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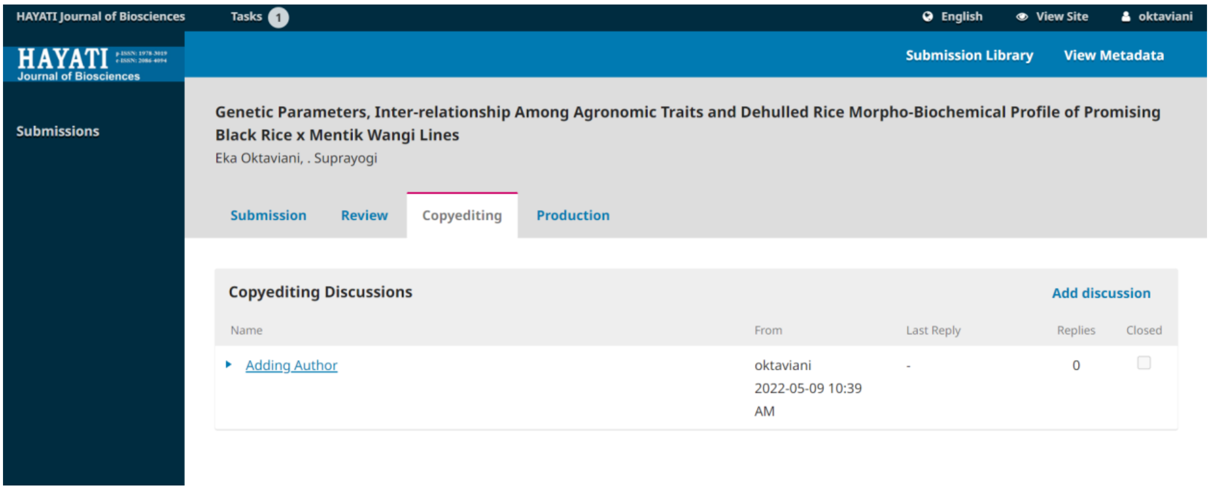
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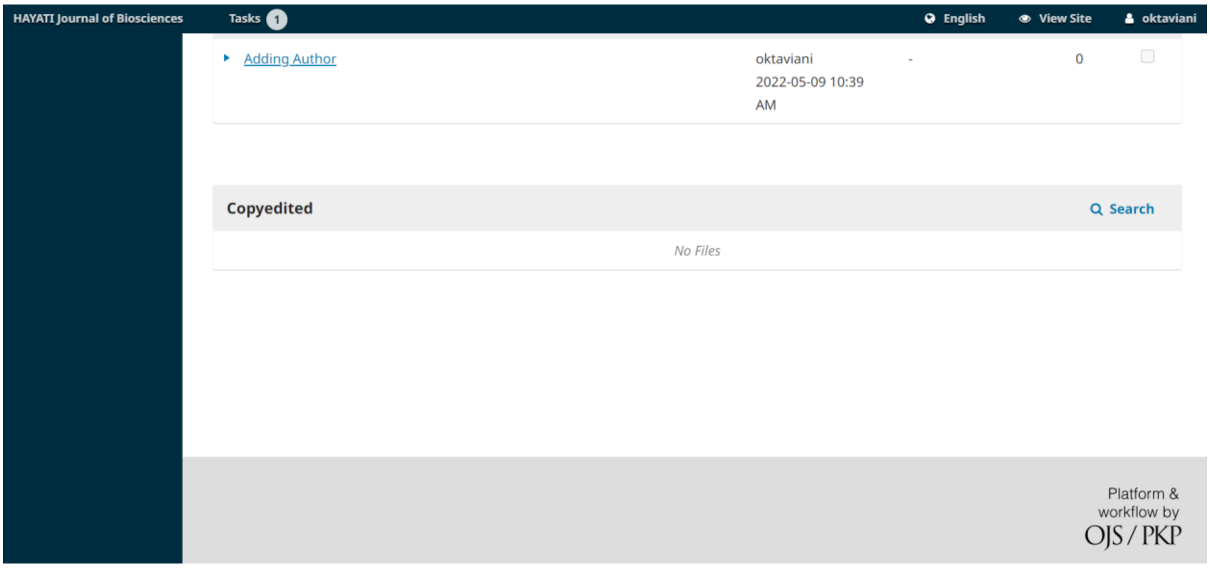
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Genetic Parameters and Inter-relationship Between Agronomic Traits of F6 Lines, and Rice Bran Morpho-Biochemical Profile of F7 Lines Derived from a Crossing of Black Rice and Mentik Wangi

Eka Oktaviani[1*] and Suprayogi[1]

[1]Department of Agrotechnology, Faculty of Agriculture, Universitas Jenderal Soedirman Jln. Dr Soeparno 61 Karangwangkal Purwokerto Utara Banyumas 53123 Jawa Tengah, Indonesia

***Corresponding author** : oktaviani@unsoed.ac.id

ABSTRACT

The development of sticky pigmented rice with high antioxidant content as superior varieties can be carried out by crossing the Black Rice with Mentik Wangi varieties. Rice breeding program to obtain rice lines with low amylose content and high antioxidants has reached the F6 lines. The purpose of this study was to determine genetic parameters and the relationship between agronomic traits of the F6 lines. Another objective of this study was to determine the morpho-biochemical profile of F7 grain, including length, cumulative color, amylose content and antioxidant activity. The results showed that all agronomic parameters had a coefficient of variance less than 20%, which indicates that phenotypic differences were caused by genotypic factors, rather than environmental ones. All the values of the Genetic Diversity Coefficient are in the low range, as well as the values of the Phenotypic Diversity Coefficient. The broad sense heritability of dry weight character was in the low range. The characters of plant height, weight of 1000 grain and weight of grain per panicle were categorized as having moderate broad sense heritability, while the characters of number of tillers, number of panicles and age of flowering had high broad sense heritability. The phenotypic variability of weight of 1000 grain and age of flowering were included in the narrow criteria, while the other characters were broad one. Path analysis showed that the number of tillers had an effect on the dry weight and grain weight per panicle, but the dry weight did not directly affect the weight of 1000 grain. Directly, the weight of 1000 grain was only significantly affected by the grain weight per panicle trait. In terms of grain length profile and amylose content, the F7 lines had difference in grain length (2 subsets) and amylose content (6 subsets) traits. The cumulative grain color of the PHMW482-17-7 and 482-17-18 lines showed the color combination of the two parents. Based on the T test conducted on F6 and F7 grain samples, 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.

Keywords: antioxidant, black rice, mentik wangi, morpho-biochemical, sticky

1. Introduction

Black rice is one type of pigmented rice, in addition to red rice and brown rice. This rice is often consumed as functional food, not as the main food ingredient (Purwanto et al., 2019). This is due to the various nutritional content of this type of rice. Black rice contains various micro and macronutrients that are important for human health (Apridamayanti et al., 2017). Protein, fat, fiber, vitamins, and minerals important for the human body are the various nutrients that this rice has (Nurhidajah, 2018; Apridamayanti et al., 2017; Kristamtini et al., 2012). Important nutrients reported in black rice are vitamin B, vitamin E, Fe ions, thaimin, magnesium, niacin, phosphorus, dietary fiber (Kristamtini et al., 2021; Murali & Kumar, 2020), Zn ions, and Mn (Kristamtini et al. et al., 2021; Murali & Kumar, 2020), Zn, and Mn ions (Kristamtini et al., 2021; Murali & Kumar, 2020), et al., 2012). Murali & Kumar (2020) also reported that black rice is free from gluten and cholesterol, low in sugar, salt and fat.

Black rice, is one of the pigmented rice classified on the basis of the color of the pericarp, aleurone, and endosperm of the rice grains (Kristamtini et al., 2012). One of the important compounds that contribute to black rice aleurone color is anthocyanin (Yoshimura et al., 2012; Palupi et al., 2020). Anthocyanins are responsible for the appearance of blue, purple, red, and orange colors in many fruits and vegetables (Miguel, 2011). Anthocyanins have high antioxidant activity and play an important role in human health (Prastiwi & 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 & Kumar, 2020; Tena et al., 2020), support health eye, anti-microbial, and prevention of neuro-degenerative diseases (Tena et al., 2020).

Anthocyanins, one of the most important types of plant flavonoid compounds, are pigments with a flavylium cation structure (AH+) that act as acids (Tena et al., 2020). This structure is directly related to antioxidant activity, because it is able to prevent or inhibit oxidation reactions by scavenging free radicals and reduce levels of oxidative stress in cells (Tena et al., 2020). Based on basic chemical reactions, anthocyanins act as donors of H atoms or as single electron transferors (Tena et al., 2020).

Although black rice is known as a functional food with the above benefits, consumer acceptance of the texture of rice prepared from this rice is low, due to the non-stickiness texture of the cooked rice (Adi et al, 2020). Non-tender/non-sticky/non-glutinous rice has a dry, hard, and separate texture, even though it has been through the cooking process. Rice texture is determined by amylose amylopectin ratio (Cameron & Wang, 2005; Adi et al., 2020; Li et al., 2016a), post-harvest processing, and cooking method (Li et al., 2016b). In addition, Cameron & Wang (2005) also found that the texture/stickiness/hardness of rice was associated with protein and crude lipid content. The higher the amylose content of rice, the more tender the texture of the rice will be, and vice versa (Khumar & Khush, 1986; Bhattacharaya et al., 1999;

Luna et al, 2015; Panesar & Kaur, 2016). Crude protein and lipid content were negatively correlated with the hardness of pasta flour and processed rice, but positively correlated with the level of rice stickiness (Cameron & Wang, 2005).

A rice plant breeding program to produce rice plants with a quality texture of soft/soft/fluffy processed rice has been carried out in Indonesia. Indonesia, through the Ministry of Agriculture, has released a rice variety with an amylose content of 19.6% in 2019, the result of a cross between Black Sticky Rice and Pandan Wangi cv Cianjur. In addition, research for the development of fluffier pigmented rice has also been carried out by researchers (Kim et al., 2010; Zhang et al., 2018; Roy & Shil, 2020). The development of pigmented rice with a fluffier rice texture in Indonesia is expected to increase the source of rice germplasm with superior characters in Indonesia.

The development of superior varieties of pigmented rice that is fluffier and has a high antioxidant content can be done by crossing the Black Rice variety with Mentik Wangi. Rice breeding research to obtain rice lines with high antioxidants and a fluffier texture of rice has reached the F6 offspring. The purpose of this study was to determine various genetic parameters and the relationship between characters based on the agronomic character of the F6 line. Another objective of this study was to determine the morpho-biochemical profile of F7 seeds, including length, cumulative color, amylose content and antioxidant activity.

2. Matrials and Methods

The genetic material used consisted of 6 (six) potential F6 lines from crosses of Black Rice and Mentik Wangi varieties and 2 (two) comparison varieties, namely Black Rice cv Cilacap and Mentik Wangi. These six lines are the results of 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, 482-17-18. Black rice and Mentik Wangi were used as comparison varieties. The F6 lines and comparison varieties were planted until harvest to obtain the F7 line. Seeds of the F7 line obtained were analyzed for bran size (seed), cumulative bran color (seed), amylose content, and antioxidant activity. Meanwhile, the seeds of the F6 line were also subjected to the same analysis.

2.1. Field Trial of F6 Lines

Field testing of the F6 line was carried out in the Experimental Farm greenhouse, Faculty of Agriculture, Universitas Jenderal Soedirman. The study was conducted from April to October 2020. The design used was a completely randomized block design, with 3 (three) blocks and 5 (five) replications in each block. The soil used is ultisol. The lines were planted in polybags, with a total of 115 polybags. Each polybag is filled with one individual rice plant. The fertilizer used consists of NPK fertilizer and manure. The planting media used consisted of ultisol soil, roasted rice husks, and manure. Weeds, pests, and diseases are controlled by conventional means and the use of chemical pesticides. The agronomic parameters observed included flowering age, plant height, dry weight, number of tillers, weight of 1000 grain, number of panicles, and weight of grain per panicle.

2.2. Morphological Characterization of F7 Rice Bran

Rice-bran morphology observed in the form of seed length, seed shape, and seed cumulative color of each line. The determination of seed size classification was determined based on parameters from the International Rice Research Institute (IRRI) (2012). The basis used is the length and the shape of the rice-bran. Cumulative rice-bran color is used to determine the segregation phenomenon.

2.3. Amylose Quantification

The amylose content of the seeds of the F7 strain 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 in the sample was then measured based on the linear regression equation in the standard curve.

2.4. Determination of Antioxidant Activity

Measurement of antioxidant activity was carried out on seeds of the F6 and F7 strains, using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method (Blois, 1958).

2.5. Data analysis

Data on agronomic parameters of the F6 line, F7 rice-bran length, and F7 amylose content of the six lines were analyzed using SAS 9.4 software. If the results of the analysis of variance indicate that there is an influence of the planted genotype on the various agronomic parameters studied, then a different test is carried out with the Least Significant Difference Test (BNT) at the 95% confidence level ($\alpha = 0.05$). The results of this analysis are also used as the basis for determining the value of genetic diversity (Coefficient of Variance/CV), phenotypic diversity, and heritability values of broad meaning, as well as phenotypic variability. The difference in antioxidant activity of the samples of the F7 strain and the F6 strain was identified by the T test. Path analysis was performed using the LISREL 8.2 software. This analysis was used to determine the direct and indirect factors determining the productivity character, namely weight of 1000 grain and weight of grain per panicle.

The estimation of gene action and number of gene control were analyzed based on Skewness and Kurtosis values, respectively, for each trait observed in the F6 generation. Skewness is the slope of the graph. Skewness shows epistasis effected expression of a trait (Lestari et al., 2015). If Skewness equals to zero, it means there is no epistasis. Skewness > 0 means there is a complementary epistasis gene action, and Skewness < 0 means there is a duplicate epistasis gene action. Kurtosis describes the shape of the distribution curve and shows several genes controlling a trait (Herawati et al., 2019). Kurtosis is the value of the taperedness of the graph. When Kurtosis > 3, has a positive value, it shows the leptokurtic graph indicates a few gene controls trait. If Kurtosis < 3, has a negative value, it shows a platykurtic diagram and its trait is controlled by many genes. Interpretation Skewness and Kurtosis value refer to scheme in Jambormias research (Jambormias, 2014). The value of the skewness ratio and the value of the kurtosis ratio can also be used to determine whether the data distribution is normal or not. If the value of the skewness ratio and the value of the kurtosis ratio are between -2 and 2, the data distribution is normal. The value of the skewness and kurtosis ratio can be obtained by dividing the skewness and kurtosis values by their respective standard errors.

The data obtained from the observations and analysis of variance (F test) were used as the basis for calculating the coefficient of variance (CV), coefficient of genetic diversity, coefficient of phenotypic diversity, and heritability values of broad meaning, as well as phenotypic variability. Based on the analysis of variance, the genotypic variance ($\sigma^2 g$), phenotypic variance ($\sigma^2 p$), coefficient of genotypic diversity (KKG) and heritability (h^2), can be estimated using the following formula (Singh & Chaudary, 1977).

$$KTe = \sigma^2 e$$

$$\sigma^2 g = KTg - KTeb$$

$$\sigma^2 p = \sigma^2 g + \sigma^2 e$$

Note :

$\sigma^2 g$ = genotip of variance

$\sigma^2 p$ = phenotip of variance

$\sigma^2 e$ = error of variance

b = replication

KTg = kuadrat tengah genotip

KTe = kuadrat tengah error

The value of the coefficient of genetic diversity can be determined by the following formula:

$$CGV = \sqrt{\sigma^2 g}\bar{X} \text{ x } 100\%$$

Note :

CGV= Coefficient of Genetic Variance

$\sigma^2 g$ = genotip of variance

$\bar{X}$ = mean of population

According to Moedjiono & Mejaya (1994) in Handayani (2018) the criteria for the coefficient of genetic diversity are determined based on absolute and relative values. The criteria for coefficients of genetic and phenotypic diversity are presented in Table 1.

Coefficient of Genetic Variance	Criteria
0 - 25% of the highest	Low
25 - 50% of the highest	Quite low
50 - 75% of the highest	Quite high
75% - 100% of the highest	High

The estimated value of broad sense heritability is determined by the following formula:

$$h^2 = \sigma^2 g \sigma^2 p \text{ x } 100\%$$

Note:

h^2 = broad sense heritability

$\sigma^2 g$ = variance of genotype

$\sigma^2 p$ = Variance of phenotype

The determination of heritability criteria follows the following criteria (McWhirter, 1979) (Table 2):

Value of broad sense heritability	Criteria
$h^2 \leq 20\%$	Low
$20\% < h^2 < 50\%$	Medium
$h^2 > 50\%$	High

Furthermore, the value of phenotypic variability is calculated by the formula:

$$\text{Variability of Phenotipic} = 2 \text{ x SD}$$

Note : SD = Standar Deviation

Determination of the criteria for phenotypic variability is calculated by comparing the value between the phenotypic variance with a value of 2 (two) times the standard deviation of the data. If the value of variability is greater than the value of SD, then it is categorized into broad criteria. And vice versa.

3. Results

3.1. Agronomic Traits of The Lines

The results of the Least Significant Difference (LSD) test to determine the difference in the response of each studied line based on agronomic characters can be seen in Table 3. Black rice and Mentik Wangi varieties were used as comparison varieties, which were excluded from the LSD test.

Table 3. Differences agronomic traits of six lines studied

Genotype	Dry weight	Plant length	Number of tillers	Number of panicles	Weight of 1000 grain	Grain weight per panicle	Days to flowering
Line 482-1-14	46.34±3.70 ab	62.43±1.38a	36.40±6.01b	16.75±1.38c	13.67±0.39ab	2.15±0.28ab	60.92±1.80a
Line 482-17-7	40.03±1.20a	70.94±3.29b	26.80±3.13a	13.95±1.31ab	12.10±1.85a	1.97±0.36ab	60.67±3.10a
Line 482-1-4	51.91±5.53b	69.93±3.97b	36.70±5.43b	16.50±2.27bc	14.21±1.63ab	1.95±0.33a	69.00±1.52c
Line 482-17-18	45.98±5.30ab	81.08±8.90c	25.10±2.27a	11.55±1.55a	15.12±1.75b	2.23±0.52ab	65.08±2.67b
Line 482-9-134	48.29±6.32b	82.83±2.61c	25.95±1.89a	16.20±2.24bc	15.65±1.31b	2.40±0.30ab	68.08±3.27bc
Line 487-24-8	46.99±6.01ab	71.53±1.86b	28.50±6.43a	14.60±2.03bc	15.77±1.48b	2.61±0.41b	61.67±1.31a
Black Rice	37.47±2.95	60.56±2.62	28.25±1.95	15.42±0.63	15.1±0.65	2.24±0.47	64.00±1.41
Mentik wangi	66.90±13.78	101.80±6.60	25.59±4.48	14.08±2.76	15.22±2.34	2.77±0.71	82.83±4.09

Note : Blue color represents the highest value and red color represents the lowest value

3.2. Gene Action and Number of Controlling Genes

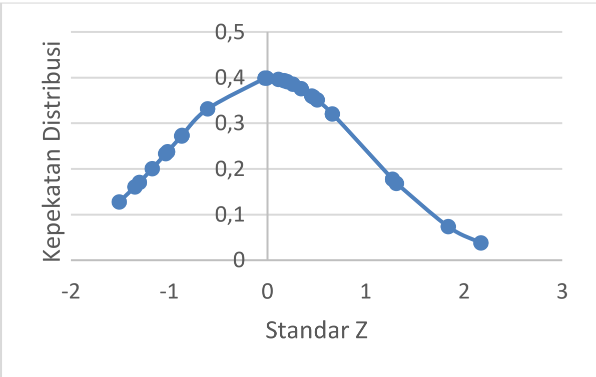
Gene action and the number of controlling genes were determined based on Skewness and Kurtosis analysis. The results of Skewness and Kurtosis analysis for dry weight, days to flowering, plant height, number of tillers, weight of 1000 grain, panicle number, and grain weight per panicle, rice-bran length, amylose content, and antioxidant activity can be seen in Table 4.

