local black rice Purwokerto have the potential to produce hope strains with a combination of superior traits, such as high productivity and harvest time efficiency. The F1 plants showed good agronomic characters, with the number of productive tillers reaching 51 tillers, the 1000-grain weight of grain of 26.93 g, and the weight of grain per clump of 140.64 g. The F1 plants showed good agronomic characteristics. These plants can be further selected to be developed as superior varieties that are highly competitive in a sustainable agricultural system.

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