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Genetic Parameters, Inter-relationship Among Agronomic Traits and Dehulled Rice Morpho-Biochemical Profile of Promising Black Rice x Mentik Wangi Lines

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ABSTRACT

A comprehensive understanding of the genetic parameters and the interrelationship among characters in the breeding population is crucial for selecting low amylose and high antioxidant black rice varieties. Meanwhile, dehulled rice morpho-biochemistry profile can be used to determine the grain quality of F6 and F7 lines of Black Rice x Mentik Wangi var. The objectives of this study were to determine the agronomic traits, figure up the genetic parameters, describe the relationship among agronomic characteristics of the F6 lines, and determine the morpho-biochemical profile of F7 dehulled rice. Agronomic traits showed a difference in each line. Genetic parameters in each trait showed various categories. Path analysis showed that the number of tillers affected the dry weight and grain weight per panicle, but the plant dry weight did not directly affect the weight of 1,000 grain. Directly, the weight of 1,000 grain was only significantly affected by the grain weight per panicle trait. The F7 lines had a difference in grain length and amylose content. The dehulled rice color of the two lines showed the combination of the two parents. Based on the student T-test conducted on F6 and F7 grain, there was no difference in antioxidant content between the two sample groups. The antioxidant activity of all lines was in the range between the antioxidant activities of the two checked varieties. Although further research is still needed, the lines have the potency to be developed as low amylose pigmented rice.

1. Introduction

Black Rice breeding program is directed to improve the quality and productivity of black rice to fulfill people's consumption needs. Research from various countries has been carried out to develop black rice with superior traits, both using conventional and modern plant breeding (genetic transformation and gene editing). For example, a research team from Korea, Kim *et al.* (2010), created black rice C3GHi variety with higher antioxidant compounds and cyanidin-3-glucoside content than local Korean black rice. Meanwhile, Wickert *et al.* (2014) released SCS120 Onix (black pericarp) and SCS119 Rubi (red pericarp) varieties in 2013, resulting from conventional breeding, which were recommended as new pigmented rice for the specialty rice market. Both varieties were recommended for their better nutritional content than white rice and high productivity in the field. Zhu *et al.* (2017) carried out a genetic transformation to produce rice germplasm with high anthocyanin content and antioxidant activity in the endosperm, which was named Zijingmi (in Chinese), rice with a purple endosperm. Zhang *et al.* (2018) also developed waxy rice through CRISPR-Cas9 targeted mutagenesis of the waxy gene in elite rice japonica XS134 and 9522 varieties. Roy and Shil (2020) reported the development of aromatic black rice through intraspecific hybridization and introgression of the *Oryza sativa* (cv. Badshabhog, Chenga, and Ranjit) and *O. rufipogon* as the donor parents and the source of the black rice gene.

One of the goals of Indonesia's black rice breeding program is to acquire black rice with low amylose content and high antioxidant. The development of black rice to obtain superior traits with low amylose content and high antioxidant has been carried out. Indonesian Ministry of Agriculture released low amylose pigmented rice variety (19.6%

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amylose content) named Jeliteng in 2019 through conventional breeding of Ketan Hitam and Pandan Wangi cv Cianjur. Amylose content is one of the biochemical criteria that determine the grain quality of rice, in addition to other physical criteria such as gelatinization temperature, viscosity, flavor, and aroma (IRRI 2013). Nevertheless, amylose content is also used to predict the tenderness or stickiness of rice texture (Avaro *et al.* 2011; Bhattacharaya and Juliano 1985; Juliano *et al.* 1965), in addition to other criteria such as post-harvest processing and cooking methods (Li *et al.* 2016). The lower the amylose content of rice, the more tender the texture of the rice, and vice versa (Bhattacharaya *et al.* 1999; Khumar and Khush 1986; Luna *et al.* 2015; Panesar and Kaur 2016). Meanwhile, rice with high antioxidant activity plays an essential role in human health (Pratiwi and Purwestri 2017). These various health benefits are anti-inflammatory, anti-obesity, prevent cardiovascular disease, anti-cancer, anti-diabetic, reduce allergies, prevent constipation, prevent anemia (Murali and Kumar 2020; Tena *et al.* 2020), support health eye, anti-microbial, and prevention of neurodegenerative diseases (Tena *et al.* 2020). Black rice with low amylose and high antioxidant has the potency as a superior variety with a fluffier texture of rice and high health benefits.

However, to obtain this superior variety, the black rice breeding program requires testing of various characters related to agronomic and post-harvest yield characters. Multi-season and multi-location tests also need to be carried out to determine the stability of the superior traits. Therefore, getting a black rice variety with those characters takes years. The development of superior varieties of pigmented rice that are fluffier and have high antioxidant can be done by crossing the Black Rice with the Mentik Wangi variety. Rice breeding to obtain lines with high antioxidant and a fluffier texture of rice has reached the 6^{th} line, which was started in 2014.