Table 4. Gene action and the number of genes controlling the characters studied

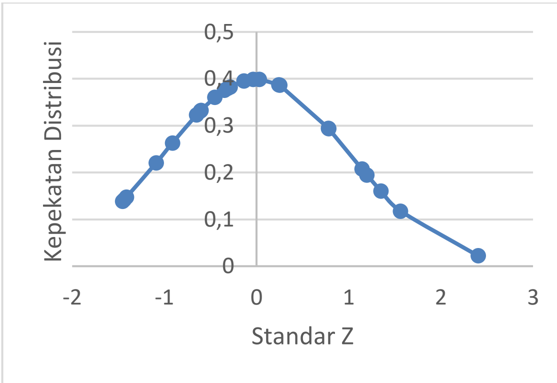
Traits	Skewness	Kurtosis	Gene action	Number of Controlling Gene
Dry weight of F6	0.358	-0.506	Additive	Many
Plant length of F6	0.558	-0.194	Additive	Many
Number of tillers F6	0.914	0.161	Additive	Many
Number of panicle F6	-0.212	-0.965	Additive	Many

Weight of 1000 grain F6	-0.560	0.077	Additive	Many
Grain weight per panicle F6	0.327	-0.694	Additive	Many
Days to flowering F6	0.246	-1.141	Additive	Many
Length of grain F7	1.192	2.213	Complementary epistasis	Many
Amylose content F7	-0.016	-0.046	Additive	Many
Antioxidant activity F7	0.809	-1.329	Additive	Many

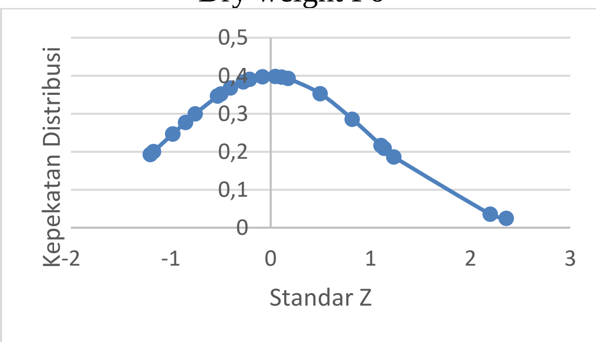
Furthermore, the normal distribution curve of the studied characters can be seen in Figure 1. All the analyzed characters show that the residual data are normally distributed.



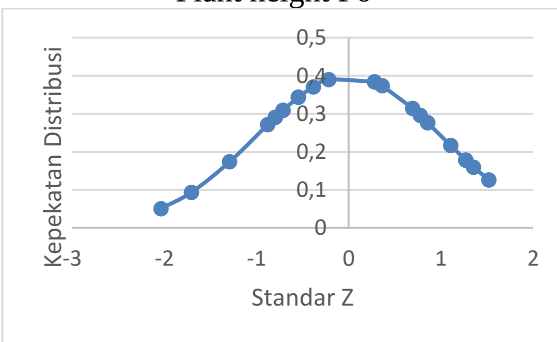
Dry weight F6



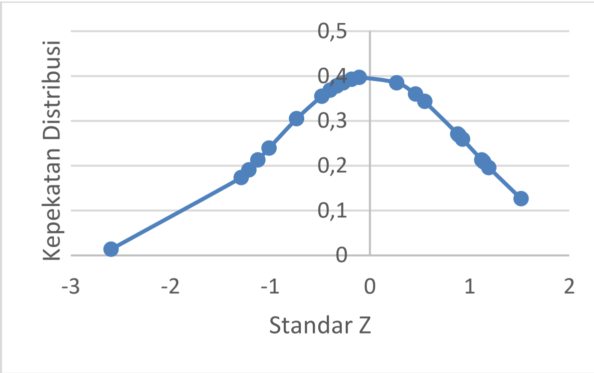
Plant height F6



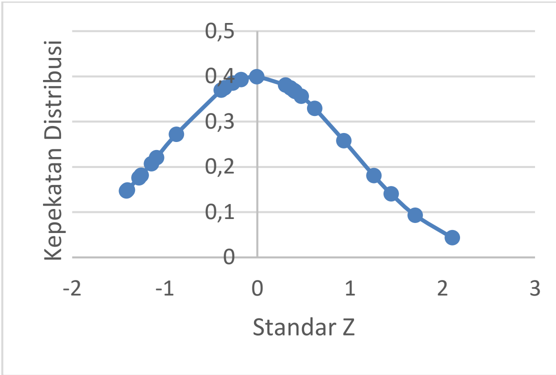
Number of tillers F6



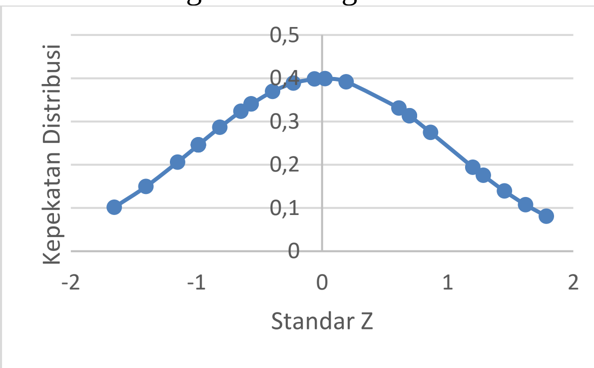
Number of panicle F6



Weight of 1000 grain F6



Grain weight per panicle F6



Days to flowering F6

Figure 1. Residual distribution curve of the data of various observed characters

3.3. Broad Sense Heritability and Phenotypic Variability

It is important to determine the value of the coefficient of diversity first to determine the cause of the differences in the appearance of the observed characters. The results of the calculation of the coefficient of diversity are presented in Table 5. All of the observed characters show a coefficient of diversity that is less than 20%.

Table 5. Coefficient of diversity of various characters studied

Traits	Mean	Max	Min	CV (%)
Dry weight	46.59	84.61	28.63	11,48
Number of tillers	29.91	54	14	15,22
Plant height	73.12	96.8	53.2	6,03
Weight of 1000 grain	14.42	22.12	7.04	9,74
Number of panicle	14.93	25	9	11,44
Grain Weight per panicle	2.22	3.64	1.1	14,63
Days to flowering	64.24	72	57	3,20

Next, the estimated value of heritability in the broad sense describes the level of similarity of the traits of the offspring to the parental varieties. The results of the analysis of heritability estimates, the coefficient of genotypic diversity and the coefficient of phenotypic diversity can be seen in Table 6. The data from the analysis show that there are various heritability estimates for each agronomic character studied. The characters of dry weight, plant height, weight of 1000 grain, and grain weight per panicle had moderate heritability estimates. Characters with high heritability estimates were number of tillers, number of panicles, and age of flowering.

The character that has the highest coefficient of genetic variation (CGV) and coefficient of phenotypic variation (CPV) is the number of tillers. Based on the data obtained, using the criteria from Hayati (2018), the character categorized as having a high CGV value is the number of tillers. Characters with high CGV values were plant height, weight of 1000 grain, number of panicles, and weight of grain per panicle. Characters with a rather low CGV were dry weight and flowering age. Meanwhile, the characters with a high CPV value were the number of tillers, the number of panicles, and the weight of seeds per panicle. Characters with a fairly high CPV value included dry weight, plant height, and weight of 1000 grain. The CGV value for the number of tillers is the same as the CPV value. The CGV value on the character of the number of panicles and the age of flowering is greater than the CPV value. In addition to these characters, the CGV has a lower value than the CPV.

Table 6. Estimated value of heritability (h2), Coefficient of Genetic Variation, and Coefficient of Phenotypic Variation of the characters studied

Traits	Mean	Phenotypic Variation	Middle Square of Error	Phenotypic Variation	h2 (%)	h2 criteria	CGV (%)	CGV criteria	CPV (%)	CPV criteria
Dry weight	46.59	7.86	28.44	36.30	21.65	Moderate	6.02	37.91% (quite low)	12.93	58.8 (quite high)
Number of tillers	29.91	22.56	20.73	43.29	52.11	High	15.88	100% (high)	21.99	100 (high)
Plant height	73.12	36.90	83.97	120.87	30.53	Moderate	8.31	52.33% (quite high)	15.04	68.4 (quite high)
Weight of 1000 grain	14.42	1.47	1.97	3.44	42.79	Moderate	8.42	53.02 (quite high)	12.87	58.53 (quite high)
Number of panicle	14.93	3.25	2.92	6.16	52.68	High	12.07	76.01 (quite high)	16.64	75.67 (high)
Grain weight per panicle	2.22	0.04	0.11	0.14	26.57	Moderate	8.80	55.42 (quite high)	17.06	77.58 (high)
Days to flowering	64.24	12.66	4.23	16.89	74.95	High	5.54	34.89 (quite low)	6.40	29.1 (quite low)

Variability is calculated based on the variance formula. The value of phenotypic variability along with the criteria for each character studied can be seen in Table 7. Based on the data below, the characters categorized as having narrow phenotypic variability are weight of 1000 grain and grain weight per panicle. Characters of dry weight, number of tillers, plant height, and number of panicles had wide phenotypic variability. Based on the calculation of the value of genetic variability, all the characters studied showed a narrow genetic variability.

Table 7. Value of phenotypic variability (PV) and genotypic variability (GV) of the traits

Traits	Mean	Variance	SD	2 X SD	PV Criteria	GV	SD GV	2 x SD	GV Criteria
Dry weight	46.59	31.21	5.59	11.17	broad	7.86	8.36	16.73	narrow
Number of tillers	29.91	38.81	6.23	12.46	broad	22.56	41.94	83.88	narrow
Plant height	73.12	62.99	7.94	15.87	broad	36.90	87.53	175.06	narrow
Weight of 1000 grain	14.42	3.29	1.81	3.63	narrow	1.47	2.97	5.95	narrow
Number of panicles	14.93	5.85	2.42	4.84	narrow	3.25	6.01	12.04	narrow
Grain weight per panicle	2.22	0.16	0.40	0.80	narrow	0.04	0.09	0.19	narrow
Days to flowering	64.24	15.76	3.97	7.94	broad	12.66	20.73	41.47	Narrow

3.4. Inter-Relationship Traits

Path analysis results with LISREL 8.2 software. The dependent variable was the weight of 1000 seeds and the weight of seeds per panicle. Another agronomic character as an influencing variable (independent variable). The path analysis diagram can be seen in Figure 2. Path analysis showed that the number of tillers had an effect on the dry weight and grain weight per panicle, but the dry weight did not directly affect the weight of 1000 seeds.

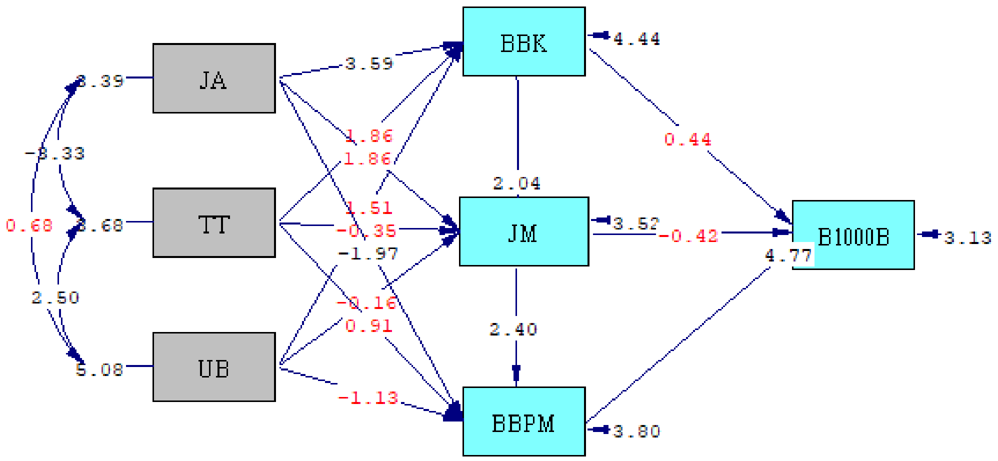


Figure 2. Path diagram of inter-relationship traits studied (P-value = 0.19579)

3.5. Morpho-Biochemical Profile of Bran Rice

The results of the analysis of the grain size of the F6 and F7 lines studied can be seen in Figure 3. This figure shows that the cumulative color of each F6 and F7 intergenerational line has similarities. There were 4 (four) with completely the same color as the black rice parents, but the other 2 (two) lines still showed the color combination between the two crossed parents.

Figure 3. Cumulative color comparison of F6 and F7 rice bran, and also the checked varieties

When viewed from the aspect of rice-bran size, based on the analysis carried out, the average rice-bran size of the 5 (five) lines studied (PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18 , 482-9-134) differed from the PHMW line 487-24-8 (Figure 4; Table 8). Meanwhile, the size of rice-bran of all lines was higher than the rice bran size of the comparison varieties used.

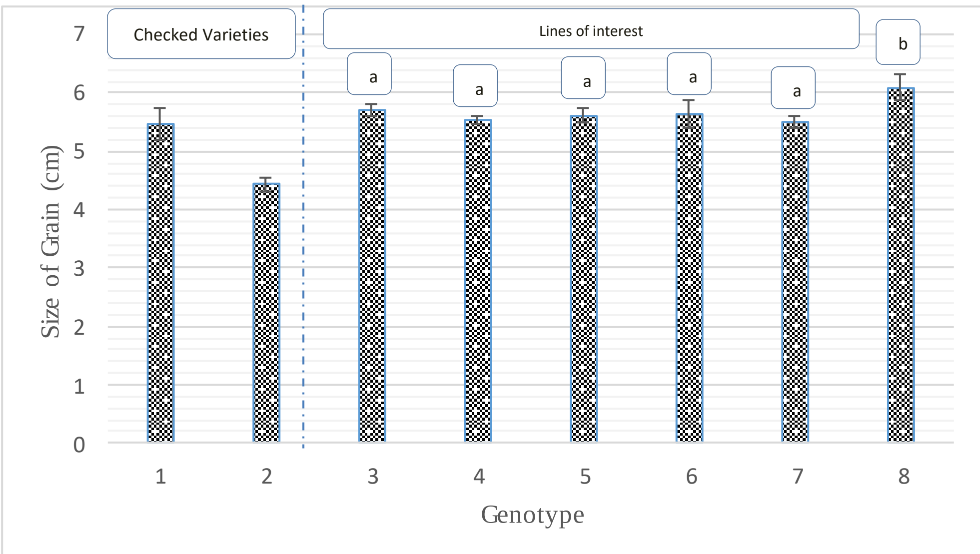


Figure 4. Size of F7 Rice Bran

The amylose profile of the rice-bran samples of the F7 lines showed that the average amylose content of each line studied was different from one another (Figure 5). Line 482-1-14 showed the lowest amylose content value 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 amylose content value compared to all lines (16.81±0.05), but it was still below the amylose content of the Black Rice comparison variety (22.37±0.04). The amylose content of all lines was in the range of amylose content of the two comparison varieties studied (Black Rice and Mentik Wangi).

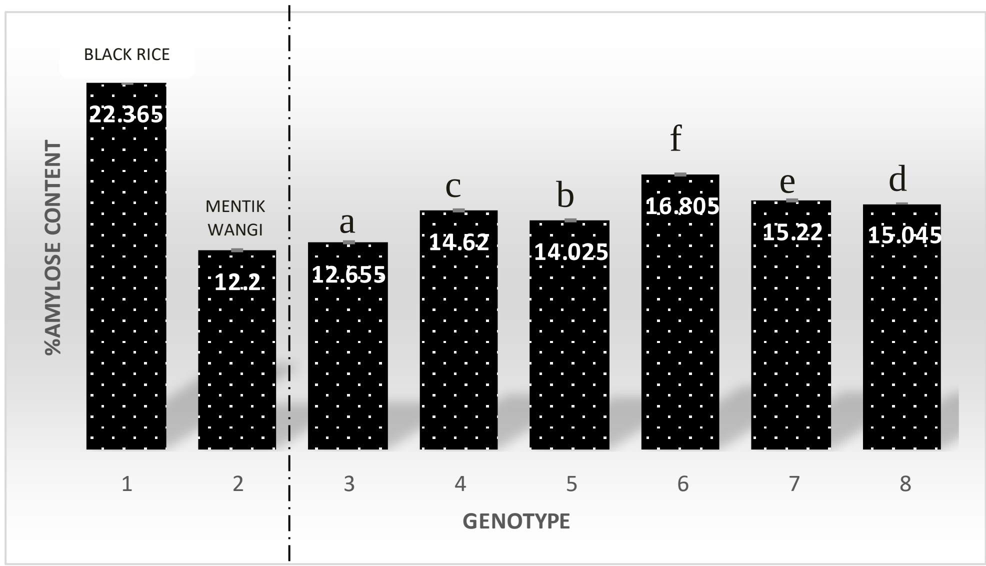


Figure 5. Amylose profile of F7 and checked varieties

For the character of antioxidant activity, independent sample T-test was conducted on 2 groups of seeds of the F6 and F7 strains. This test aims 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.

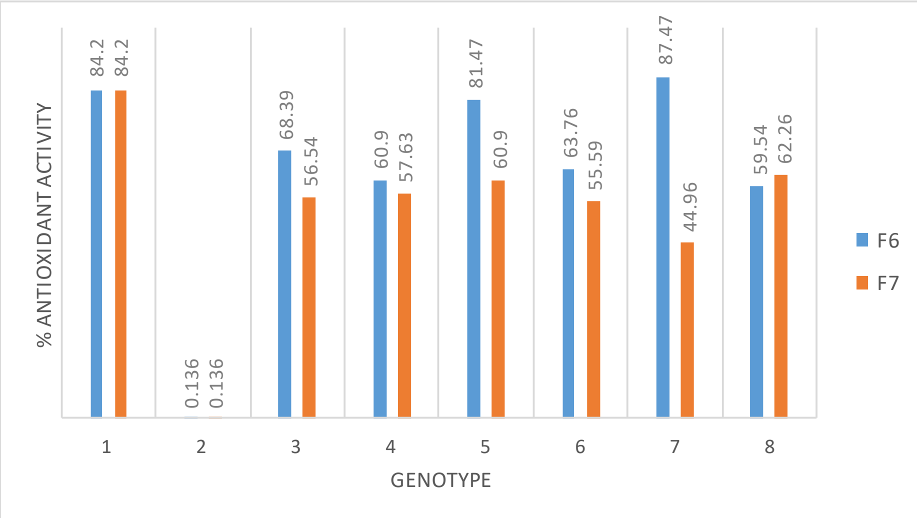


Figure 6. Antioxidant activity of F6 and F7 rice bran samples

4. Discussion

The purpose of this study was to determine genetic parameters based on agronomic data of F6 lines. Another objective of this research is to determine the agronomic character that determines the character of agricultural productivity, through path analysis. These genetic parameters can be used as the basis for determining selection criteria in plant breeding programs, so that the selection is more effective and efficient (Yudilastari et al., 2018). In addition, a morpho-biochemical profile that is suitable for the purpose of developing low amylose pigmented rice is also carried out as a basis for the selection process for potential lines.

4.1. Agronomic Traits

All the traits observed in each line showed a different effect in each line studied. The dry weight of all lines was between the dry weight of the two comparison varieties. There were two subsets of dry weight trait for the 6 (six) lines. The same analysis was applied to the trait of plant height. The plant height of all lines was between the plant heights of the two comparison varieties. There were 3 (three) subsets of plant height characters for the 6 (six) lines studied. Line 482-1-4 has differences with other strains. Each line 482-1-7, 482-1-4, and 487-24-8 had no difference in plant height. Meanwhile, lines 482-17-18 and 482-9-134 also did not have differences in plant height, because they were in one subset (group). For the number of tillers, there were lines that had a higher number of tillers than the comparison varieties, namely lines 482-1-14 and 482-1-4. The number of tillers of the other lines was the same as in the comparison variety. The grouping of panicle numbers is more varied. There are 3 (three) subsets that group the lines based on the character of the number of panicles. The number of panicles of the 482-1-14 line

was higher and statistically different compared to the checked varieties. Meanwhile, lines 482-1-4, 482-9-134, 487-24-8 and black rice varieties did not have statistical differences in the number of panicles. The number of panicles of the Mentik Wangi variety did not differ from that of the 482-17-7 strain. For weight of 1000 grain, there were only 2 (two) subsets that grouped each line. The weight of 1000 grain of the comparison varieties did not differ from those of 482-17-18, 482-9-134, and 487-24-8. The other three lines had 1000 seed weights lower than the two comparison varieties. For the character of grain weight per panicle, there are 2 (two) subsets formed from the statistical analysis. Lines 482-1-14, 482-17-7, 482-17-18, and 4829-134 had no difference in grain weight per panicle of black rice varieties. Meanwhile, the grain weight per panicle of the Mentik Wangi variety was not different from that of the 487-24-8 line, which indicated the highest value of grain weight per panicle among all other lines and the Black Rice variety. Flowering age characters resulted in 3 (three) subsets of the genotypes studied. The flowering age of the Mentik Wangi variety was different from all the studied lines, because the flowering age was the longest compared to the Black Rice lines and varieties. Flowering age of lines 482-1-14, 482-17-7, and 487-24-8 were not significantly different. For the Black Rice variety, it has the same flowering age as the 482-17-18 line.

Research by Kartahadimaja et al. (2021) reported that statistical analysis of agronomic characters 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 variations. Meanwhile, research by Kasim et al. (2020) showed that the rice plant height of the 17 rice varieties studied ranged from 95-160 cm. The plant heights 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 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 heights are preferred by farmers and researchers. While the parameters of flowering age, varied between 104-151 DAS. This indicates that these varieties have a longer flowering life than the lines used in this study. The earlier flowering age of rice varieties is preferred by farmers and researchers, because varieties with an earlier flowering age have a shorter life cycle, thus allowing a higher frequency of harvesting per year than varieties with a longer flowering age. This can have an impact on farmers' annual income. Meanwhile, the number of panicles varied between 12-24. The range of genotypic panicles in this study was in the range of the number of panicles studied by Kasim

et al. (2020). Meanwhile, the total number of grain per plant also varied, between 128 to 305. The weight of 100 seeds of the spadi variety also varied, between 1.4 – 2.56 grams.

Based on the agronomic data of the cross lines studied, characters still appear that are outside the range of character values of the two comparison varieties used. This is in accordance with the opinion of Welsh (1981) which states that the action of duplicate genes and additive genes can cause transgressive segregation. Transgressive segregation is segregation that causes offspring to have characters with measurement ranges that are below or even above their parents, so that it can provide opportunities for breeders to get the desired segregate (Nugraha & Suwarno, 2007).

4.2. Action and Number of Gene Controllers

Agronomic character is a phenotype, whose expression is determined by the interaction between genotypic factors and environmental factors. The characters studied in this study are quantitative characters, or some call them complex characters (Mackay, 2009). According to Ikram & Chardon (2010), quantitative characters are controlled by a complex genetic system, because it involves several genes (polygenic), with each gene having a minor influence, can be in the form of a small additive effect, dominant or epistatic, and sensitive to environmental conditions. (Mackay, 2009; Ikram & Chardon, 2010). Genetic complexity arises from alleles that experience segregation at multiple loci (Mackay, 2009).

The genetic variation of quantitative characters is assumed to be controlled by the collective influence (together) of the Quantitative Trait Loci (QTL), epistasis (interaction between QTL-QTL), environment, and interactions between QTL and environment (Semagn et al., 2010). Because of this complexity, many genotype characters can produce the same phenotype, and the same genotype can express different phenotypes (Mackay, 2009). Therefore, there is no clear relationship between genotype and phenotype in the expression of this trait. Semagn et al. (2010) wrote that unlike monogenic controlled characters, these polygenic characters do not follow the Mendelian inheritance pattern as in qualitative characters.

The analysis of skewness and kurtosis can provide information about the nature of gene action and the number of genes that control a character (Samak et al., 2011). The analysis of skewness and kurtosis plays an important role in determining the presence or absence of epistasis in the cross zuriat (Ramadhan et al., 2018). The results showed that most of the agronomic characters of the studied lines were controlled by additive gene action, with only seed length being controlled by the complementary gene action of epistasis. Yudilastari et al. (2018) wrote that

gene action in controlling a character can be divided into two, namely additive and non-additive (dominant gene action and epistasis). Allard (1964) and Crowder (1981) wrote that the term additive gene action is used in relation to genes affecting trait expression, where each allele contributes to the trait's phenotype. These contributions are known as additive effects, because the phenotype is determined by the sum of the effects of each allele of the gene loci involved. Changes caused by allelic substitution at each locus are not affected by alleles at other loci. The effect of additive genes from each allele can be passed from parents to offspring, because the contribution of each allele does not depend on allelic interactions (Yudilastari et al., 2018; Nugraha & Suwarno, 2007). Characters controlled by additive gene action indicate that selection can take place in the early generations because these characters can be inherited in the next generation (Ramadhan et al., 2018). On the other hand, for characters controlled by dominant or epistatic gene action, selection is carried out in the next generation (Mahalingam et al., 2011; Sulistyowati et al., 2015).

Based on this research, the characters affected by the action of additive genes, which can be passed on to the lines, were dry weight, plant height, number of tillers, number of panicles, weight of 1000 grain, and grain weight per panicle, amylose content, and antioxidant activity. On the other hand, the character of the length of the rice-bran is controlled by the action of non-additive genes, in this case it is epistasis, so that there is a tendency that it cannot be passed on to the lines. The same thing was reported by Anis et al. (2016), that there is a tendency for the character of plant height and harvest time to be influenced by the action of additive genes in their inheritance. In addition, the research of Ramadhan et al (2018) reported that there was additive gene action that affected the character of the number of primary branches in the zuriat population of IPB 3S/IPB160-F-36 rice crosses and almost all of the characters whose panicle architecture studied were controlled by additive gene action on zuriat population of IPB160-F-36/IPB 5R crosses, except for the length of the primary branches.

The results showed that all the characters studied were controlled by many controlling genes. This supports the etymology of the quantitative character itself, which is a polygenic (many gene) controlled character. Compared with the research of Ramadhan et al. (2018), the characters controlled by multiple genes in all cross populations studied were panicle length, number of primary branches, and grain density. The length of the primary branch and the number of branches/primary branches in the IPB160-F-36/IPB 5R cross zuriat were also controlled by many genes. Riyanto et al (2021) also reported that the characters of plant height,

flowering age, harvest age, panicle length, and number of grain per panicle were controlled by many genes.

4.3. Heritability, Coefficient of Variation, and Variability

Yudilastari et al. (2018) wrote that heritability is a genetic parameter that can be used to determine the role of genetics in the inheritance of a character from parents to offspring/lines. Heritability is used as the basis for estimating the relative contribution of differences in the magnitude of genetic and non-genetic factors to the total phenotypic diversity in a population (Ene et al., 2015; Konate et al., 2016). Information about heritability can be used by breeders to determine the extent to which the intensity of selection is carried out to distinguish environmental influences on the phenotype of a plant (Zehra et al., 2017). Heritability is an important concept in quantitative genetics, especially in selection in plant breeding programs (Konate et al., 2018).

The value of heritability in the broad sense of this study is in the range that varies for each character. The data from the analysis showed that there were various heritability estimates for each agronomic character studied. The characters of dry weight, plant height, weight of 1000 grain, and grain weight per panicle had moderate broad-sense heritability. Characters with high heritability estimates were number of tillers, number of panicles, and age of flowering. Adhikari et al. (2018) wrote that the low heritability value indicates that the character appears due to variations in environmental factors involved in the expression of the character, and vice versa. A high broad sense heritability indicates a high selection response for a particular character. A good character to be used as a selection character is a character that has a high heritability value (Begum et al., 2015). A small heritability value will have an impact on a small selection progress value (Mursito, 2003).

The results of this study are in line with the research of Adhikari et al. (2018), that the flowering age also has a high broad-sense heritability. Meanwhile, the character of weight of 1000 grain and grain weight per panicle, which can be used as yield component parameters, also had moderate borad-sense heritability, compared to research by Adhikari et al. (2018). Research by Ogunbayo et al. (2014) also showed results in accordance with this study. The characters of flowering age and number of tillers also had high broad-sense heritability. This suggests that these characters are primarily under genetic control, rather than the environment. Similar results were confirmed in the study of Konate et al. (2016), that flowering age, number of tillers, and

number of panicles are also categorized as characters with high broad-sense heritability. The weight of 1000 grain character also had a moderate broad-sense heritability.

The results showed that the entire value of the coefficient of phenotypic variation of all characters was higher than the coefficient of genotypic variation. Adhikari et al. (2018) wrote that this indicates the influence of the environment on these characters. The magnitude of the influence of the plant growth environment on the observed characters is explained by the level of difference value between those two parameters. Furthermore, Adhikari et al. (2018) wrote that the large difference in the values of these parameters indicates a large environmental influence on the expression of certain characters. The CPV value for all characters in this study showed a higher tendency than the GPV. This is in line with the research results of Bagati et al. (2016), that the value of the coefficient of phenotypic diversity is higher than the coefficient of genotype diversity in all the characters studied.

Based on the data above, the characters categorized as having narrow phenotypic variability were weight of 1000 grain and grain weight per panicle. Characters of dry weight, number of tillers, plant height, and number of panicles had broad phenotypic variability. Based on the calculation of the value of genotypic variability, all the characters studied showed a narrow genotypic variability. Hayati (2018) wrote that characters with narrow phenotypic variability are not effective for selection. Therefore, characters with dry weight, number of tillers, plant height, and number of panicles can be used as selection criteria. A high coefficient of variability indicated a favorable selection range for the desired character, while a low coefficient of variability indicated a need to create variability and conduct selection (Adhikari et al., 2018).

4.4. Inter-Relationship Between Characters

Path analysis showed that the number of tillers had an effect on 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. This analysis is used to determine the magnitude of the direct or indirect effect on the yiled component towards the yiled (Akhmadi et al., 2017). A good selection character is one that has a large real correlation value and a high direct or indirect effect value on the yield (Boer, 2011). Yield is a complex character and depends on a number of related characters. Therefore, crop yields usually depend on the actions and interactions of a number of important characters. Knowledge of the various characters that determine crop yields is important for examining various yield components (growth parameters) and paying more attention to yield

components that have the greatest influence on crop yields (Kinfe et al, 2015). A good selection character is one that has a large real correlation value and a high direct or indirect effect value on the results (Boer, 2011).

The results of this study have differences when compared with the research of Akhmadi et al. (2017). The results of the research Akhmadi et al. (2017) showed that characters that had a direct effect on high yields were length of panicle, weight of 1000 filled grain, number of filled grains per panicle and grain filling period. The character of the generative plant height and the total number of grain per panicle had a high negative direct effect on yield, but the indirect effect through panicle length was quite high. Several studies that have been carried out using this analysis showed the characteristics of flowering age, 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 100 grains which have a direct effect on high yield on some rice plant populations (Aryana et al., 2011; Rachmawati et al., 2014; Safitri et al. (2011).

4.5. Morpho-Biochemical Profile

The rice-bran cumulative color of the F6 and F7 lines showed that there were 4 (four) lines with completely the same color as the black rice parent, but the other 2 (two) lines still showed the color combination between the two parents. The research of Laokuldilak et al. (2011) reported that pigmented rice contains high Cyanidin-3-Glucoside (58-95%). C3G is part of the anthocyanin group of compounds. The appearance of rice-bran color in pigmented rice is thought to be related to the content of Cyanidin-3-Glucoside compound, although the relationship between these two is not clear, due to the complexity of the existing genetic system. According to research Ham et al. (2015), there was a significant positive correlation on C3G content towards the brightness and yellow color of rice bran. However, the genetic system responsible for rice-bran pigmentation and Cyanidin-3-Glucoside content is not same, but is related through a specific pathway with anthocyanin metabolism. Mackon 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.

When viewed from the aspect of rice-bran size, based on the analysis carried out, the average rice-bran size of the 5 (five) lines studied (PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18 , 482-9-134) differ from the PHMW 487-24-8. Meanwhile, the rice-bran size of all lines was higher than that of the comparison varieties. The length of the rice bran of the lines was above

the length of the rice-bran of the parent, Mentik Wangi, and had a size that was not significantly different from the length of the rice bran of the Black Rice variety. This condition could be caused by transgressive segregation of alleles responsible for the expression of rice bran length characters. Based on the analysis of Skewness and Kurtosis, it was known that this character is controlled by complementary epistasis gene action and controlled by a large number of genes (polygenic). According to IRRI (2013), the length of the rice bran in all these lines was grouped into the medium classification, except for the 482-17-7 line, which was grouped under the short criteria. The length of the rice-bran is part of determining the shape of the rice grain. The shape of rice grain is one of the determinants of the quality of rice grain. Grain quality is one of the selection parameters in plant breeding program (Kush and Cruz, 2000; Kartahadimaja et al., 2021). When compared with previous studies (Oktaviani et al., 2021), F7 rice bran size did not have a significant difference with F6 rice-bran size. All the lines studied in the F6 generation had higher rice-bran size compared to the rice-bran size of the Padi Hitam and Mentik Wangi varieties. All F6 and F7 lines were also categorized into the moderate, based on the standards of the International Rise Research Institute (2013). Based on the research of Kartahadimaja et al. (2021), rice-bran size of the 12 genotypes studied varied in length, width, and thickness. B3 line is a new line with the shortest length (8.3 mm) but the widest among all lines. Meanwhile, the other 9 lines varied between 9.04 – 10.31 mm, and were included in the long seed criteria. Other lines, such as B2, B4, B7, F2, F3, F4, H1, H4, L2, and two varieties IR 64 and Ciherang, were included in the criteria for length with a narrower width. The IR64 variety is the variety with the smallest width (2.55 mm).

Amylose content is one of the criteria that determines grain quality (IRRI, 2012). In addition, amylose content is also one of the parameters used to predict the quality of processed rice (Juliano et al., 1965; Bhattacharaya and Juliano, 1985). The amylose content of the studied lines was between the amylose content of the comparison varieties. In addition, each line showed a significant difference in amylose content. Based on the criteria of Khush & Cruz (2000), the amylose content of grain could be grouped into 5 (five) criteria, waxy rice (0% to 2%), very low amylose rice (3% to 9%), low amylose rice (10% to 19 %), medium amylose rice (20 % to 25 %), and rice with high amylose (> 25 %). The amylose content of the studied lines was at a low criteria. The results of this study are a continuation of previous studies that examined the amylose content in the F6 line. The F6 generation PHMW lines were in various criteria. The classification includes very low amylose (lines 482-1-14), low amylose (lines 487-24-8, 482-

9-134, 482-1-4, and 482-17-7) and medium amylose (lines 487-24-8, 482-9-134, 482-1-4, and 482-17-7) and medium amylose (lines 482-17-18) (Oktaviani et al., 2021).

It is important to measure the antioxidant profile of the lines to map the antioxidant profile of the lines compared to checked varieties. The results showed that there was 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 a greater reducing power than the long white rice bran extract. The main antioxidant compounds detected by High Performance Liquid Chromatography (HPLC) were oryzanol (39-63%) and phenolic acids (33-43%). In addition, Laokuldilok et al. (2011) also found that black rice had 18-26% anthocyanin content. Ferulic acid was the dominant phenolic acid in the rice samples studied. Black rice contained higher levels of gallic acid, hydroxybenzoic acid, and protocatechoic acid compared to red rice and white rice. In addition, the research of Jun et al. (2011) reported that antioxidant activity of 40% pigmented rice-bran acetone extract, at an antioxidant concentration of 500 g/mL, red rice with the highest total phenolic and total flavonoids showed highest antioxidant activity (83.6% based on the radical DPPH test). In addition, there was an interesting study by Setyaningsih et al. (2015) who studied the positive correlation between amylose content and levels of antioxidant compounds, such as melatonin and phenolics.

Conclusions

All agronomic parameters studied were thought to be controlled by many genes (polygenic) and additive gene action, except for rice-bran length. The data from the analysis showed that there were various broad-sense heritability for each agronomic character. The characters of dry weight, plant height, weight of 1000 grain, and grain weight per panicle had moderate broad-sense heritability. Characters with high broad-sense heritability were number of tillers, number of panicles, and days to flowering. Based on the results of the study, all the characters observed in each line showed a different effect in each line studied. The value of broad-sense heritability this study varied for each character. Path analysis showed that the number of tillers had an effect on 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 seeds of the F6 and F7 lines showed that there were 4 (four) lines with completely the same color as the black rice, but the

other 2 (two) lines still showed the color combination between the two checked varieties. When viewed from the aspect of rice-bran size, the rice-bran size mean of the 5 (five) lines studied (PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18 , 482-9-134) differ from the PHMW 487-24-8. Meanwhile, the rice-bran size of all studied lines was higher than the rice-bran size of the comparison varieties. The amylose content of lines was between the amylose content of the comparison 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 sticky rice traits (low amylose content) with multi-location and multi-season field trial, selection based on molecular markers related to genes associated with low amylose content and high antioxidant content, as well as organoleptic testing of processed rice from these lines.

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Genetic Parameters and Inter-relationship Between Agronomic Traits of F6 Lines, and Rice Bran Morpho-Biochemical Profile of F7 Lines Derived from a Crossing of Black Rice and Mentik Wangi

ABSTRACT

The development of sticky pigmented rice with high antioxidant content as superior varieties can be carried out by crossing the Black Rice with Mentik Wangi varieties. Rice breeding program to obtain rice lines with low amylose content and high antioxidants has reached the F6 lines. The purpose of this study was to determine genetic parameters and the relationship between agronomic traits of the F6 lines. Another objective of this study was to determine the morpho-biochemical profile of F7 grain, including length, cumulative color, amylose content and antioxidant activity. The results showed that all agronomic parameters had a coefficient of variance less than 20%, which indicates that phenotypic differences were caused by genotypic factors, rather than environmental ones. All the values of the Genetic Diversity Coefficient are in the low range, as well as the values of the Phenotypic Diversity Coefficient. The broad sense heritability of dry weight character was in the low range. The characters of plant height, weight of 1000 grain and weight of grain per panicle were categorized as having moderate broad sense heritability, while the characters of number of tillers, number of panicles and age of flowering had high broad sense heritability. The phenotypic variability of weight of 1000 grain and age of flowering were included in the narrow criteria, while the other characters were broad one. Path analysis showed that the number of tillers had an effect on the dry weight and grain weight per panicle, but the dry weight did not directly affect the weight of 1000 grain. Directly, the weight of 1000 grain was only significantly affected by the grain weight per panicle trait. In terms of grain length profile and amylose content, the F7 lines had difference in grain length (2 subsets) and amylose content (6 subsets) traits. The cumulative grain color of the PHMW482-17-7 and 482-17-18 lines showed the color combination of the two parents. Based on the T test conducted on F6 and F7 grain samples, 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.