The objectives of this study were to determine the agronomic traits, figure up the genetic parameters, describe the relationship among agronomic traits of the F6 lines, and determine the morpho-biochemical profile of F7 dehulled rice. Analysis of agronomic traits was conducted to compare the agronomic characteristics of lines with the checked varieties. The determination of genetic parameters was directed to define the influence of environmental and genetic factors on phenotypic traits. The relationship among characters was analyzed to determine the characters that have a direct and indirect effect on the yield components. In addition, the determination of the morpho-biochemical profile of the dehulled rice was carried out to determine the size of the length of the dehulled rice to predict the stickiness through the analysis of amylose content and to predict the level of free radical scavenging through the determination of antioxidant activity. This research is part of a rice plant breeding program to get low amylose pigmented rice before heading to the field test at multi-location and multi-season. The development of pigmented rice plays an important role in increasing the diversity of rice germplasm. This research gives scientific information about developing low amylose pigmented rice and related studies.

2. Materials and Methods

The genetic materials consisted of 6 (six) potential F6 lines (from conventional breeding of Black Rice cv Purworejo and var. Mentik Wangi) and 2 (two) checked varieties. These lines resulted from the development of pigmented rice, which began in 2014. The lines are PH/MW 482-1-14, 482-24-8, 482-9-134, 482-1-4, 482-17-7, and 482-17-18. The F6 lines and checked varieties were cultivated until harvest to obtain the F7 lines. Dehulled rice of the F7 lines was analyzed for size, cumulative color, amylose content, and antioxidant activity. Meanwhile, the dehulled rice of the F6 lines was also subjected to the same analysis.

2.1. Field Trial

Field trial of the F6 lines and the checked varieties was carried out in the Experimental Farm greenhouse, Faculty of Agriculture, Universitas Jenderal Soedirman. The study was conducted from April to October 2020. The experiment used Completely Randomized Block Design (CRBD), with 3 (three) replications, so that there were 24 experimental units. The growth media contained ultisols soil, rice husks, and cow manure (4:1:1). The growth media was applied with NPK (15 grams per polybag). Conventional techniques and chemical pesticides were employed to control weeds, pests, and diseases. The agronomic traits observed were heading date, plant height, plant dry weight, number of tillers, the weight of 1,000 grain, number of panicles, and grain weight per panicle.

2.2. Morphology Characterization of Dehulled Rice

Dehulled rice morphology was observed in each line's length and cumulative color. The determination of size classification was based on the International Rice Research Institute (IRRI) (2013). Cumulative dehulled rice color was used to determine the uniformity of dehulled rice pigment in each line.

2.3. Amylose Quantification of The Lines

The amylose content of the dehulled rice of the F7 lines was determined based on the iodo-colorimetric method (Juliano 1971). Analytical repetition was carried out two times. Quantitative analysis of amylose was measured by making a standard amylose curve first. The amylose quantification was then measured based on the linear regression equation in the standard curve.

2.4. Determination of Antioxidant Activity

Determination of antioxidant activity was measured on dehulled rice of the F6 and F7 lines using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method (Blois 1958). The DPPH assay is based on both electron transfer (SET) and hydrogen atom transfer (HAT) reactions (Liang and Kitts 2014).

2.5. Data Analysis

Data on agronomic traits of the F6 lines, dehulled rice length of the F7 lines, and amylose content of the F7 lines was analyzed using SAS 9.4 software. The Least Significant Difference (LSD) test at the 95% confidence level (α = 0.05) was carried out to indicate the influence of the lines on agronomic traits. The analysis of variance (F test) results were used to quantify the value of the Coefficient of Variance/CV, broad-sense heritability, Coefficient of Genotypic and Phenotypic Variance. Broad sense heritability was calculated using the formula suggested by Allard (1960). The determination of heritability criteria follows The determination of heritability criteria followed McWhirter (1979). of McWhirter (1979). The Coefficient of Genotypic and Phenotypic Variance was calculated using Singh and Chaudhary's (1977) formula. The genotypic and phenotypic variation coefficient was categorized as proposed by Sivasubramanian and Madhavamenon (1973). The student T-test identified the difference in antioxidant activity of the F7 and the F6 lines. Path analysis was performed using the LISREL 8.2 software. Path analysis was used to determine the yield component's direct and indirect factors, namely weight of 1,000 grain and weight of grain per panicle.

3. Results

3.1. Agronomic Traits of The Lines

The Least Significant Difference (LSD) test results to determine the difference in the response of each line based on agronomic traits can be seen in Table 1. In addition, the agronomic traits of the lines could also be compared with those of the checked varieties (Black Rice and Mentik Wangi var.).