Keywords: antioxidant, black rice, mentik wangi, morpho-biochemical, sticky

1. Introduction

Black rice is one type of pigmented rice, in addition to red rice and brown rice. This rice is often consumed as functional food, not as the main food ingredient (Purwanto et al., 2019). This is due to the various nutritional content of this type of rice. Black rice contains various micro and macronutrients that are important for human health (Apridamayanti et al., 2017). Protein, fat, fiber, vitamins, and minerals important for the human body are the various nutrients that this rice has (Nurhidajah, 2018; Apridamayanti et al., 2017; Kristamtini et al., 2012). Important nutrients reported in black rice are vitamin B, vitamin E, Fe ions, thaimin, magnesium, niacin, phosphorus, dietary fiber (Kristamtini et al., 2021; Murali & Kumar, 2020), Zn ions, and Mn (Kristamtini et al. et al., 2021; Murali & Kumar, 2020), Zn, and Mn ions (Kristamtini et al., 2021; Murali & Kumar, 2020), et al., 2012). Murali & Kumar (2020) also reported that black rice is free from gluten and cholesterol, low in sugar, salt and fat.

Black rice, is one of the pigmented rice classified on the basis of the color of the pericarp, aleurone, and endosperm of the rice grains (Kristamtini et al., 2012). One of the important compounds that contribute to black rice aleurone color is anthocyanin (Yoshimura et al., 2012; Palupi et al., 2020). Anthocyanins are responsible for the appearance of blue, purple, red, and orange colors in many fruits and vegetables (Miguel, 2011). Anthocyanins have high antioxidant activity and play an important role in human health (Prastiwi & 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 & Kumar, 2020; Tena et al., 2020), support health eye, anti-microbial, and prevention of neuro-degenerative diseases (Tena et al., 2020).

Anthocyanins, one of the most important types of plant flavonoid compounds, are pigments with a flavylium cation structure (AH+) that act as acids (Tena et al., 2020). This structure is directly related to antioxidant activity, because it is able to prevent or inhibit oxidation reactions by scavenging free radicals and reduce levels of oxidative stress in cells (Tena et al., 2020). Based on basic chemical reactions, anthocyanins act as donors of H atoms or as single electron transferors (Tena et al., 2020).

Although black rice is known as a functional food with the above benefits, consumer acceptance of the texture of rice prepared from this rice is low, due to the non-stickiness texture of the cooked rice (Adi et al, 2020). Non-tender/non-sticky/non-glutinous rice has a dry, hard, and separate texture, even though it has been through the cooking process. Rice texture is determined by amylose amylopectin ratio (Cameron & Wang, 2005; Adi et al., 2020; Li et al., 2016a), post-harvest processing, and cooking method (Li et al., 2016b). In addition, Cameron & Wang (2005) also found that the texture/stickiness/hardness of rice was associated with protein and crude lipid content. The higher the amylose content of rice, the more tender the texture of the rice will be, and vice versa (Khumar & Khush, 1986; Bhattacharaya et al., 1999; Luna et al, 2015; Panesar & Kaur, 2016). Crude protein and lipid content were negatively correlated with the hardness of pasta flour and processed rice, but positively correlated with the level of rice stickiness (Cameron & Wang, 2005).

A rice plant breeding program to produce rice plants with a quality texture of soft/soft/fluffy processed rice has been carried out in Indonesia. Indonesia, through the Ministry of Agriculture, has released a rice variety with an amylose content of 19.6% in 2019, the result of a cross between Black Sticky Rice and Pandan Wangi cv Cianjur. In addition, research for the development of fluffier pigmented rice has also been carried out by researchers (Kim et al., 2010; Zhang et al., 2018; Roy & Shil, 2020). The development of pigmented rice with a fluffier rice texture in Indonesia is expected to increase the source of rice germplasm with superior characters in Indonesia.

The development of superior varieties of pigmented rice that is fluffier and has a high antioxidant content can be done by crossing the Black Rice variety with Mentik Wangi. Rice breeding research to obtain rice lines with high antioxidants and a fluffier texture of rice has reached the F6 offspring. The purpose of this study was to determine various genetic parameters and the relationship between characters based on the agronomic character of the F6 line. Another objective of this study was to determine the morpho-biochemical profile of F7 seeds, including length, cumulative color, amylose content and antioxidant activity.

2. Matrials and Methods

The genetic material used consisted of 6 (six) potential F6 lines from crosses of Black Rice and Mentik Wangi varieties and 2 (two) comparison varieties, namely Black Rice cv Cilacap and Mentik Wangi. These six lines are the results of 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, 482-17-18. Black rice and Mentik Wangi were used as comparison varieties. The F6 lines and comparison varieties were planted until harvest to obtain the F7 line. Seeds of the F7 line obtained were analyzed for bran size (seed), cumulative bran color (seed), amylose content, and antioxidant activity. Meanwhile, the seeds of the F6 line were also subjected to the same analysis.

2.1. Field Trial of F6 Lines

Field testing of the F6 line was carried out in the Experimental Farm greenhouse, Faculty of Agriculture, Universitas Jenderal Soedirman. The study was conducted from April to October 2020. The design used was a completely randomized block design, with 3 (three) blocks and 5 (five) replications in each block. The soil used is ultisol. The lines were planted in polybags, with a total of 115 polybags. Each polybag is filled with one individual rice plant. The fertilizer used consists of NPK fertilizer and manure. The planting media used consisted of ultisol soil, roasted rice husks, and manure. Weeds, pests, and diseases are controlled by conventional means and the use of chemical pesticides. The agronomic parameters observed included flowering age, plant height, dry weight, number of tillers, weight of 1000 grain, number of panicles, and weight of grain per panicle.

2.2. Morphological Characterization of F7 Rice Bran

Rice-bran morphology observed in the form of seed length, seed shape, and seed cumulative color of each line. The determination of seed size classification was determined based on parameters from the International Rice Research Institute (IRRI) (2012). The basis used is the length and the shape of the rice-bran. Cumulative rice-bran color is used to determine the segregation phenomenon.

2.3. Amylose Quantification

The amylose content of the seeds of the F7 strain 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 in the sample was then measured based on the linear regression equation in the standard curve.

2.4. Determination of Antioxidant Activity

Measurement of antioxidant activity was carried out on seeds of the F6 and F7 strains, using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method (Blois, 1958).

2.5. Data analysis

Data on agronomic parameters of the F6 line, F7 rice-bran length, and F7 amylose content of the six lines were analyzed using SAS 9.4 software. If the results of the analysis of variance indicate that there is an influence of the planted genotype on the various agronomic parameters studied, then a different test is carried out with the Least Significant Difference Test (BNT) at the 95% confidence level ($\alpha = 0.05$). The results of this analysis are also used as the basis for determining the value of genetic diversity (Coefficient of Variance/CV), phenotypic diversity, and heritability values of broad meaning, as well as phenotypic variability. The difference in antioxidant activity of the samples of the F7 strain and the F6 strain was identified by the T test. Path analysis was performed using the LISREL 8.2 software. This analysis was used to determine the direct and indirect factors determining the productivity character, namely weight of 1000 grain and weight of grain per panicle.

The estimation of gene action and number of gene control were analyzed based on Skewness and Kurtosis values, respectively, for each trait observed in the F6 generation. Skewness is the slope of the graph. Skewness shows epistasis effected expression of a trait (Lestari et al., 2015).

If Skewness equals to zero, it means there is no epistasis. Skewness > 0 means there is a complementary epistasis gene action, and Skewness < 0 means there is a duplicate epistasis gene action. Kurtosis describes the shape of the distribution curve and shows several genes controlling a trait (Herawati et al., 2019). Kurtosis is the value of the taperedness of the graph. When Kurtosis > 3, has a positive value, it shows the leptokurtic graph indicates a few gene controls trait. If Kurtosis < 3, has a negative value, it shows a platykurtic diagram and its trait is controlled by many genes. Interpretation Skewness and Kurtosis value refer to scheme in Jambormias research (Jambormias, 2014). The value of the skewness ratio and the value of the kurtosis ratio can also be used to determine whether the data distribution is normal or not. If the value of the skewness ratio and the value of the kurtosis ratio are between -2 and 2, the data distribution is normal. The value of the skewness and kurtosis ratio can be obtained by dividing the skewness and kurtosis values by their respective standard errors.

The data obtained from the observations and analysis of variance (F test) were used as the basis for calculating the coefficient of variance (CV), coefficient of genetic diversity, coefficient of phenotypic diversity, and heritability values of broad meaning, as well as phenotypic variability. Based on the analysis of variance, the genotypic variance (σ^2g), phenotypic variance (σ^2p), coefficient of genotypic diversity (KKG) and heritability (h^2), can be estimated using the following formula (Singh & Chaudary, 1977).

$$KTe = \sigma^2e$$

$$\sigma^2g = KTg - KTeb$$

$$\sigma^2p = \sigma^2g + \sigma^2e$$

Note :

σ^2g = genotip of variance

σ^2p = phenotip of variance

σ^2e = error of variance

b = replication

KTg = kuadrat tengah genotip

KTe = kuadrat tengah error

The value of the coefficient of genetic diversity can be determined by the following formula:

$$CGV = \sqrt{\sigma^2g}\bar{X} \text{ x } 100\%$$

Note :

CGV= Coefficient of Genetic Variance

$\sigma^2 g$ = genotip of variance

$\bar{X}$ = mean of population

According to Moedjiono & Mejaya (1994) in Handayani (2018) the criteria for the coefficient of genetic diversity are determined based on absolute and relative values. The criteria for coefficients of genetic and phenotypic diversity are presented in Table 1.

Coefficient of Genetic Variance	Criteria
0 - 25% of the highest	Low
25 - 50% of the highest	Quite low
50 - 75% of the highest	Quite high
75% - 100% of the highest	High

The estimated value of broad sense heritability is determined by the following formula:

$$h^2 = \sigma^2 g \sigma^2 p \text{ x } 100\%$$

Note:

h^2 = broad sense heritability

$\sigma^2 g$ = variance of genotype

$\sigma^2 p$ = Variance of phenotype

The determination of heritability criteria follows the following criteria (McWhirter, 1979) (Table 2):

Value of broad sense heritability	Criteria
$h^2 \leq 20\%$	Low
$20\% < h^2 < 50\%$	Medium
$h^2 > 50\%$	High

Furthermore, the value of phenotypic variability is calculated by the formula:

Variability of Phenotipic = 2 x SD

Note : SD = Standar Deviation

Determination of the criteria for phenotypic variability is calculated by comparing the value between the phenotypic variance with a value of 2 (two) times the standard deviation of the data. If the value of variability is greater than the value of SD, then it is categorized into broad criteria. And vice versa.

3. Results

3.1. Agronomic Traits of The Lines

The results of the Least Significant Difference (LSD) test to determine the difference in the response of each studied line based on agronomic characters can be seen in Table 3. Black rice and Mentik Wangi varieties were used as comparison varieties, which were excluded from the LSD test.

Table 3. Differences agronomic traits of six lines studied

Genotype	Dry weight	Plant length	Number of tillers	Number of panicles	Weight of 1000 grain	Grain weight per panicle	Days to flowering
Line 482-1-14	46.34±3.70 ab	62.43±1.38a	36.40±6.01b	16.75±1.38c	13.67±0.39ab	2.15±0.28ab	60.92±1.80a
Line 482-17-7	40.03±1.20a	70.94±3.29b	26.80±3.13a	13.95±1.31ab	12.10±1.85a	1.97±0.36ab	60.67±3.10a
Line 482-1-4	51.91±5.53b	69.93±3.97b	36.70±5.43b	16.50±2.27bc	14.21±1.63ab	1.95±0.33a	69.00±1.52c
Line 482-17-18	45.98±5.30ab	81.08±8.90c	25.10±2.27a	11.55±1.55a	15.12±1.75b	2.23±0.52ab	65.08±2.67b
Line 482-9-134	48.29±6.32b	82.83±2.61c	25.95±1.89a	16.20±2.24bc	15.65±1.31b	2.40±0.30ab	68.08±3.27bc
Line 487-24-8	46.99±6.01ab	71.53±1.86b	28.50±6.43a	14.60±2.03bc	15.77±1.48b	2.61±0.41b	61.67±1.31a
Black Rice	37.47±2.95	60.56±2.62	28.25±1.95	15.42±0.63	15.1±0.65	2.24±0.47	64.00±1.41
Mentik wangi	66.90±13.78	101.80±6.60	25.59±4.48	14.08±2.76	15.22±2.34	2.77±0.71	82.83±4.09

Note : Blue color represents the highest value and red color represents the lowest value

3.2. Gene Action and Number of Controlling Genes

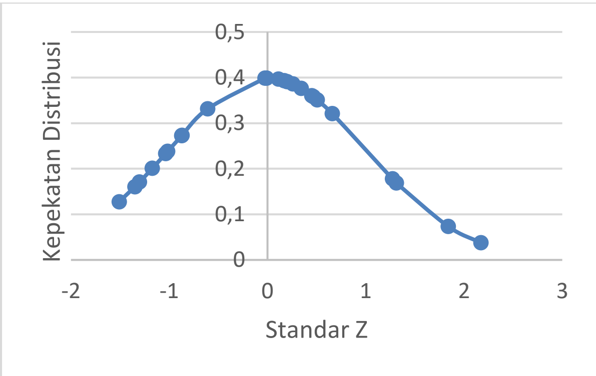
Gene action and the number of controlling genes were determined based on Skewness and Kurtosis analysis. The results of Skewness and Kurtosis analysis for dry weight, days to flowering, plant height, number of tillers, weight of 1000 grain, panicle number, and grain weight per panicle, rice-bran length, amylose content, and antioxidant activity can be seen in Table 4.

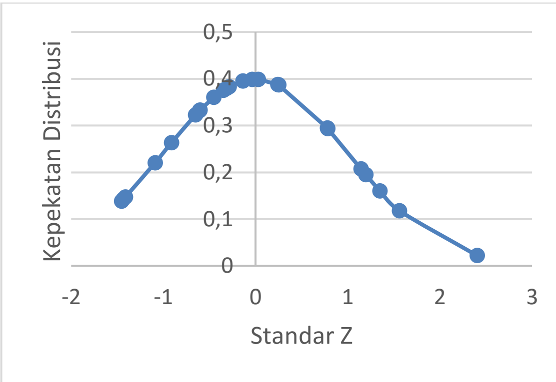
Table 4. Gene action and the number of genes controlling the characters studied

Traits	Skewness	Kurtosis	Gene action	Number of Controlling Gene
Dry weight of F6	0.358	-0.506	Additive	Many
Plant length of F6	0.558	-0.194	Additive	Many
Number of tillers F6	0.914	0.161	Additive	Many
Number of panicle F6	-0.212	-0.965	Additive	Many
Weight of 1000 grain F6	-0.560	0.077	Additive	Many
Grain weight per panicle F6	0.327	-0.694	Additive	Many
Days to flowering F6	0.246	-1.141	Additive	Many
Length of grain F7	1.192	2.213	Complementary epistasis	Many

Amylose content F7	-0.016	-0.046	Additive	Many
Antioxidant activity F7	0.809	-1.329	Additive	Many

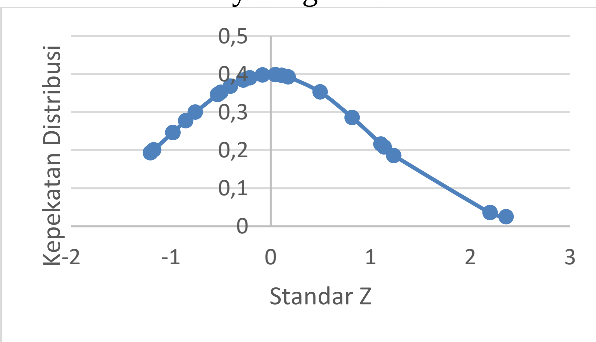
Furthermore, the normal distribution curve of the studied characters can be seen in Figure 1. All the analyzed characters show that the residual data are normally distributed.

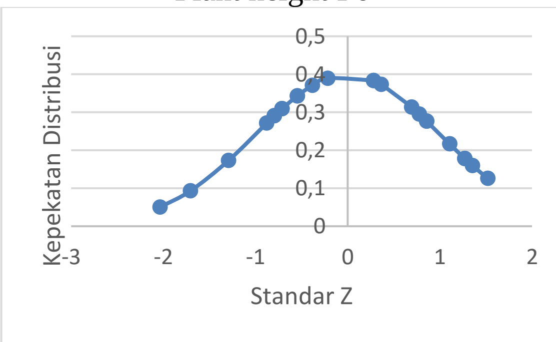




Dry weight F6

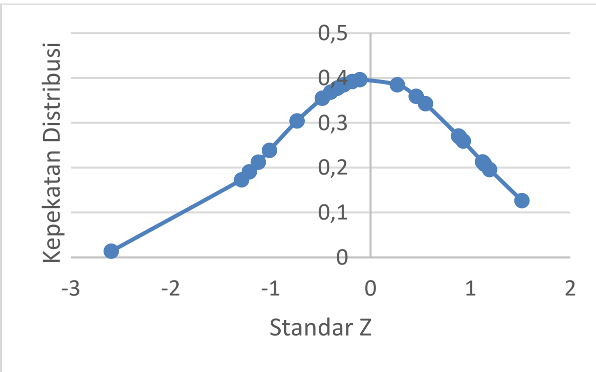
Plant height F6

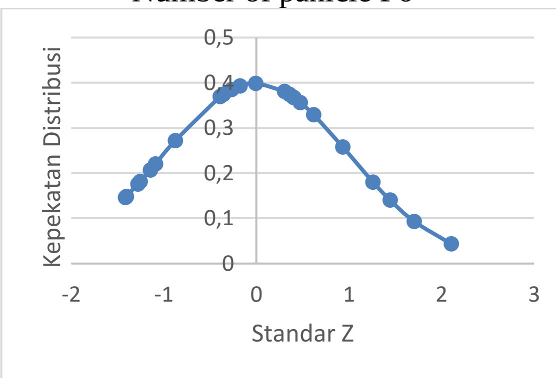




Number of tillers F6

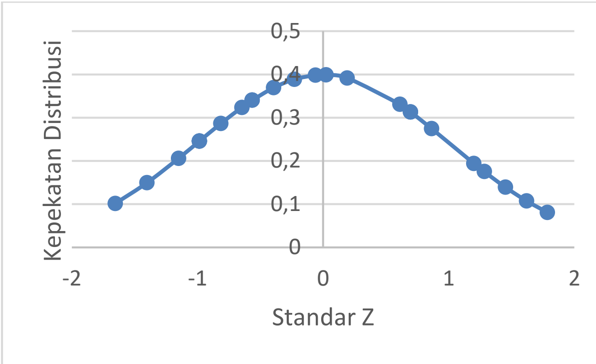
Number of panicle F6





Weight of 1000 grain F6

Grain weight per panicle F6



Days to flowering F6

Figure 1. Residual distribution curve of the data of various observed characters

3.3. Broad Sense Heritability and Phenotypic Variability

It is important to determine the value of the coefficient of diversity first to determine the cause of the differences in the appearance of the observed characters. The results of the calculation of the coefficient of diversity are presented in Table 5. All of the observed characters show a coefficient of diversity that is less than 20%.

Table 5. Coefficient of diversity of various characters studied

Traits	Mean	Max	Min	CV (%)
Dry weight	46.59	84.61	28.63	11,48
Number of tillers	29.91	54	14	15,22
Plant height	73.12	96.8	53.2	6,03
Weight of 1000 grain	14.42	22.12	7.04	9,74
Number of panicle	14.93	25	9	11,44
Grain Weight per panicle	2.22	3.64	1.1	14,63
Days to flowering	64.24	72	57	3,20

Next, the estimated value of heritability in the broad sense describes the level of similarity of the traits of the offspring to the parental varieties. The results of the analysis of heritability estimates, the coefficient of genotypic diversity and the coefficient of phenotypic diversity can be seen in Table 6. The data from the analysis show that there are various heritability estimates for each agronomic character studied. The characters of dry weight, plant height, weight of 1000 grain, and grain weight per panicle had moderate heritability estimates. Characters with high heritability estimates were number of tillers, number of panicles, and age of flowering.

The character that has the highest coefficient of genetic variation (CGV) and coefficient of phenotypic variation (CPV) is the number of tillers. Based on the data obtained, using the criteria from Hayati (2018), the character categorized as having a high CGV value is the number of tillers. Characters with high CGV values were plant height, weight of 1000 grain, number of panicles, and weight of grain per panicle. Characters with a rather low CGV were dry weight and flowering age. Meanwhile, the characters with a high CPV value were the number of tillers, the number of panicles, and the weight of seeds per panicle. Characters with a fairly high CPV value included dry weight, plant height, and weight of 1000 grain. The CGV value for the number of tillers is the same as the CPV value. The CGV value on the character of the number of panicles and the age of flowering is greater than the CPV value. In addition to these characters, the CGV has a lower value than the CPV.

Table 6. Estimated value of heritability (h2), Coefficient of Genetic Variation, and Coefficient of Phenotypic Variation of the characters studied

Traits	Mean	Phenotypic Variation	Middle Square of Error	Phenotypic Variation	h2 (%)	h2 criteria	CGV (%)	CGV criteria	CPV (%)	CPV criteria
Dry weight	46.59	7.86	28.44	36.30	21.65	Moderate	6.02	37.91% (quite low)	12.93	58.8 (quite high)
Number of tillers	29.91	22.56	20.73	43.29	52.11	High	15.88	100% (high)	21.99	100 (high)
Plant height	73.12	36.90	83.97	120.87	30.53	Moderate	8.31	52.33% (quite high)	15.04	68.4 (quite high)
Weight of 1000 grain	14.42	1.47	1.97	3.44	42.79	Moderate	8.42	53.02 (quite high)	12.87	58.53 (quite high)
Number of panicle	14.93	3.25	2.92	6.16	52.68	High	12.07	76.01 (quite high)	16.64	75.67 (high)
Grain weight per panicle	2.22	0.04	0.11	0.14	26.57	Moderate	8.80	55.42 (quite high)	17.06	77.58 (high)
Days to flowering	64.24	12.66	4.23	16.89	74.95	High	5.54	34.89 (quite low)	6.40	29.1 (quite low)

Variability is calculated based on the variance formula. The value of phenotypic variability along with the criteria for each character studied can be seen in Table 7. Based on the data below, the characters categorized as having narrow phenotypic variability are weight of 1000 grain and grain weight per panicle. Characters of dry weight, number of tillers, plant height, and number of panicles had wide phenotypic variability. Based on the calculation of the value of genetic variability, all the characters studied showed a narrow genetic variability.

Table 7. Value of phenotypic variability (PV) and genotypic variability (GV) of the traits

Traits	Mean	Variance	SD	2 X SD	PV Criteria	GV	SD GV	2 x SD	GV Criteria
Dry weight	46.59	31.21	5.59	11.17	broad	7.86	8.36	16.73	narrow
Number of tillers	29.91	38.81	6.23	12.46	broad	22.56	41.94	83.88	narrow
Plant height	73.12	62.99	7.94	15.87	broad	36.90	87.53	175.06	narrow
Weight of 1000 grain	14.42	3.29	1.81	3.63	narrow	1.47	2.97	5.95	narrow
Number of panicles	14.93	5.85	2.42	4.84	narrow	3.25	6.01	12.04	narrow
Grain weight per panicle	2.22	0.16	0.40	0.80	narrow	0.04	0.09	0.19	narrow
Days to flowering	64.24	15.76	3.97	7.94	broad	12.66	20.73	41.47	Narrow

3.4. Inter-Relationship Traits

Path analysis results with LISREL 8.2 software. The dependent variable was the weight of 1000 seeds and the weight of seeds per panicle. Another agronomic character as an influencing variable (independent variable). The path analysis diagram can be seen in Figure 2. Path analysis showed that the number of tillers had an effect on the dry weight and grain weight per panicle, but the dry weight did not directly affect the weight of 1000 seeds.

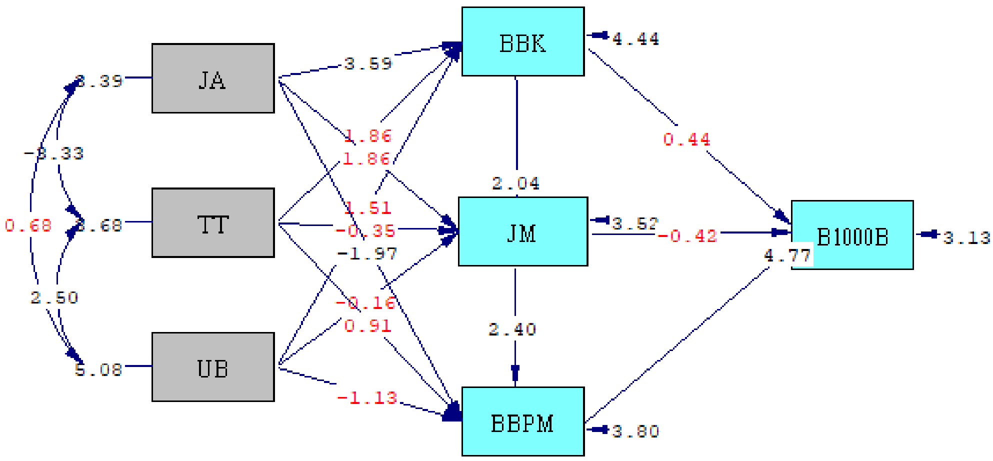


Figure 2. Path diagram of inter-relationship traits studied (P-value = 0.19579)

3.5. Morpho-Biochemical Profile of Bran Rice

The results of the analysis of the grain size of the F6 and F7 lines studied can be seen in Figure 3. This figure shows that the cumulative color of each F6 and F7 intergenerational line has similarities. There were 4 (four) with completely the same color as the black rice parents, but the other 2 (two) lines still showed the color combination between the two crossed parents.

Figure 3. Cumulative color comparison of F6 and F7 rice bran, and also the checked varieties

When viewed from the aspect of rice-bran size, based on the analysis carried out, the average rice-bran size of the 5 (five) lines studied (PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18 , 482-9-134) differed from the PHMW line 487-24-8 (Figure 4; Table 8). Meanwhile, the size of rice-bran of all lines was higher than the rice bran size of the comparison varieties used.

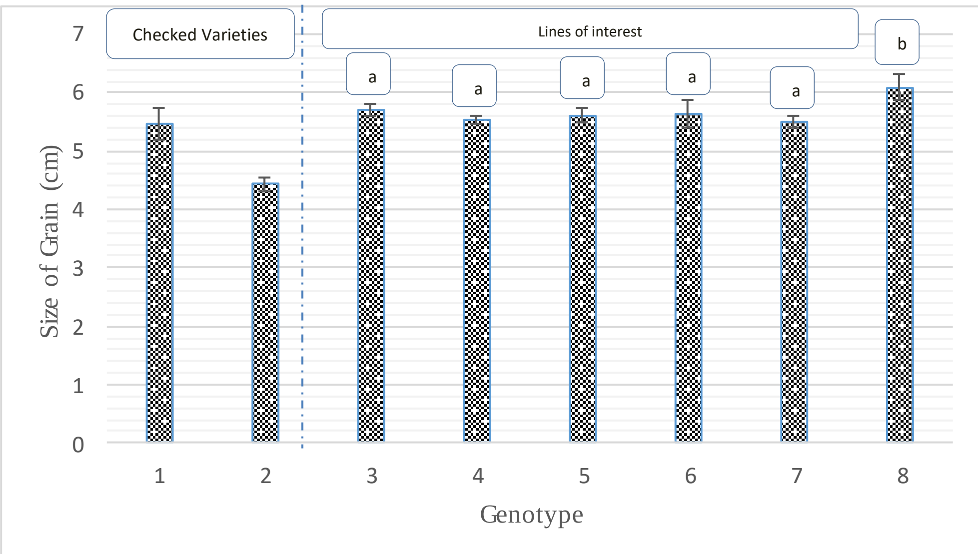


Figure 4. Size of F7 Rice Bran

The amylose profile of the rice-bran samples of the F7 lines showed that the average amylose content of each line studied was different from one another (Figure 5). Line 482-1-14 showed the lowest amylose content value 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 amylose content value compared to all lines (16.81±0.05), but it was still below the amylose content of the Black Rice comparison variety (22.37±0.04). The amylose content of all lines was in the range of amylose content of the two comparison varieties studied (Black Rice and Mentik Wangi).

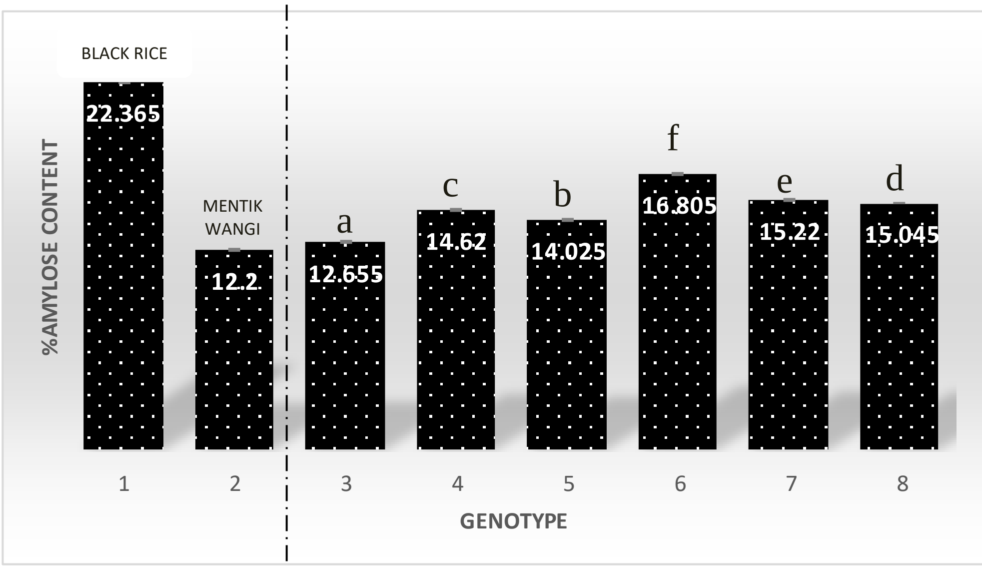


Figure 5. Amylose profile of F7 and checked varieties

For the character of antioxidant activity, independent sample T-test was conducted on 2 groups of seeds of the F6 and F7 strains. This test aims 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.

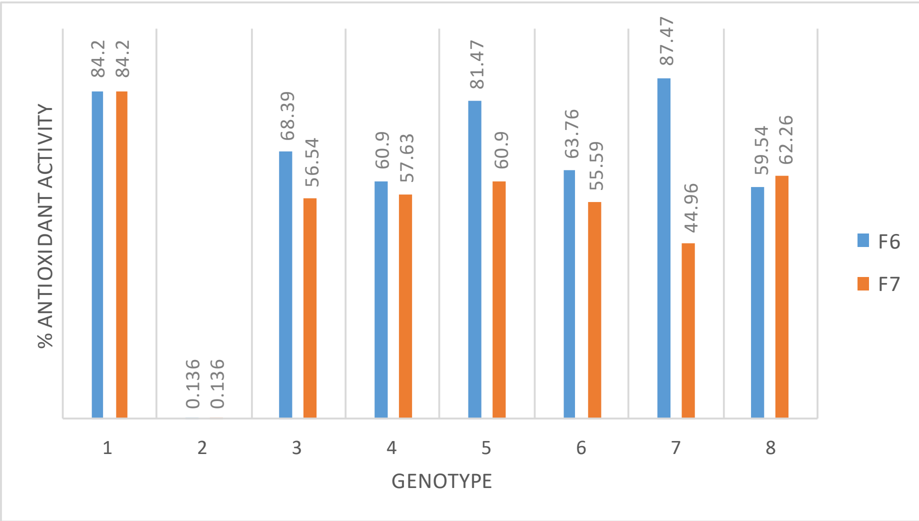


Figure 6. Antioxidant activity of F6 and F7 rice bran samples

4. Discussion

The purpose of this study was to determine genetic parameters based on agronomic data of F6 lines. Another objective of this research is to determine the agronomic character that determines the character of agricultural productivity, through path analysis. These genetic parameters can be used as the basis for determining selection criteria in plant breeding programs, so that the selection is more effective and efficient (Yudilastari et al., 2018). In addition, a morpho-biochemical profile that is suitable for the purpose of developing low amylose pigmented rice is also carried out as a basis for the selection process for potential lines.

4.1. Agronomic Traits

All the traits observed in each line showed a different effect in each line studied. The dry weight of all lines was between the dry weight of the two comparison varieties. There were two subsets of dry weight trait for the 6 (six) lines. The same analysis was applied to the trait of plant height. The plant height of all lines was between the plant heights of the two comparison varieties. There were 3 (three) subsets of plant height characters for the 6 (six) lines studied. Line 482-1-4 has differences with other strains. Each line 482-1-7, 482-1-4, and 487-24-8 had no difference in plant height. Meanwhile, lines 482-17-18 and 482-9-134 also did not have differences in plant height, because they were in one subset (group). For the number of tillers, there were lines that had a higher number of tillers than the comparison varieties, namely lines 482-1-14 and 482-1-4. The number of tillers of the other lines was the same as in the comparison variety. The grouping of panicle numbers is more varied. There are 3 (three) subsets that group the lines based on the character of the number of panicles. The number of panicles of the 482-1-14 line

was higher and statistically different compared to the checked varieties. Meanwhile, lines 482-1-4, 482-9-134, 487-24-8 and black rice varieties did not have statistical differences in the number of panicles. The number of panicles of the Mentik Wangi variety did not differ from that of the 482-17-7 strain. For weight of 1000 grain, there were only 2 (two) subsets that grouped each line. The weight of 1000 grain of the comparison varieties did not differ from those of 482-17-18, 482-9-134, and 487-24-8. The other three lines had 1000 seed weights lower than the two comparison varieties. For the character of grain weight per panicle, there are 2 (two) subsets formed from the statistical analysis. Lines 482-1-14, 482-17-7, 482-17-18, and 4829-134 had no difference in grain weight per panicle of black rice varieties. Meanwhile, the grain weight per panicle of the Mentik Wangi variety was not different from that of the 487-24-8 line, which indicated the highest value of grain weight per panicle among all other lines and the Black Rice variety. Flowering age characters resulted in 3 (three) subsets of the genotypes studied. The flowering age of the Mentik Wangi variety was different from all the studied lines, because the flowering age was the longest compared to the Black Rice lines and varieties. Flowering age of lines 482-1-14, 482-17-7, and 487-24-8 were not significantly different. For the Black Rice variety, it has the same flowering age as the 482-17-18 line.

Research by Kartahadimaja et al. (2021) reported that statistical analysis of agronomic characters 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 variations. Meanwhile, research by Kasim et al. (2020) showed that the rice plant height of the 17 rice varieties studied ranged from 95-160 cm. The plant heights 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 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 heights are preferred by farmers and researchers. While the parameters of flowering age, varied between 104-151 DAS. This indicates that these varieties have a longer flowering life than the lines used in this study. The earlier flowering age of rice varieties is preferred by farmers and researchers, because varieties with an earlier flowering age have a shorter life cycle, thus allowing a higher frequency of harvesting per year than varieties with a longer flowering age. This can have an impact on farmers' annual income. Meanwhile, the number of panicles varied between 12-24. The range of genotypic panicles in this study was in the range of the number of panicles studied by Kasim

et al. (2020). Meanwhile, the total number of grain per plant also varied, between 128 to 305. The weight of 100 seeds of the spadi variety also varied, between 1.4 – 2.56 grams.

Based on the agronomic data of the cross lines studied, characters still appear that are outside the range of character values of the two comparison varieties used. This is in accordance with the opinion of Welsh (1981) which states that the action of duplicate genes and additive genes can cause transgressive segregation. Transgressive segregation is segregation that causes offspring to have characters with measurement ranges that are below or even above their parents, so that it can provide opportunities for breeders to get the desired segregate (Nugraha & Suwarno, 2007).

4.2. Action and Number of Gene Controllers

Agronomic character is a phenotype, whose expression is determined by the interaction between genotypic factors and environmental factors. The characters studied in this study are quantitative characters, or some call them complex characters (Mackay, 2009). According to Ikram & Chardon (2010), quantitative characters are controlled by a complex genetic system, because it involves several genes (polygenic), with each gene having a minor influence, can be in the form of a small additive effect, dominant or epistatic, and sensitive to environmental conditions. (Mackay, 2009; Ikram & Chardon, 2010). Genetic complexity arises from alleles that experience segregation at multiple loci (Mackay, 2009).

The genetic variation of quantitative characters is assumed to be controlled by the collective influence (together) of the Quantitative Trait Loci (QTL), epistasis (interaction between QTL-QTL), environment, and interactions between QTL and environment (Semagn et al., 2010). Because of this complexity, many genotype characters can produce the same phenotype, and the same genotype can express different phenotypes (Mackay, 2009). Therefore, there is no clear relationship between genotype and phenotype in the expression of this trait. Semagn et al. (2010) wrote that unlike monogenic controlled characters, these polygenic characters do not follow the Mendelian inheritance pattern as in qualitative characters.

The analysis of skewness and kurtosis can provide information about the nature of gene action and the number of genes that control a character (Samak et al., 2011). The analysis of skewness and kurtosis plays an important role in determining the presence or absence of epistasis in the cross zuriat (Ramadhan et al., 2018). The results showed that most of the agronomic characters of the studied lines were controlled by additive gene action, with only seed length being controlled by the complementary gene action of epistasis. Yudilastari et al. (2018) wrote that

gene action in controlling a character can be divided into two, namely additive and non-additive (dominant gene action and epistasis). Allard (1964) and Crowder (1981) wrote that the term additive gene action is used in relation to genes affecting trait expression, where each allele contributes to the trait's phenotype. These contributions are known as additive effects, because the phenotype is determined by the sum of the effects of each allele of the gene loci involved. Changes caused by allelic substitution at each locus are not affected by alleles at other loci. The effect of additive genes from each allele can be passed from parents to offspring, because the contribution of each allele does not depend on allelic interactions (Yudilastari et al., 2018; Nugraha & Suwarno, 2007). Characters controlled by additive gene action indicate that selection can take place in the early generations because these characters can be inherited in the next generation (Ramadhan et al., 2018). On the other hand, for characters controlled by dominant or epistatic gene action, selection is carried out in the next generation (Mahalingam et al., 2011; Sulistyowati et al., 2015).

Based on this research, the characters affected by the action of additive genes, which can be passed on to the lines, were dry weight, plant height, number of tillers, number of panicles, weight of 1000 grain, and grain weight per panicle, amylose content, and antioxidant activity. On the other hand, the character of the length of the rice-bran is controlled by the action of non-additive genes, in this case it is epistasis, so that there is a tendency that it cannot be passed on to the lines. The same thing was reported by Anis et al. (2016), that there is a tendency for the character of plant height and harvest time to be influenced by the action of additive genes in their inheritance. In addition, the research of Ramadhan et al (2018) reported that there was additive gene action that affected the character of the number of primary branches in the zuriat population of IPB 3S/IPB160-F-36 rice crosses and almost all of the characters whose panicle architecture studied were controlled by additive gene action on zuriat population of IPB160-F-36/IPB 5R crosses, except for the length of the primary branches.