The traits observed in each line showed a difference. The plant dry weight and the plant height of all lines were in the range of the two checked varieties. The number of tillers of the other lines was the same as in the checked varieties. Meanwhile, the grouping of panicle numbers was more varied. There were 3 (three) subsets that group the lines based on the character of the number of panicles. For the weight of 1,000 grain trait, there were only 2 (two) subsets. For the character of grain weight per panicle, there are 2 (two) subsets. Meanwhile, the heading date was categorized into 3 (three) subsets. The heading date of the Mentik Wangi variety was different from all lines.

3.2. Genetic Parameters of The Lines

It was crucial to determine the value of the Coefficient of Variance (CV) first to determine the cause of the differences in the traits. The CV values are presented in Table 2. All of the traits showed a CV value of less than 20%.

Next, the broad-sense heritability, the Coefficient of Genotypic Variance (CGV) and the Coefficient of Phenotypic Variance (CPV) can be seen in Table 3. The broad-sense heritability of all traits showed various categories. The traits of plant dry weight, plant height, the weight of 1,000 grain, and grain weight per panicle were in the moderate range of broad-sense heritability. Traits with a high value of broad-sense heritability were the number of tillers, number of panicles, and heading date.

3.3. Inter-Relationship Among Traits

The dependent variables as the yield component were the weight of 1000 grain and the weight of

Table 1. Differences in agronomic traits of six lines

Genotype	Plant dry weight (g)	Plant length (cm)	Number of tillers	Number of panicles	Weight of 1,000 grain (g)	Grain weight per panicle (g)	Heading date (days)
Line 482-1-14	46.34±3.70^{ab}	62.43±1.38^{a}	36.40±6.01^{b}	16.75±1.38^{c}	13.67±0.39^{ab}	2.15±0.28^{ab}	60.92±1.80^{a}
Line 482-17-7	40.03±1.20^{a}	70.94±3.29^{b}	26.80±3.13^{a}	13.95±1.31^{ab}	12.10±1.85^{a}	1.97±0.36^{ab}	60.67±3.10^{a}
Line 482-1-4	51.91±5.53^{b}	69.93±3.97b	36.70±5.43^{b}	16.50±2.27^{bc}	14.21±1.63^{ab}	1.95±0.33^{a}	69.00±1.52^{c}
Line 482-17-18	45.98±5.30^{ab}	81.08±8.90^{c}	25.10±2.27^{a}	11.55±1.55^{a}	15.12±1.75^{b}	2.23±0.52^{ab}	65.08±2.67^{b}
Line 482-9-134	48.29±6.32^{b}	82.83±2.61^{c}	25.95±1.89^{a}	16.20±2.24^{bc}	15.65±1.31^{b}	2.40±0.30^{ab}	68.08±3.27^{bc}
Line 487-24-8	46.99±6.01^{ab}	71.53±1.86^{b}	28.50±6.43^{a}	14.60±2.03^{bc}	15.77±1.48^{b}	2.61±0.41^{b}	61.67±1.31^{a}
Black Rice	37.47±2.95^{a}	60.56±2.62^{a}	28.25±1.95^{a}	15.42±0.63^{bc}	15.1±0.65^{b}	2.24±0.47^{ab}	64.00±1.41^{b}
Mentik Wangi	66.90±13.78^{c}	101.80±6.60^{d}	25.59±4.48^{a}	14.08±2.76^{b}	15.22±2.34^{b}	2.77±0.71^{b}	82.83±4.09^{d}

The numbers followed by the same letter in the same column are not significantly different according to the 5% LSD test

Table 2. Coefficient of Variance (CV) of the Traits

Traits	Mean	Max	Min	CV (%)
Plant dry weight (g)	46.59	84.61	28.63	11.48
Number of tillers	29.91	54.00	14.00	15.22
Plant height (cm)	73.12	96.80	53.20	6.03
Weight of 1,000 grain (g)	14.42	22.12	7.04	9.74
Number of panicles	14.93	25.00	9.00	11.44
Grain weight per panicle (g)	2.22	3.64	1.10	14.63
Heading date (days)	64.24	72.00	57.00	3.20

Table 3. Broad sense heritability (h2), coefficient of genetic variance, and coefficient of phenotypic variance of the traits