The results showed that all the characters studied were controlled by many controlling genes. This supports the etymology of the quantitative character itself, which is a polygenic (many gene) controlled character. Compared with the research of Ramadhan et al. (2018), the characters controlled by multiple genes in all cross populations studied were panicle length, number of primary branches, and grain density. The length of the primary branch and the number of branches/primary branches in the IPB160-F-36/IPB 5R cross zuriat were also controlled by many genes. Riyanto et al (2021) also reported that the characters of plant height,

flowering age, harvest age, panicle length, and number of grain per panicle were controlled by many genes.

4.3. Heritability, Coefficient of Variation, and Variability

Yudilastari et al. (2018) wrote that heritability is a genetic parameter that can be used to determine the role of genetics in the inheritance of a character from parents to offspring/lines. Heritability is used as the basis for estimating the relative contribution of differences in the magnitude of genetic and non-genetic factors to the total phenotypic diversity in a population (Ene et al., 2015; Konate et al., 2016). Information about heritability can be used by breeders to determine the extent to which the intensity of selection is carried out to distinguish environmental influences on the phenotype of a plant (Zehra et al., 2017). Heritability is an important concept in quantitative genetics, especially in selection in plant breeding programs (Konate et al., 2018).

The value of heritability in the broad sense of this study is in the range that varies for each character. The data from the analysis showed that there were various heritability estimates for each agronomic character studied. The characters of dry weight, plant height, weight of 1000 grain, and grain weight per panicle had moderate broad-sense heritability. Characters with high heritability estimates were number of tillers, number of panicles, and age of flowering. Adhikari et al. (2018) wrote that the low heritability value indicates that the character appears due to variations in environmental factors involved in the expression of the character, and vice versa. A high broad sense heritability indicates a high selection response for a particular character. A good character to be used as a selection character is a character that has a high heritability value (Begum et al., 2015). A small heritability value will have an impact on a small selection progress value (Mursito, 2003).

The results of this study are in line with the research of Adhikari et al. (2018), that the flowering age also has a high broad-sense heritability. Meanwhile, the character of weight of 1000 grain and grain weight per panicle, which can be used as yield component parameters, also had moderate borad-sense heritability, compared to research by Adhikari et al. (2018). Research by Ogunbayo et al. (2014) also showed results in accordance with this study. The characters of flowering age and number of tillers also had high broad-sense heritability. This suggests that these characters are primarily under genetic control, rather than the environment. Similar results were confirmed in the study of Konate et al. (2016), that flowering age, number of tillers, and

number of panicles are also categorized as characters with high broad-sense heritability. The weight of 1000 grain character also had a moderate broad-sense heritability.

The results showed that the entire value of the coefficient of phenotypic variation of all characters was higher than the coefficient of genotypic variation. Adhikari et al. (2018) wrote that this indicates the influence of the environment on these characters. The magnitude of the influence of the plant growth environment on the observed characters is explained by the level of difference value between those two parameters. Furthermore, Adhikari et al. (2018) wrote that the large difference in the values of these parameters indicates a large environmental influence on the expression of certain characters. The CPV value for all characters in this study showed a higher tendency than the GPV. This is in line with the research results of Bagati et al. (2016), that the value of the coefficient of phenotypic diversity is higher than the coefficient of genotype diversity in all the characters studied.

Based on the data above, the characters categorized as having narrow phenotypic variability were weight of 1000 grain and grain weight per panicle. Characters of dry weight, number of tillers, plant height, and number of panicles had broad phenotypic variability. Based on the calculation of the value of genotypic variability, all the characters studied showed a narrow genotypic variability. Hayati (2018) wrote that characters with narrow phenotypic variability are not effective for selection. Therefore, characters with dry weight, number of tillers, plant height, and number of panicles can be used as selection criteria. A high coefficient of variability indicated a favorable selection range for the desired character, while a low coefficient of variability indicated a need to create variability and conduct selection (Adhikari et al., 2018).

4.4. Inter-Relationship Between Characters

Path analysis showed that the number of tillers had an effect on 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. This analysis is used to determine the magnitude of the direct or indirect effect on the yiled component towards the yiled (Akhmadi et al., 2017). A good selection character is one that has a large real correlation value and a high direct or indirect effect value on the yield (Boer, 2011). Yield is a complex character and depends on a number of related characters. Therefore, crop yields usually depend on the actions and interactions of a number of important characters. Knowledge of the various characters that determine crop yields is important for examining various yield components (growth parameters) and paying more attention to yield

components that have the greatest influence on crop yields (Kinfe et al, 2015). A good selection character is one that has a large real correlation value and a high direct or indirect effect value on the results (Boer, 2011).

The results of this study have differences when compared with the research of Akhmadi et al. (2017). The results of the research Akhmadi et al. (2017) showed that characters that had a direct effect on high yields were length of panicle, weight of 1000 filled grain, number of filled grains per panicle and grain filling period. The character of the generative plant height and the total number of grain per panicle had a high negative direct effect on yield, but the indirect effect through panicle length was quite high. Several studies that have been carried out using this analysis showed the characteristics of flowering age, 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 100 grains which have a direct effect on high yield on some rice plant populations (Aryana et al., 2011; Rachmawati et al., 2014; Safitri et al. (2011).

4.5. Morpho-Biochemical Profile

The rice-bran cumulative color of the F6 and F7 lines showed that there were 4 (four) lines with completely the same color as the black rice parent, but the other 2 (two) lines still showed the color combination between the two parents. The research of Laokuldilak et al. (2011) reported that pigmented rice contains high Cyanidin-3-Glucoside (58-95%). C3G is part of the anthocyanin group of compounds. The appearance of rice-bran color in pigmented rice is thought to be related to the content of Cyanidin-3-Glucoside compound, although the relationship between these two is not clear, due to the complexity of the existing genetic system. According to research Ham et al. (2015), there was a significant positive correlation on C3G content towards the brightness and yellow color of rice bran. However, the genetic system responsible for rice-bran pigmentation and Cyanidin-3-Glucoside content is not same, but is related through a specific pathway with anthocyanin metabolism. Mackon 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.

When viewed from the aspect of rice-bran size, based on the analysis carried out, the average rice-bran size of the 5 (five) lines studied (PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18 , 482-9-134) differ from the PHMW 487-24-8. Meanwhile, the rice-bran size of all lines was higher than that of the comparison varieties. The length of the rice bran of the lines was above

the length of the rice-bran of the parent, Mentik Wangi, and had a size that was not significantly different from the length of the rice bran of the Black Rice variety. This condition could be caused by transgressive segregation of alleles responsible for the expression of rice bran length characters. Based on the analysis of Skewness and Kurtosis, it was known that this character is controlled by complementary epistasis gene action and controlled by a large number of genes (polygenic). According to IRRI (2013), the length of the rice bran in all these lines was grouped into the medium classification, except for the 482-17-7 line, which was grouped under the short criteria. The length of the rice-bran is part of determining the shape of the rice grain. The shape of rice grain is one of the determinants of the quality of rice grain. Grain quality is one of the selection parameters in plant breeding program (Kush and Cruz, 2000; Kartahadimaja et al., 2021). When compared with previous studies (Oktaviani et al., 2021), F7 rice bran size did not have a significant difference with F6 rice-bran size. All the lines studied in the F6 generation had higher rice-bran size compared to the rice-bran size of the Padi Hitam and Mentik Wangi varieties. All F6 and F7 lines were also categorized into the moderate, based on the standards of the International Rise Research Institute (2013). Based on the research of Kartahadimaja et al. (2021), rice-bran size of the 12 genotypes studied varied in length, width, and thickness. B3 line is a new line with the shortest length (8.3 mm) but the widest among all lines. Meanwhile, the other 9 lines varied between 9.04 – 10.31 mm, and were included in the long seed criteria. Other lines, such as B2, B4, B7, F2, F3, F4, H1, H4, L2, and two varieties IR 64 and Ciherang, were included in the criteria for length with a narrower width. The IR64 variety is the variety with the smallest width (2.55 mm).

Amylose content is one of the criteria that determines grain quality (IRRI, 2012). In addition, amylose content is also one of the parameters used to predict the quality of processed rice (Juliano et al., 1965; Bhattacharaya and Juliano, 1985). The amylose content of the studied lines was between the amylose content of the comparison varieties. In addition, each line showed a significant difference in amylose content. Based on the criteria of Khush & Cruz (2000), the amylose content of grain could be grouped into 5 (five) criteria, waxy rice (0% to 2%), very low amylose rice (3% to 9%), low amylose rice (10% to 19 %), medium amylose rice (20 % to 25 %), and rice with high amylose (> 25 %). The amylose content of the studied lines was at a low criteria. The results of this study are a continuation of previous studies that examined the amylose content in the F6 line. The F6 generation PHMW lines were in various criteria. The classification includes very low amylose (lines 482-1-14), low amylose (lines 487-24-8, 482-

9-134, 482-1-4, and 482-17-7) and medium amylose (lines 487-24-8, 482-9-134, 482-1-4, and 482-17-7) and medium amylose (lines 482-17-18) (Oktaviani et al., 2021).

It is important to measure the antioxidant profile of the lines to map the antioxidant profile of the lines compared to checked varieties. The results showed that there was 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 a greater reducing power than the long white rice bran extract. The main antioxidant compounds detected by High Performance Liquid Chromatography (HPLC) were oryzanol (39-63%) and phenolic acids (33-43%). In addition, Laokuldilok et al. (2011) also found that black rice had 18-26% anthocyanin content. Ferulic acid was the dominant phenolic acid in the rice samples studied. Black rice contained higher levels of gallic acid, hydroxybenzoic acid, and protocatechoic acid compared to red rice and white rice. In addition, the research of Jun et al. (2011) reported that antioxidant activity of 40% pigmented rice-bran acetone extract, at an antioxidant concentration of 500 g/mL, red rice with the highest total phenolic and total flavonoids showed highest antioxidant activity (83.6% based on the radical DPPH test). In addition, there was an interesting study by Setyaningsih et al. (2015) who studied the positive correlation between amylose content and levels of antioxidant compounds, such as melatonin and phenolics.

Conclusions

All agronomic parameters studied were thought to be controlled by many genes (polygenic) and additive gene action, except for rice-bran length. The data from the analysis showed that there were various broad-sense heritability for each agronomic character. The characters of dry weight, plant height, weight of 1000 grain, and grain weight per panicle had moderate broad-sense heritability. Characters with high broad-sense heritability were number of tillers, number of panicles, and days to flowering. Based on the results of the study, all the characters observed in each line showed a different effect in each line studied. The value of broad-sense heritability this study varied for each character. Path analysis showed that the number of tillers had an effect on 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 seeds of the F6 and F7 lines showed that there were 4 (four) lines with completely the same color as the black rice, but the

other 2 (two) lines still showed the color combination between the two checked varieties. When viewed from the aspect of rice-bran size, the rice-bran size mean of the 5 (five) lines studied (PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18 , 482-9-134) differ from the PHMW 487-24-8. Meanwhile, the rice-bran size of all studied lines was higher than the rice-bran size of the comparison varieties. The amylose content of lines was between the amylose content of the comparison 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 sticky rice traits (low amylose content) with multi-location and multi-season field trial, selection based on molecular markers related to genes associated with low amylose content and high antioxidant content, as well as organoleptic testing of processed rice from these lines.

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Genetic Parameters and Inter-relationship Between Agronomic Traits of F6 Lines, and Rice Bran Morpho-Biochemical Profile of F7 Lines Derived from a Crossing of Black Rice and Mentik Wangi

Eka Oktaviani[1*] and Suprayogi[1]
[1]Department of Agrotechnology, Faculty of Agriculture, Universitas Jenderal Soedirman
Jln. Dr Soeparno 61 Karangwangkal Purwokerto Utara Banyumas 53123 Jawa Tengah, Indonesia

***Corresponding author** : oktaviani@unsoed.ac.id

ABSTRACT

The development of sticky pigmented rice with high antioxidant content as superior varieties can be carried out by crossing the Black Rice with Mentik Wangi varieties. Rice breeding program to obtain rice lines with low amylose content and high antioxidants has reached the F6 lines. The purpose of this study was to determine genetic parameters and the relationship between agronomic traits of the F6 lines. Another objective of this study was to determine the morpho-biochemical profile of F7 grain, including length, cumulative color, amylose content and antioxidant activity. The results showed that all agronomic parameters had a coefficient of variance less than 20%, which indicates that phenotypic differences were caused by genotypic factors, rather than environmental ones. All the values of the Genetic Diversity Coefficient are in the low range, as well as the values of the Phenotypic Diversity Coefficient. The broad sense heritability of dry weight character was in the low range. The characters of plant height, weight of 1000 grain and weight of grain per panicle were categorized as having moderate broad sense heritability, while the characters of number of tillers, number of panicles and age of flowering had high broad sense heritability. The phenotypic variability of weight of 1000 grain and age of flowering were included in the narrow criteria, while the other characters were broad one. Path analysis showed that the number of tillers had an effect on the dry weight and grain weight per panicle, but the dry weight did not directly affect the weight of 1000 grain. Directly, the weight of 1000 grain was only significantly affected by the grain weight per panicle trait. In terms of grain length profile and amylose content, the F7 lines had difference in grain length (2 subsets) and amylose content (6 subsets) traits. The cumulative grain color of the PHMW482-17-7 and 482-17-18 lines showed the color combination of the two parents. Based on the T test conducted on F6 and F7 grain samples, 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.

Keywords: antioxidant, black rice, mentik wangi, morpho-biochemical, sticky

1. Introduction

Black rice is one type of pigmented rice, in addition to red rice and brown rice. This kind of rice is often consumed as functional food, not as the main food ingredient (Purwanto et al., 2019). This is due to the various nutritional content of this type of rice. Black rice contains various micro and macronutrients that are important for human health (Apridamayanti et al., 2017). Protein, fat, fiber, vitamins, and minerals important for the human body are the various nutrients in this rice (Nurhidajah, 2018; Apridamayanti et al., 2017; Kristamtini et al., 2012). Important nutrients reported in black rice are vitamin B, vitamin E, Fe ion, thiamin, magnesium, niacin, phosphorus, dietary fiber (Kristamtini et al., 2021; Murali & Kumar, 2020), Zn ion, and Mn ion (Kristamtini et al. et al., 2021; Murali & Kumar, 2020). Murali & Kumar (2020) also reported that black rice is free from gluten and cholesterol, low in sugar, salt and fat.

Black rice, is one of the pigmented rice classified on the basis of the color of the pericarp, aleurone, and endosperm of the rice grains (Kristamtini et al., 2012). One of the important compounds that contribute to black rice aleurone color is anthocyanin (Yoshimura et al., 2012; Palupi et al., 2020). Anthocyanins are responsible for the appearance of blue, purple, red, and orange colors in many fruits and vegetables (Miguel, 2011). Anthocyanins have high antioxidant activity and play an important role in human health (Prastiwi & 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 & Kumar, 2020; Tena et al., 2020), support health eye, anti-microbial, and prevention of neuro-degenerative diseases (Tena et al., 2020).

Anthocyanins, one of the most important types of plant flavonoid compounds, are pigments with a flavylium cation structure (AH+) that act as acids (Tena et al., 2020). This structure is directly related to antioxidant activity, because it is able to prevent or inhibit oxidation reactions by scavenging free radicals and reduce levels of oxidative stress in cells (Tena et al., 2020). Based on basic chemical reactions, anthocyanins act as donors of H atoms or as single electron transferors (Tena et al., 2020).

Although black rice is known as a functional food with the above benefits, consumer acceptance of the texture of rice processed from this rice is low, due to the non-stickiness texture of the cooked rice (Adi et al, 2020). The non-tender/non-sticky/non-glutinous rice has a dry, hard, and separate texture, even though it has been through the cooking process. Based on the previous researches, rice texture is determined by amylose amylopectin ratio (Cameron & Wang, 2005; Adi et al., 2020; Li et al., 2016a), post-harvest processing, and cooking method (Li et al., 2016b). In addition, Cameron & Wang (2005) also found that the texture/stickiness/hardness of rice was associated with protein and crude lipid content. The higher the amylose content of rice, the more tender the texture of the rice will be, and vice versa (Khumar & Khush, 1986; Bhattacharaya et al., 1999; Luna et al, 2015; Panesar & Kaur, 2016). Crude protein and lipid content were negatively correlated with the hardness of pasta flour and processed rice, but positively correlated with the level of rice stickiness (Cameron & Wang, 2005).

A rice plant breeding program to produce variety of rice with a quality texture of soft/sticky/fluffy processed rice has been carried out in Indonesia. Indonesia, through the Ministry of Agriculture, has released a rice variety with an amylose content of 19.6% in 2019, the result of a cross between Black Sticky Rice and Pandan Wangi cv Cianjur. In addition, research for the development of fluffier/stickier pigmented rice has also been carried out by researchers (Kim et al., 2010; Zhang et al., 2018; Roy & Shil, 2020). The development of pigmented rice with a fluffier/stickier rice texture in Indonesia is expected to increase the source of rice germplasm with superior characters in Indonesia.

The development of superior varieties of pigmented rice that is stickier and has a high antioxidant content can be done by crossing the Black Rice variety with Mentik Wangi. Rice breeding research to obtain rice lines with high antioxidants and a fluffier texture of rice has reached the F6 line. The purpose of this study was to determine various genetic parameters and the relationship between characters based on the agronomic character of the F6 line. Another objective of this study was to determine the morpho-biochemical profile of F7 rice-bran, including length, cumulative color, amylose content and antioxidant activity.

2. Matrials and Methods

The genetic material used consisted of 6 (six) potential F6 lines derived from a crossing of Black Rice and Mentik Wangi varieties and 2 (two) comparison varieties, i.e Black Rice cv Cilacap and Mentik Wangi. These six lines are the results of 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, 482-17-18. Black rice and Mentik Wangi were used as comparison varieties. The F6 lines and comparison varieties were planted until harvest to obtain the F7 line. Grain of the F7 lines obtained were analyzed for bran size, cumulative bran color, amylose content, and antioxidant activity. Meanwhile, the grain of the F6 lines were also subjected to the same analysis.

2.1. Field Trial of F6 Lines

Field testing of the F6 line was carried out in the Experimental Farm greenhouse, Faculty of Agriculture, Universitas Jenderal Soedirman. The study was conducted from April to October 2020. The design used was a completely randomized block design, with 3 (three) blocks and 5 (five) replications in each block. The soil used is ultisol. The lines were planted in polybags, with a total of 115 polybags. Each polybag is filled with one individual rice plant. The fertilizer used consists of NPK fertilizer and manure. The planting media used consisted of ultisol soil, roasted rice husks, and manure. Weeds, pests, and diseases are controlled by conventional means and the use of chemical pesticides. The agronomic parameters observed included flowering age, plant height, dry weight, number of tillers, weight of 1000 grain, number of panicles, and weight of grain per panicle.

2.2. Morphological Characterization of F7 Rice Bran

Rice-bran morphology observed in the form of rice-bran length, rice-bran shape, and rice-bran cumulative color of each line. The determination of grain size classification was determined based on parameters from the International Rice Research Institute (IRRI) (2012). The basis used is the length and the shape of the rice-bran. Cumulative rice-bran color is used to determine the segregation phenomenon.

2.3. Amylose Quantification

The amylose content of the seeds of the F7 line 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 in the sample was then measured based on the linear regression equation in the standard curve.