Traits	Mean	Phenotypic variation	Middle square of error	Phenotypic variation	h2 (%)	h2 criteria	CGV (%)	CGV criteria	CPV (%)	CPV criteria
Plant dry weight (g)	46.59	7.86	28.44	36.30	21.65	Moderate	6.02	quite low	12.93	quite high
Number of tillers	29.91	22.56	20.73	43.29	52.11	High	15.88	high	21.99	high
Plant height (cm)	73.12	36.90	83.97	120.87	30.53	Moderate	8.31	quite high	15.04	quite high
Weight of 1,000 grain (g)	14.42	1.47	1.97	3.44	42.79	Moderate	8.42	quite high	12.87	quite high
Number of panicles	14.93	3.25	2.92	6.16	52.68	High	12.07	quite high	16.64	high
Grain weight per panicle (g)	2.22	0.04	0.11	0.14	26.57	Moderate	8.80	quite high	17.06	high
Heading date (days)	64.24	12.66	4.23	16.89	74.95	High	5.54	quite low	6.40	quite low

grain per panicle. Other agronomic traits as an independent variable. The path analysis diagram could be seen in Figure 1. Path analysis showed that the number of tillers directly affected the plant dry weight and grain weight per panicle, but the plant dry weight did not directly affect the weight of 1000 grain.

3.4. Morpho-Biochemical Profile of Dehulled Rice

Figure 2 showed that the cumulative color of F6 line same with these of F7. Four lines had the same color as the Black Rice variety, but the other lines showed the color combination between the checked varieties.

Based on the aspect of dehulled rice size, the average of 5 (five) lines (482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134) differed from the line 487-24-8 (Figure 3). Meanwhile, dehulled rice of all lines was larger than the checked varieties.

The amylose profile of the dehulled rice of the F7 lines showed that the average amylose content of each line was different from one another (Figure 4). Line 482-1-14 showed the lowest amylose content value

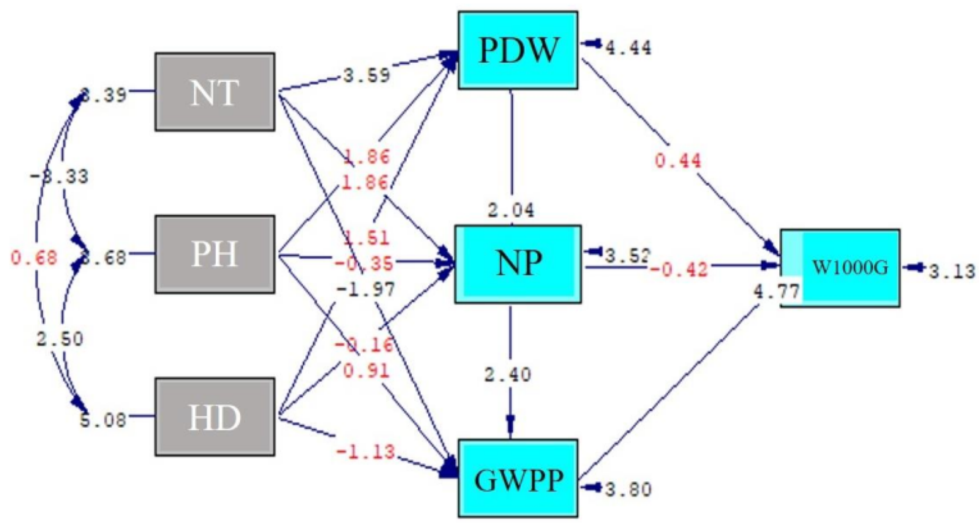

Figure 1. Path diagram of inter-relationship traits (p-value = 0.19579). (Notes: NT: number of tillers, PH: plant height, HD: heading date, PDW: plant dry weight, NP: number of panicles, GWPP: grain weight per panicle, W1000G: weight of 1,000 grain)

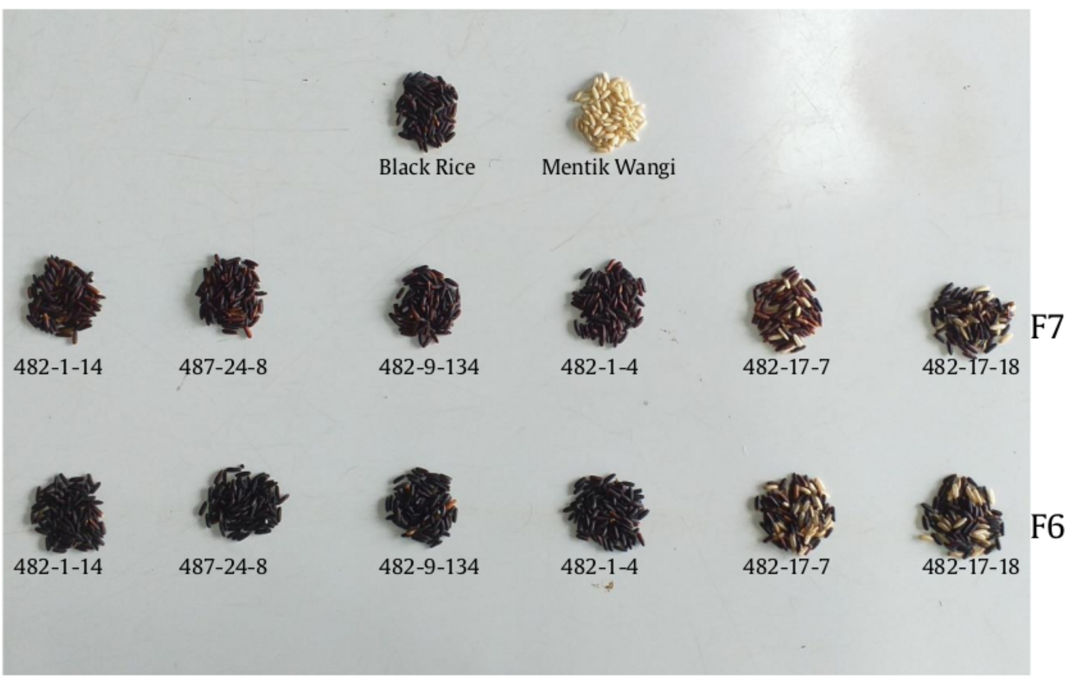

Figure 2. Color of the dehulled rice of lines and checked varieties

compared to other lines (12.66±0.06). This line also had the same amylose content as the Mentik Wangi variety. Meanwhile, the 482-17-18 line showed the highest value compared to all lines (16.81±0.05), but it was still below the Black Rice variety (22.37±0.04). The amylose content of all lines was in the range of amylose content of the checked varieties (Black Rice and Mentik Wangi).

For the trait of antioxidant activity, an independent sample T-test was conducted on 2 (two)

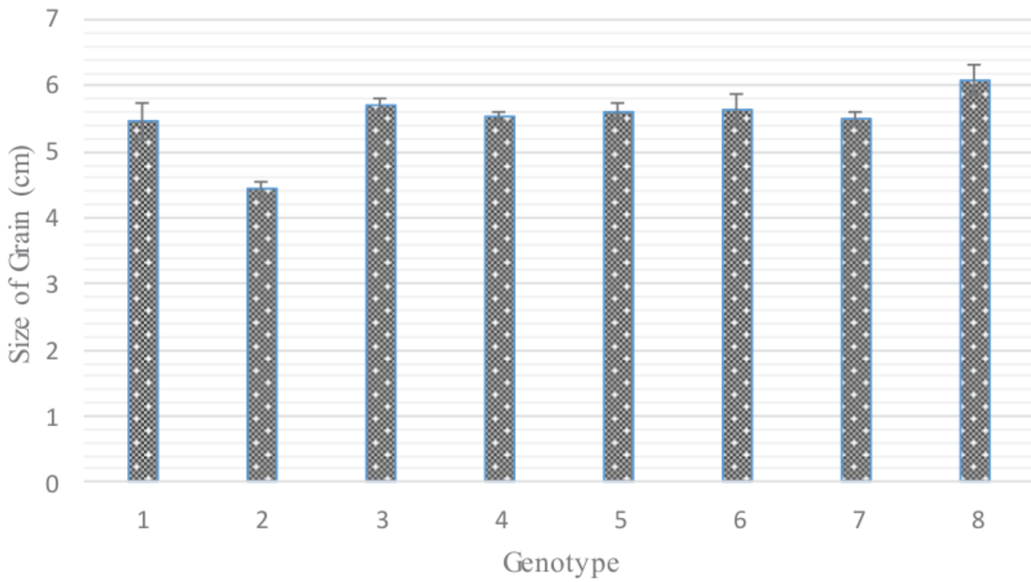

Figure 3. Length Size of F7 Dehulled Rice (Note: No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; while no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134, 487-24-8 lines, respectively)