2.4. Determination of Antioxidant Activity

Measurement of antioxidant activity was carried out on rice-bran of the F6 and F7 lines, using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method (Blois, 1958).

2.5. Data Analysis

Data on agronomic parameters of the F6 line, F7 rice-bran length, and F7 amylose content of the six lines were analyzed using SAS 9.4 software. If the results of the analysis of variance indicate that there is an influence of the planted genotype on the various agronomic parameters studied, then a different test is carried out with the Least Significant Difference Test (BNT) at the 95% confidence level ($\alpha = 0.05$). The results of this analysis are also used as the basis for determining the value of genetic diversity (Coefficient of Variance/CV), phenotypic diversity, and heritability values of broad meaning, as well as phenotypic variability. The difference in antioxidant activity of the samples of the F7 line and the F6 line was identified by the T test. Path analysis was performed using the LISREL 8.2 software. This analysis was used to determine the direct and indirect factors determining the productivity character, namely weight of 1000 grain and weight of grain per panicle.

The estimation of gene action and number of gene control were analyzed based on Skewness and Kurtosis values, respectively, for each trait observed in the F6 generation. Skewness is the slope of the graph. Skewness shows epistasis effected expression of a trait (Lestari et al., 2015). If Skewness equals to zero, it means there is no epistasis. Skewness > 0 means there is a complementary epistasis gene action, and Skewness < 0 means there is a duplicate epistasis gene action. Kurtosis describes the shape of the distribution curve and shows several genes controlling a trait (Herawati et al., 2019). Kurtosis is the value of the taperedness of the graph. When Kurtosis > 3, has a positive value, it shows the leptokurtic graph indicates a few gene controls trait. If Kurtosis < 3, has a negative value, it shows a platykurtic diagram and its trait is controlled by many genes. Interpretation Skewness and Kurtosis value refer to scheme in Jambormias research (Jambormias, 2014). The value of the skewness ratio and the value of the kurtosis ratio can also be used to determine whether the data distribution is normal or not. If the value of the skewness ratio and the value of the kurtosis ratio are between -2 and 2, the data distribution is normal. The value of the skewness and kurtosis ratio can be obtained by dividing the skewness and kurtosis values by their respective standard errors.

The data obtained from the observations and analysis of variance (F test) were used as the basis for calculating the coefficient of variance (CV), coefficient of genetic diversity, coefficient of phenotypic diversity, and heritability values of broad meaning, as well as phenotypic variability. Based on the analysis of variance, the genotypic variance (σ^2g), phenotypic variance (σ^2p), coefficient of genotypic diversity (KKG) and heritability (h^2), can be estimated using the following formula (Singh & Chaudary, 1977).

$$KTe = \sigma^2 e$$

$$\sigma^2 g = KTg - KTeb$$

$$\sigma^2 p = \sigma^2 g + \sigma^2 e$$

Note :

σ^2g = genotip of variance

σ^2p = phenotip of variance

σ^2e = error of variance

b = replication

KTg = kuadrat tengah genotip

KTe = kuadrat tengah error

The value of the coefficient of genetic diversity can be determined by the following formula:

$$CGV = \sqrt{\sigma^2 g}\bar{X} \text{ x } 100\%$$

Note :

CGV= Coefficient of Genetic Variance

$\sigma^2 g$ = genotip of variance

$\bar{X}$ = mean of population

According to Moedjiono & Mejaya (1994) in Handayani (2018) the criteria for the coefficient of genetic diversity are determined based on absolute and relative values. The criteria for coefficients of genetic and phenotypic diversity are presented in Table 1.

Coefficient of Genetic Variance	Criteria
0 - 25% of the highest	Low
25 - 50% of the highest	Quite low
50 - 75% of the highest	Quite high
75% - 100% of the highest	High

The estimated value of broad sense heritability is determined by the following formula:

$$h^2 = \sigma^2 g \sigma^2 p \text{ x } 100\%$$

Note:

h^2 = broad sense heritability

$\sigma^2 g$ = variance of genotype

$\sigma^2 p$ = Variance of phenotype

The determination of heritability criteria follows the following criteria (McWhirter, 1979) (Table 2):

Value of broad sense heritability	Criteria
$h^2 \leq 20\%$	Low
$20\% < h^2 < 50\%$	Medium
$h^2 > 50\%$	High

Furthermore, the value of phenotypic variability is calculated by the formula:

$$\text{Variability of Phenotipic} = 2 \text{ x SD}$$

Note : SD = Standar Deviation

Determination of the criteria for phenotypic variability is calculated by comparing the value between the phenotypic variance with a value of 2 (two) times the standard deviation of the data. If the value of variability is greater than the value of SD, then it is categorized into broad criteria. And vice versa.

3. Results

3.1. Agronomic Traits of The Lines

The results of the Least Significant Difference (LSD) test to determine the difference in the response of each studied line based on agronomic characters can be seen in Table 3. Black rice and Mentik Wangi varieties were used as comparison varieties, which were excluded from the LSD test.

Table 3. Differences agronomic traits of six lines studied

Genotype	Dry weight	Plant length	Number of tillers	Number of panicles	Weight of 1000 grain	Grain weight per panicle	Days to flowering
Line 482-1-14	46.34±3.70 ab	62.43±1.38a	36.40±6.01b	16.75±1.38c	13.67±0.39ab	2.15±0.28ab	60.92±1.80a
Line 482-17-7	40.03±1.20a	70.94±3.29b	26.80±3.13a	13.95±1.31ab	12.10±1.85a	1.97±0.36ab	60.67±3.10a
Line 482-1-4	51.91±5.53b	69.93±3.97b	36.70±5.43b	16.50±2.27bc	14.21±1.63ab	1.95±0.33a	69.00±1.52c
Line 482-17-18	45.98±5.30ab	81.08±8.90c	25.10±2.27a	11.55±1.55a	15.12±1.75b	2.23±0.52ab	65.08±2.67b
Line 482-9-134	48.29±6.32b	82.83±2.61c	25.95±1.89a	16.20±2.24bc	15.65±1.31b	2.40±0.30ab	68.08±3.27bc
Line 487-24-8	46.99±6.01ab	71.53±1.86b	28.50±6.43a	14.60±2.03bc	15.77±1.48b	2.61±0.41b	61.67±1.31a
Black Rice	37.47±2.95	60.56±2.62	28.25±1.95	15.42±0.63	15.1±0.65	2.24±0.47	64.00±1.41
Mentik wangi	66.90±13.78	101.80±6.60	25.59±4.48	14.08±2.76	15.22±2.34	2.77±0.71	82.83±4.09

Note : Blue color represents the highest value and red color represents the lowest value

3.2. Gene Action and Number of Controlling Genes

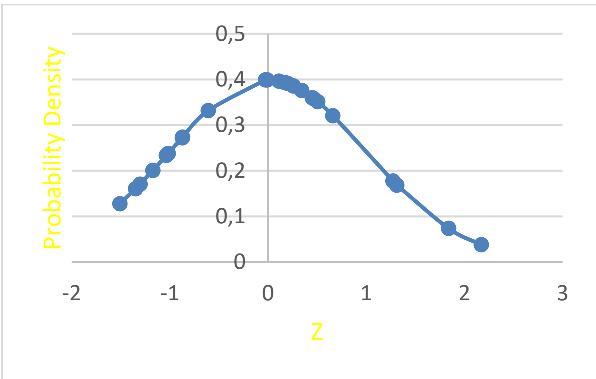
Gene action and the number of controlling genes were determined based on Skewness and Kurtosis analysis. The results of Skewness and Kurtosis analysis for dry weight, days to flowering, plant height, number of tillers, weight of 1000 grain, panicle number, and grain weight per panicle, rice-bran length, amylose content, and antioxidant activity can be seen in Table 4.

Table 4. Gene action and the number of genes controlling the characters studied

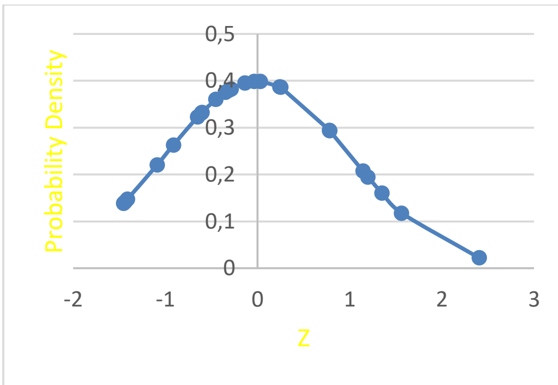
Traits	Skewness	Kurtosis	Gene action	Number of Controlling Gene
Dry weight of F6	0.358	-0.506	Additive	Many
Plant length of F6	0.558	-0.194	Additive	Many
Number of tillers F6	0.914	0.161	Additive	Many
Number of panicle F6	-0.212	-0.965	Additive	Many

Weight of 1000 grain F6	-0.560	0.077	Additive	Many
Grain weight per panicle F6	0.327	-0.694	Additive	Many
Days to flowering F6	0.246	-1.141	Additive	Many
Length of grain F7	1.192	2.213	Complementary epistasis	Many
Amylose content F7	-0.016	-0.046	Additive	Many
Antioxidant activity F7	0.809	-1.329	Additive	Many

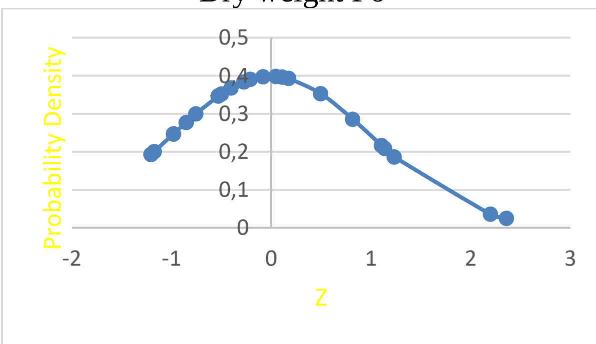
Furthermore, the normal distribution curve of the studied characters can be seen in Figure 1. All the analyzed characters show that the residual data are normally distributed.



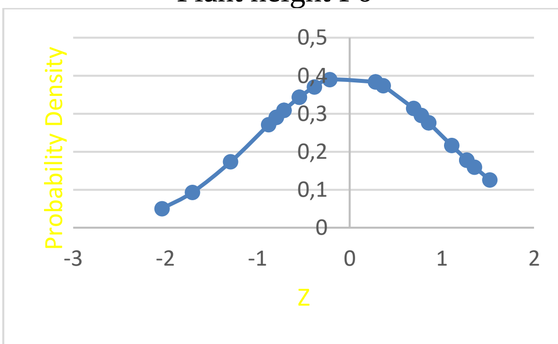
Dry weight F6



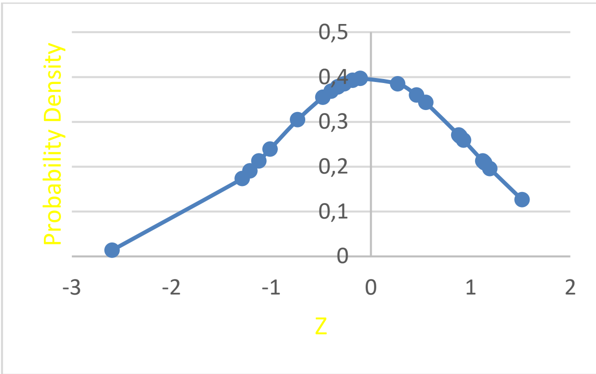
Plant height F6



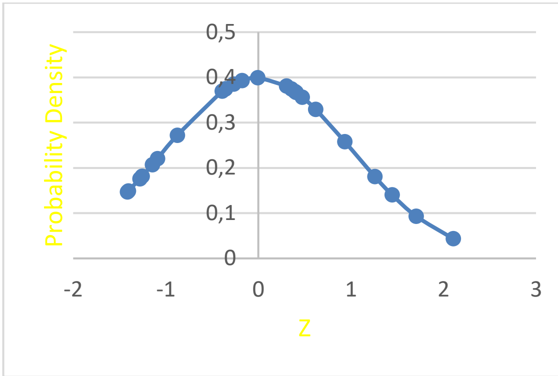
Number of tillers F6



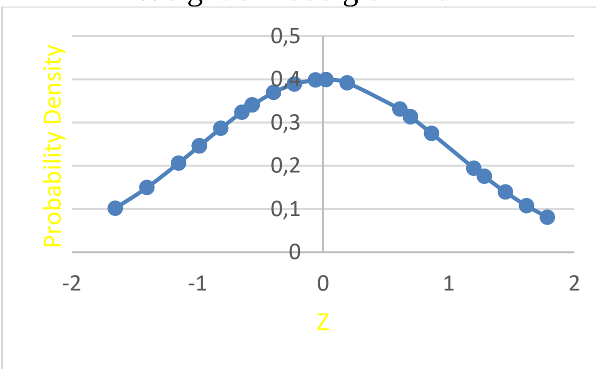
Number of panicle F6



Weight of 1000 grain F6



Grain weight per panicle F6



Days to flowering F6

Figure 1. Residual distribution curve of the data of various observed characters

3.3. Broad Sense Heritability and Phenotypic Variability

It is important to determine the value of the coefficient of diversity first to determine the cause of the differences in the appearance of the observed characters. The results of the calculation of the coefficient of diversity are presented in Table 5. All of the observed characters show a coefficient of diversity that is less than 20%.

Table 5. Coefficient of diversity of various characters studied

Traits	Mean	Max	Min	CV (%)
Dry weight	46.59	84.61	28.63	11,48
Number of tillers	29.91	54	14	15,22
Plant height	73.12	96.8	53.2	6,03
Weight of 1000 grain	14.42	22.12	7.04	9,74
Number of panicle	14.93	25	9	11,44
Grain Weight per panicle	2.22	3.64	1.1	14,63
Days to flowering	64.24	72	57	3,20

Next, the estimated value of heritability in the broad sense describes the level of similarity of the traits of the offspring to the parental varieties. The results of the analysis of heritability estimates, the coefficient of genotypic diversity and the coefficient of phenotypic diversity can be seen in Table 6. The data from the analysis show that there are various heritability estimates for each agronomic character studied. The characters of dry weight, plant height, weight of 1000 grain, and grain weight per panicle had moderate heritability estimates. Characters with high heritability estimates were number of tillers, number of panicles, and age of flowering.

The character that has the highest coefficient of genetic variation (CGV) and coefficient of phenotypic variation (CPV) is the number of tillers. Based on the data obtained, using the criteria from Hayati (2018), the character categorized as having a high CGV value is the number of tillers. Characters with high CGV values were plant height, weight of 1000 grain, number of panicles, and weight of grain per panicle. Characters with a rather low CGV were dry weight and flowering age. Meanwhile, the characters with a high CPV value were the number of tillers, the number of panicles, and the weight of seeds per panicle. Characters with a fairly high CPV value included dry weight, plant height, and weight of 1000 grain. The CGV value for the number of tillers is the same as the CPV value. The CGV value on the character of the number of panicles and the age of flowering is greater than the CPV value. In addition to these characters, the CGV has a lower value than the CPV.

Table 6. Estimated value of heritability (h2), Coefficient of Genetic Variation, and Coefficient of Phenotypic Variation of the characters studied

Traits	Mean	Phenotypic Variation	Middle Square of Error	Phenotypic Variation	h2 (%)	h2 criteria	CGV (%)	CGV criteria	CPV (%)	CPV criteria
Dry weight	46.59	7.86	28.44	36.30	21.65	Moderate	6.02	37.91% (quite low)	12.93	58.8 (quite high)
Number of tillers	29.91	22.56	20.73	43.29	52.11	High	15.88	100% (high)	21.99	100 (high)
Plant height	73.12	36.90	83.97	120.87	30.53	Moderate	8.31	52.33% (quite high)	15.04	68.4 (quite high)
Weight of 1000 grain	14.42	1.47	1.97	3.44	42.79	Moderate	8.42	53.02 (quite high)	12.87	58.53 (quite high)
Number of panicle	14.93	3.25	2.92	6.16	52.68	High	12.07	76.01 (quite high)	16.64	75.67 (high)
Grain weight per panicle	2.22	0.04	0.11	0.14	26.57	Moderate	8.80	55.42 (quite high)	17.06	77.58 (high)
Days to flowering	64.24	12.66	4.23	16.89	74.95	High	5.54	34.89 (quite low)	6.40	29.1 (quite low)

Variability is calculated based on the variance formula. The value of phenotypic variability along with the criteria for each character studied can be seen in Table 7. Based on the data below, the characters categorized as having narrow phenotypic variability are weight of 1000 grain and grain weight per panicle. Characters of dry weight, number of tillers, plant height, and number of panicles had wide phenotypic variability. Based on the calculation of the value of genetic variability, all the characters studied showed a narrow genetic variability.

Table 7. Value of phenotypic variability (PV) and genotypic variability (GV) of the traits

Traits	Mean	Variance	SD	2 X SD	PV Criteria	GV	SD GV	2 x SD	GV Criteria
Dry weight	46.59	31.21	5.59	11.17	broad	7.86	8.36	16.73	narrow
Number of tillers	29.91	38.81	6.23	12.46	broad	22.56	41.94	83.88	narrow
Plant height	73.12	62.99	7.94	15.87	broad	36.90	87.53	175.06	narrow
Weight of 1000 grain	14.42	3.29	1.81	3.63	narrow	1.47	2.97	5.95	narrow
Number of panicles	14.93	5.85	2.42	4.84	narrow	3.25	6.01	12.04	narrow
Grain weight per panicle	2.22	0.16	0.40	0.80	narrow	0.04	0.09	0.19	narrow
Days to flowering	64.24	15.76	3.97	7.94	broad	12.66	20.73	41.47	Narrow

3.4. Inter-Relationship Traits

Path analysis results with LISREL 8.2 software. The dependent variable was the weight of 1000 seeds and the weight of seeds per panicle. Another agronomic character as an influencing variable (independent variable). The path analysis diagram can be seen in Figure 2. Path analysis showed that the number of tillers had an effect on the dry weight and grain weight per panicle, but the dry weight did not directly affect the weight of 1000 seeds.

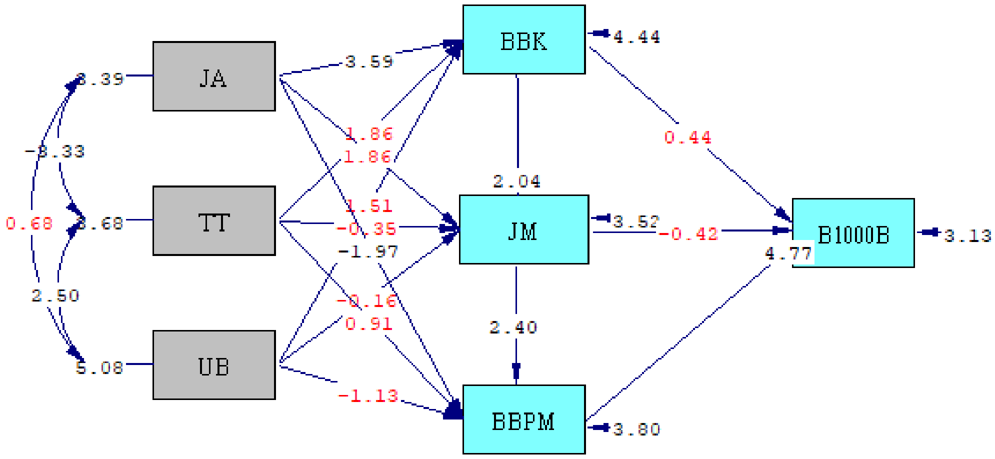


Figure 2. Path diagram of inter-relationship traits studied (P-value = 0.19579)

3.5. Morpho-Biochemical Profile of Bran Rice

The results of the analysis of the grain size of the F6 and F7 lines studied can be seen in Figure 3. This figure shows that the cumulative color of each F6 and F7 intergenerational line has similarities. There were 4 (four) with completely the same color as the black rice parents, but the other 2 (two) lines still showed the color combination between the two crossed parents.