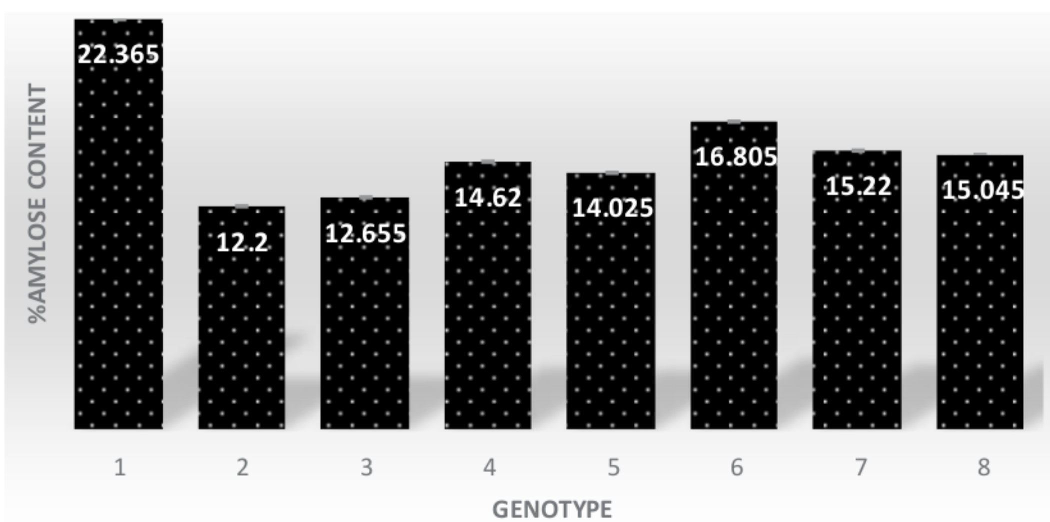

Figure 4. Amylose profile of F7 lines and checked varieties (Note: No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; while no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134, 487-24-8 lines, respectively)

groups of dehulled rice of the F6 and F7 lines. This test aimed to determine the difference in the average of two unpaired samples/not the same sample. The results of the analysis obtained the value of Sig. (2 tailed) >0.05, so it was concluded that there was no difference in antioxidant activity between the average F6 and F7 lines (Figure 5).

4. Discussion

4.1. Agronomic Traits of The Lines

Based on the data analysis, the traits observed in each line showed a different effect in each line. Compared to another study, research by Kartahadimaja *et al.* (2021) reported that statistical analysis of agronomic traits in 12 (twelve) rice genotypes showed that plant height, number of tillers, number of productive tillers, flag leaf width, flag leaf length, and flag leaf angle showed significant variation. Meanwhile, Kasim *et al.* (2020) showed that the rice plant height of the 17 rice varieties ranged from 95-160 cm. The plant height of the studied lines were below the plant height range of the rice varieties studied by Kasim *et al.* (2020). Plant height is related to the internode elongation of plants (Zhang *et al.* 2017). According to Kasim *et al.* (2020), reducing plant height can increase crop resistance to rain stress and reduce the risk of yield reduction due to rain stress. Thus, varieties with shorter height are preferred by the farmers and researchers. In this study, the height of the lines was between 62.43±1.38 cm to 82.83±2.61 cm, which indicates a shorter plant height than in the study of Kasim *et al.* (2020). Then, compared with the heading date varieties used in Kasim *et al.* (2020) research, the lines had a shorter heading date. Farmers prefer the earlier heading date allowing a higher harvesting frequency per year than varieties with a longer heading date. It can have an impact on farmers' annual income. The earlier heading date varieties used by researchers can accelerate the process of conventional breeding through hybridization with other varieties, thus speeding up the rice breeding program.

Meanwhile, the number of panicles varied between 12-24. The number of panicles in this study was in the range of those studied by Kasim *et al.* (2020). Meanwhile, the total number of grains per plant also varied, between 128 to 305. The weight of 1000 grain also varied, between 1.4–2.56 grams. The number of panicles, grain weight, and the number

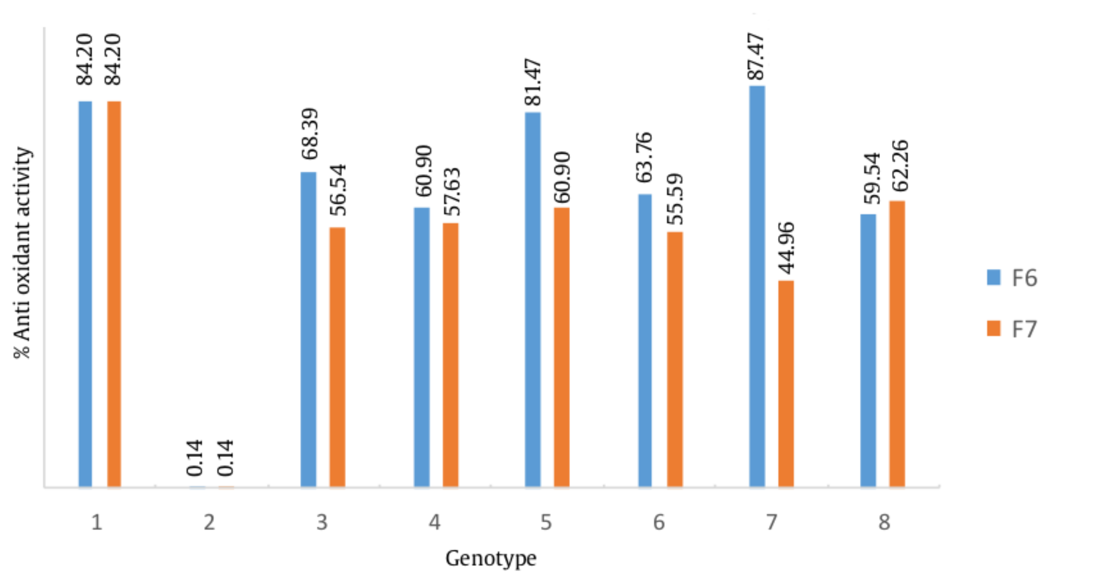

Figure 5. Antioxidant activity of F6 and F7 dehulled rice. (Note: No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134, 487-24-8 lines, respectively)

of grains per panicle determined grain yield in rice (Xing and Zhang 2010). Plant breeders prefer to choose the lines with the large panicles with a higher grain number of panicles because it indicates the new rice types with higher yields (Khush 2000).

4.2. Genetic Parameters of The Lines

The value of the broad-sense heritability of this study was in the range that varies for each character. The data from the analysis showed that there were various values of broad-sense heritability for each trait. The characters of dry weight, plant height, the weight of 1,000 grain, and grain weight per panicle had moderate broad-sense heritability. Traits with high heritability estimates were the number of tillers, number of panicles, and heading date. Adhikari *et al.* (2018) wrote that the low heritability value indicates that the traits appear due to variations in environmental factors and vice versa. A high broad-sense heritability indicates a high selection response for a particular trait. A good trait used as a basis of selection was a character with a high broad-sense heritability value (Begum *et al.* 2015). A low broad-sense heritability value would impact an insufficient genetic advance (Mursito 2003).

The results of this study were in line with the research of Adhikari *et al.* (2018) that the heading date also had a high broad-sense heritability. Meanwhile, the traits of the weight of 1,000 grain and grain weight per panicle, which can be used as yield component parameters, also had moderate broad-sense heritability compared to research by Adhikari *et al.* (2018). This data followed the results of Ogunbayo *et al.* (2014) research. The traits of heading date and number of tillers also had high broad-sense heritability. This finding suggests that these traits were primarily under genetic control rather than an environmental one. Traits with high broad-sense heritability can be passed to the next generation. Similar results were confirmed in Konate *et al.* (2016) study, that heading date, number of tillers, and number of panicles are also categorized as traits with high broad-sense heritability. The weight of 1,000 grain traits also had a moderate broad-sense heritability.

The results showed that the entire value of the CPV of all traits was higher than these of CGV. It is in line with Bagati *et al.* (2016) research that the value of the CPV was higher than the CGV in all the traits studied. Adhikari *et al.* (2018) wrote that this indicates the influence of the environment on these traits. The magnitude of the effect of the plant growth environment on the observed traits is explained by the level of a difference value between those two parameters.

4.3. Inter-Relationship Among Traits

Path analysis showed that the number of tillers affected the dry weight and grain weight per panicle, but the dry weight had no direct effect on the weight of 1000 grain. Directly, the weight of 1000 grain was only significantly affected by the trait of grain weight per panicle. This analysis was used to determine the magnitude of the direct or indirect effect on the yield component towards the yield (Akhmadi *et al.* 2017). Yield is a complex character and depends on several related traits. Therefore, crop yields usually depend on the actions and interactions of several important traits. Knowledge of the various traits that determine crop yields is essential for examining various yield components (growth parameters) and paying more attention to yield components that have the most significant influence on crop yields (Kinfe *et al.* 2015). A good selection trait has a large real correlation value and a high direct or indirect effect on the yield (Boer 2011).

The results of this study differed from the research of Akhmadi *et al.* (2017).The research by Akhmadi *et al.* (2017) showed that traits that directly affected high yields were the length of the panicle, the weight of 1000 filled grain, the number of filled grains per panicle, and the grain-filling period. The character of the generative plant height and the total number of grains per panicle had a high negative direct effect on yield. Still, the indirect effect through panicle length was relatively high. Several studies showed that heading date, harvesting age, plant height, number of tillers, number of productive tillers, number of filled grain per panicle, total grain number per panicle, panicle length, and weight of 1000 grain have a direct effect on the high yield on some rice populations (Rachmawati *et al.* 2014; Safitri *et al.* 2011). If a specific trait is known to affect the yield component directly, then the yield determination can be understood by looking at the profile of the trait. Analyzing the inter-relationship among traits can give information related to characters that correlate with the yield. Therefore, knowledge on these can be used as initial information before yield or productivity are known.

4.4. Morpho-Biochemical Profile of The Lines

The cumulative color of the dehulled rice of F6 and F7 lines showed 4 (four) lines that completely had the same color as the Black Rice variety, but the other 2 (two) lines still showed the color combination between the parents. Laokuldilak *et al.* (2011) reported that pigmented rice contains high Cyanidin-3-Glucoside (58-95%). Moreover, Laokuldilak *et al.* (2011) stated that C3G is part of the anthocyanin group. The appearance of rice-bran color in pigmented rice is thought to be related to the content of the Cyanidin-3-Glucoside compound. However, the relationship between these two is not clear due to the complexity of the existing genetic system. According to research by Ham *et al.* (2015), there was a significant positive correlation between C3G content towards rice bran's brightness and yellow color. However, the genetic system responsible for rice-bran pigmentation and Cyanidin-3-Glucoside content is not the same but is related through a specific pathway with anthocyanin metabolism. Macron *et al.* (2021) wrote that the biosynthesis of anthocyanins and their storage in rice bran is a complex process due to the involvement of structural and regulatory genes.

Based on the dehulled rice size, the average size of the 5 (five) lines (PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134) differed from the PHMW 487-24-8. The length of the dehulled rice of the lines was above the length of the checked variety, Mentik Wangi, and had a size that was not significantly different from the length of the dehulled rice of the Black Rice variety. This phenomenon could be caused by the transgressive segregation (Nugraha and Suwarno 2007) of alleles responsible for expressing rice length characters. According to IRRI (2013), the length of the rice in all lines studied was grouped into the medium classification, except for the 482-17-7 line, the short criteria. The length of the rice is part of determining the shape of the rice (IRRI 2013). The same reference also stated that the shape of the rice grain is one of the determinants of rice grain quality. Grain quality is one of the selection parameters in plant breeding programs (Kush and Cruz 2000; Kartahadimaja *et al.* 2021). Compared with the same line in the previous study (Oktaviani *et al.* 2021), the dehulled rice size of F7 lines did not significantly differ from the F6 ones. All the lines studied in the F6 generation had larger dehulled rice sizes than the Black Rice and Mentik Wangi varieties. All F6 and F7 lines were also categorized as moderate, based on the International Rise Research Institute (2013). Based on the research of Kartahadimaja *et al.* (2021), the grain size of the 12 genotypes studied varied in length, width, and thickness.

Amylose content is one of the criteria that determines grain quality (IRRI 2013). The amylose content of the studied lines was between these of the checked varieties. In addition, each line showed a significant difference in amylose content. The amylose content of the studied lines was at low criteria so that they could be used to create a fluffier/glutinous texture of pigmented rice. The low amylose content of the studied lines follows the objectives of the black rice breeding program.

It was essential to measure the antioxidant activity of the lines to map the antioxidant profile of the lines compared to checked varieties. The results showed no difference in the average antioxidant activity between the F6 and F7 lines. The antioxidant activity of lines showed various values. Laokuldilok *et al.* (2011) wrote that the pigmented rice bran extract, which had the outer shell removed, had greater reducing power than the long white rice bran extract. However, an interesting study by Setyaningsih *et al.* (2015) studied the positive correlation between amylose content and levels of antioxidant compounds, such as melatonin and phenolics. This finding can provide an essential basis of information if one of the biochemical compounds is known, although the case in each variety will be specific.

In conclusion, the data from the analysis showed that there was various broad-sense heritability for each agronomic character. The characters of dry weight, plant height, the weight of 1,000 grain, and grain weight per panicle had moderate broad-sense heritability. Characters with high broad-sense heritability were the number of tillers, panicles, and days to flower. Based on the study results, all the characters observed in each line showed a different effect in each line studied. The value of broad-sense heritability in this study varied for each character. Path analysis showed that the number of tillers affected the dry weight and grain weight per panicle, but the dry weight had no direct effect on the weight of 1000 grain. Directly, the weight of 1000 grain was only significantly affected by the character of grain weight per panicle. The cumulative color of the dehulled rice showed that 4 (four) lines with completely like the black rice, but the other 2 (two) lines still showed the color combination between the two checked varieties. Based on the dehulled rice size, the mean of the 5 (five) lines (PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134) differed from the PHMW 487-24-8. Meanwhile, the dehulled rice size of all lines was more significant than these of the checked varieties. The amylose content of lines was between the amylose content of the checked varieties. In addition, each line showed a significant difference in amylose content. The antioxidant activity of the studied lines showed various values. Further research is needed to check the stability of low amylose traits with multi-location and multi-season field trials, selection based on molecular markers related to genes associated with low amylose content and high antioxidant content, and organoleptic testing of processed rice from these lines. The results of this study can provide important information related to studies needed in the development of low amylose pigmented rice in Indonesia. In addition, the results of further research in the form of potential rice lines with fluffier texture and high antioxidant can be used as candidates for rice varieties with superior characters. It can enrich the collection of superior rice germplasm in Indonesia.

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