Figure 3. Cumulative color comparison of F6 and F7 rice bran, and also the checked varieties

When viewed from the aspect of rice-bran size, based on the analysis carried out, the average rice-bran size of the 5 (five) lines studied (PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18 , 482-9-134) differed from the PHMW line 487-24-8 (Figure 4). Meanwhile, the size of rice-bran of all lines was higher than the rice bran size of the comparison varieties used.

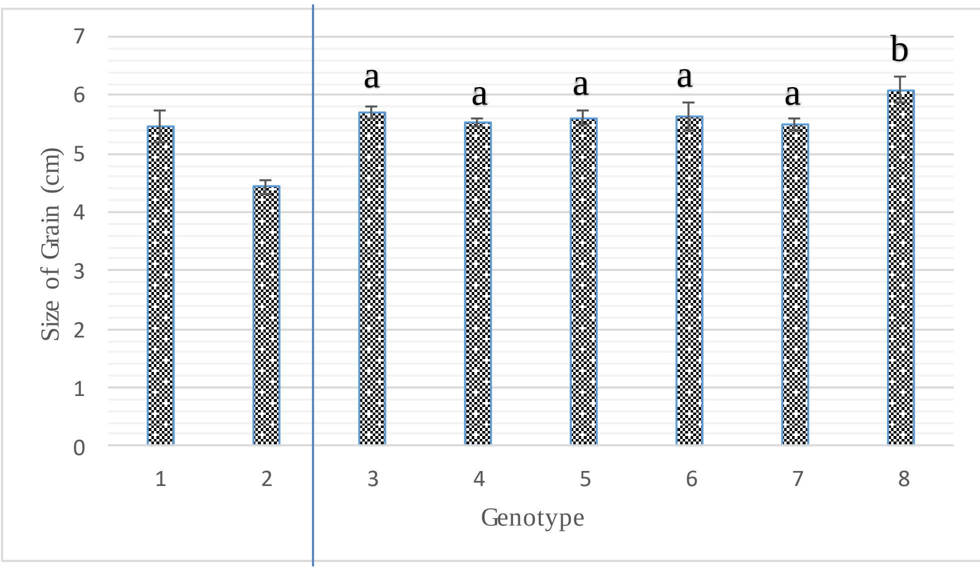


Figure 4. Size of F7 Rice Bran (Note : No. 1 and 2 are Black Rice and Mentik Wangi, respectively; while no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18 , 482-9-134, 487-24-8 lines, respectively)

The amylose profile of the rice-bran samples of the F7 lines showed that the average amylose content of each line studied was different from one another (Figure 5). Line 482-1-14 showed the lowest amylose content value 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 amylose content value compared to all lines (16.81±0.05), but it was still below the amylose content of the Black Rice comparison variety (22.37±0.04). The amylose content of all lines was in the range of amylose content of the two comparison varieties studied (Black Rice and Mentik Wangi).

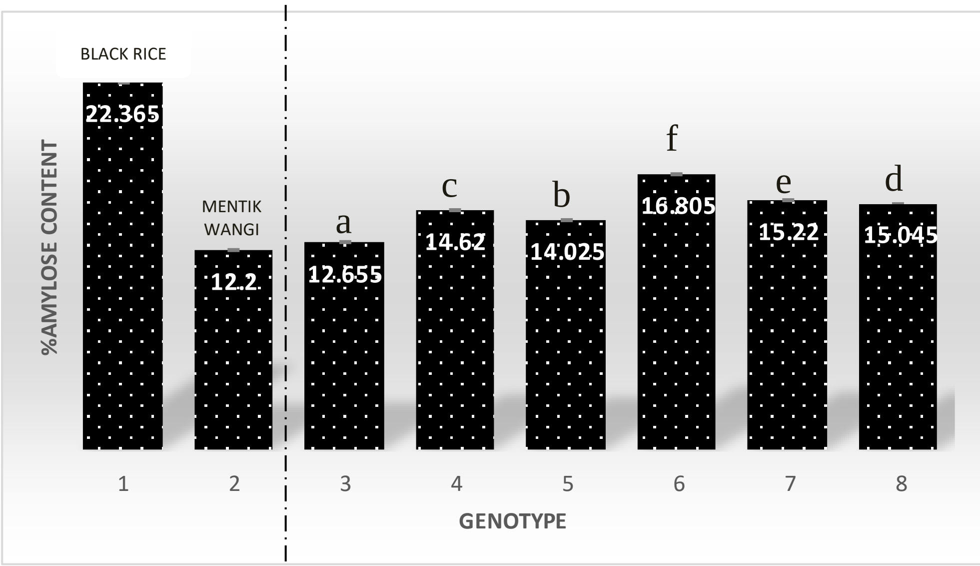


Figure 5. Amylose profile of F7 and checked varieties

For the character of antioxidant activity, independent sample T-test was conducted on 2 groups of seeds of the F6 and F7 lines. This test aims 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.

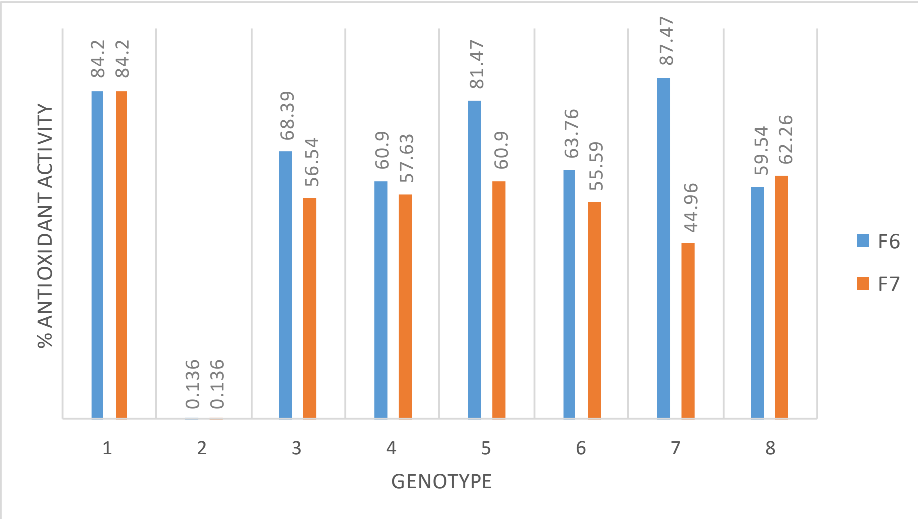


Figure 6. Antioxidant activity of F6 and F7 rice bran samples

4. Discussion

The purpose of this study was to determine genetic parameters based on agronomic data of F6 lines. Another objective of this research is to determine the agronomic character that determines the character of agricultural productivity, through path analysis. These genetic parameters can be used as the basis for determining selection criteria in plant breeding programs, so that the selection is more effective and efficient (Yudilastari et al., 2018). In addition, a morpho-biochemical profile that is suitable for the purpose of developing low amylose pigmented rice is also carried out as a basis for the selection process for potential lines.

4.1. Agronomic Traits

All the traits observed in each line showed a different effect in each line studied. The dry weight of all lines was between the dry weight of the two comparison varieties. There were two subsets of dry weight trait for the 6 (six) lines. The same analysis was applied to the trait of plant height.

The plant height of all lines was between the plant heights of the two comparison varieties. There were 3 (three) subsets of plant height characters for the 6 (six) lines studied. Line 482-1-4 has differences with other lines. Each line 482-1-7, 482-1-4, and 487-24-8 had no difference in plant height. Meanwhile, lines 482-17-18 and 482-9-134 also did not have differences in plant height, because they were in one subset (group). For the number of tillers, there were lines that had a higher number of tillers than the comparison varieties, namely lines 482-1-14 and 482-1-4. The number of tillers of the other lines was the same as in the comparison variety. The grouping of panicle numbers is more varied. There are 3 (three) subsets that group the lines based on the character of the number of panicles. The number of panicles of the 482-1-14 line was higher and statistically different compared to the checked varieties. Meanwhile, lines 482-1-4, 482-9-134, 487-24-8 and black rice varieties did not have statistical differences in the number of panicles. The number of panicles of the Mentik Wangi variety did not differ from that of the 482-17-7 line. For weight of 1000 grain, there were only 2 (two) subsets that grouped each line. The weight of 1000 grain of the comparison varieties did not differ from those of 482-17-18, 482-9-134, and 487-24-8. The other three lines had 1000 seed weights lower than the two comparison varieties. For the character of grain weight per panicle, there are 2 (two) subsets formed from the statistical analysis. Lines 482-1-14, 482-17-7, 482-17-18, and 4829-134 had no difference in grain weight per panicle of black rice varieties. Meanwhile, the grain weight per panicle of the Mentik Wangi variety was not different from that of the 487-24-8 line, which indicated the highest value of grain weight per panicle among all other lines and the Black Rice variety. Flowering age characters resulted in 3 (three) subsets of the genotypes studied. The flowering age of the Mentik Wangi variety was different from all the studied lines, because the flowering age was the longest compared to the Black Rice lines and varieties. Flowering age of lines 482-1-14, 482-17-7, and 487-24-8 were not significantly different. For the Black Rice variety, it has the same flowering age as the 482-17-18 line.

Research by Kartahadimaja et al. (2021) reported that statistical analysis of agronomic characters 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 variations. Meanwhile, research by Kasim et al. (2020) showed that the rice plant height of the 17 rice varieties studied ranged from 95-160 cm. The plant heights 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 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 heights are preferred by farmers and researchers. While the parameters of flowering age, varied between 104-151 DAS. This indicates that these varieties have a longer flowering life than the lines used in this study. The earlier flowering age of rice varieties is preferred by farmers and researchers, because varieties with an earlier flowering age have a shorter life cycle, thus allowing a higher frequency of harvesting per year than varieties with a longer flowering age. This can have an impact on farmers' annual income. Meanwhile, the number of panicles varied between 12-24. The range of genotypic panicles in this study was in the range of the number of panicles studied by Kasim et al. (2020). Meanwhile, the total number of grain per plant also varied, between 128 to 305. The weight of 100 seeds of the spadi variety also varied, between 1.4 – 2.56 grams.

Based on the agronomic data of the cross lines studied, characters still appear that are outside the range of character values of the two comparison varieties used. This is in accordance with the opinion of Welsh (1981) which states that the action of duplicate genes and additive genes can cause transgressive segregation. Transgressive segregation is segregation that causes offspring to have characters with measurement ranges that are below or even above their parents, so that it can provide opportunities for breeders to get the desired segregate (Nugraha & Suwarno, 2007).

4.2. Action and Number of Gene Controllers

Agronomic character is a phenotype, whose expression is determined by the interaction between genotypic factors and environmental factors. The characters studied in this study are quantitative characters, or some call them complex characters (Mackay, 2009). According to Ikram & Chardon (2010), quantitative characters are controlled by a complex genetic system, because it involves several genes (polygenic), with each gene having a minor influence, can be in the form of a small additive effect, dominant or epistatic, and sensitive to environmental conditions. (Mackay, 2009; Ikram & Chardon, 2010). Genetic complexity arises from alleles that experience segregation at multiple loci (Mackay, 2009).

The genetic variation of quantitative characters is assumed to be controlled by the collective influence (together) of the Quantitative Trait Loci (QTL), epistasis (interaction between QTL-QTL), environment, and interactions between QTL and environment (Semagn et al., 2010). Because of this complexity, many genotype characters can produce the same phenotype, and the same genotype can express different phenotypes (Mackay, 2009). Therefore, there is no clear relationship between genotype and phenotype in the expression of this trait. Semagn et al.

(2010) wrote that unlike monogenic controlled characters, these polygenic characters do not follow the Mendelian inheritance pattern as in qualitative characters.

The analysis of skewness and kurtosis can provide information about the nature of gene action and the number of genes that control a character (Samak et al., 2011). The analysis of skewness and kurtosis plays an important role in determining the presence or absence of epistasis in the cross zuriat (Ramadhan et al., 2018). The results showed that most of the agronomic characters of the studied lines were controlled by additive gene action, with only seed length being controlled by the complementary gene action of epistasis. Yudilastari et al. (2018) wrote that gene action in controlling a character can be divided into two, namely additive and non-additive (dominant gene action and epistasis). Allard (1964) and Crowder (1981) wrote that the term additive gene action is used in relation to genes affecting trait expression, where each allele contributes to the trait's phenotype. These contributions are known as additive effects, because the phenotype is determined by the sum of the effects of each allele of the gene loci involved. Changes caused by allelic substitution at each locus are not affected by alleles at other loci. The effect of additive genes from each allele can be passed from parents to offspring, because the contribution of each allele does not depend on allelic interactions (Yudilastari et al., 2018; Nugraha & Suwarno, 2007). Characters controlled by additive gene action indicate that selection can take place in the early generations because these characters can be inherited in the next generation (Ramadhan et al., 2018). On the other hand, for characters controlled by dominant or epistatic gene action, selection is carried out in the next generation (Mahalingam et al., 2011; Sulistyowati et al., 2015).

Based on this research, the characters affected by the action of additive genes, which can be passed on to the lines, were dry weight, plant height, number of tillers, number of panicles, weight of 1000 grain, and grain weight per panicle, amylose content, and antioxidant activity. On the other hand, the character of the length of the rice-bran is controlled by the action of non-additive genes, in this case it is epistasis, so that there is a tendency that it cannot be passed on to the lines. The same thing was reported by Anis et al. (2016), that there is a tendency for the character of plant height and harvest time to be influenced by the action of additive genes in their inheritance. In addition, the research of Ramadhan et al (2018) reported that there was additive gene action that affected the character of the number of primary branches in the zuriat population of IPB 3S/IPB160-F-36 rice crosses and almost all of the characters whose panicle architecture studied were controlled by additive gene action on zuriat population of IPB160-F-36/IPB 5R crosses, except for the length of the primary branches.

The results showed that all the characters studied were controlled by many controlling genes. This supports the etymology of the quantitative character itself, which is a polygenic (many gene) controlled character. Compared with the research of Ramadhan et al. (2018), the characters controlled by multiple genes in all cross populations studied were panicle length, number of primary branches, and grain density. The length of the primary branch and the number of branches/primary branches in the IPB160-F-36/IPB 5R cross zuriat were also controlled by many genes. Riyanto et al (2021) also reported that the characters of plant height, flowering age, harvest age, panicle length, and number of grain per panicle were controlled by many genes.

4.3. Heritability, Coefficient of Variation, and Variability

Yudilastari et al. (2018) wrote that heritability is a genetic parameter that can be used to determine the role of genetics in the inheritance of a character from parents to offspring/lines. Heritability is used as the basis for estimating the relative contribution of differences in the magnitude of genetic and non-genetic factors to the total phenotypic diversity in a population (Ene et al., 2015; Konate et al., 2016). Information about heritability can be used by breeders to determine the extent to which the intensity of selection is carried out to distinguish environmental influences on the phenotype of a plant (Zehra et al., 2017). Heritability is an important concept in quantitative genetics, especially in selection in plant breeding programs (Konate et al., 2018).

The value of heritability in the broad sense of this study is in the range that varies for each character. The data from the analysis showed that there were various heritability estimates for each agronomic character studied. The characters of dry weight, plant height, weight of 1000 grain, and grain weight per panicle had moderate broad-sense heritability. Characters with high heritability estimates were number of tillers, number of panicles, and age of flowering. Adhikari et al. (2018) wrote that the low heritability value indicates that the character appears due to variations in environmental factors involved in the expression of the character, and vice versa. A high broad sense heritability indicates a high selection response for a particular character. A good character to be used as a selection character is a character that has a high heritability value (Begum et al., 2015). A small heritability value will have an impact on a small selection progress value (Mursito, 2003).

The results of this study are in line with the research of Adhikari et al. (2018), that the flowering age also has a high broad-sense heritability. Meanwhile, the character of weight of 1000 grain and grain weight per panicle, which can be used as yield component parameters, also had moderate borad-sense heritability, compared to research by Adhikari et al. (2018). Research by Ogunbayo et al. (2014) also showed results in accordance with this study. The characters of flowering age and number of tillers also had high broad-sense heritability. This suggests that these characters are primarily under genetic control, rather than the environment. Similar results were confirmed in the study of Konate et al. (2016), that flowering age, number of tillers, and number of panicles are also categorized as characters with high broad-sense heritability. The weight of 1000 grain character also had a moderate broad-sense heritability.

The results showed that the entire value of the coefficient of phenotypic variation of all characters was higher than the coefficient of genotypic variation. Adhikari et al. (2018) wrote that this indicates the influence of the environment on these characters. The magnitude of the influence of the plant growth environment on the observed characters is explained by the level of difference value between those two parameters. Furthermore, Adhikari et al. (2018) wrote that the large difference in the values of these parameters indicates a large environmental influence on the expression of certain characters. The CPV value for all characters in this study showed a higher tendency than the GPV. This is in line with the research results of Bagati et al. (2016), that the value of the coefficient of phenotypic diversity is higher than the coefficient of genotype diversity in all the characters studied.

Based on the data above, the characters categorized as having narrow phenotypic variability were weight of 1000 grain and grain weight per panicle. Characters of dry weight, number of tillers, plant height, and number of panicles had broad phenotypic variability. Based on the calculation of the value of genotypic variability, all the characters studied showed a narrow genotypic variability. Hayati (2018) wrote that characters with narrow phenotypic variability are not effective for selection. Therefore, characters with dry weight, number of tillers, plant height, and number of panicles can be used as selection criteria. A high coefficient of variability indicated a favorable selection range for the desired character, while a low coefficient of variability indicated a need to create variability and conduct selection (Adhikari et al., 2018).

4.4. Inter-Relationship Between Characters

Path analysis showed that the number of tillers had an effect on 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. This analysis is used to determine the magnitude of the direct or indirect effect on the yiled component towards the yiled (Akhmadi et al., 2017). A good selection character is one that has a large real correlation value and a high direct or indirect effect value on the yield (Boer, 2011). Yield is a complex character and depends on a number of related characters. Therefore, crop yields usually depend on the actions and interactions of a number of important characters. Knowledge of the various characters that determine crop yields is important for examining various yield components (growth parameters) and paying more attention to yield components that have the greatest influence on crop yields (Kinfe et al, 2015). A good selection character is one that has a large real correlation value and a high direct or indirect effect value on the results (Boer, 2011).

The results of this study have differences when compared with the research of Akhmadi et al. (2017). The results of the research Akhmadi et al. (2017) showed that characters that had a direct effect on high yields were length of panicle, weight of 1000 filled grain, number of filled grains per panicle and grain filling period. The character of the generative plant height and the total number of grain per panicle had a high negative direct effect on yield, but the indirect effect through panicle length was quite high. Several studies that have been carried out using this analysis showed the characteristics of flowering age, 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 100 grains which have a direct effect on high yield on some rice plant populations (Aryana et al., 2011; Rachmawati et al., 2014; Safitri et al. (2011).

4.5. Morpho-Biochemical Profile

The rice-bran cumulative color of the F6 and F7 lines showed that there were 4 (four) lines with completely the same color as the black rice parent, but the other 2 (two) lines still showed the color combination between the two parents. The research of Laokuldilak et al. (2011) reported that pigmented rice contains high Cyanidin-3-Glucoside (58-95%). C3G is part of the anthocyanin group of compounds. The appearance of rice-bran color in pigmented rice is thought to be related to the content of Cyanidin-3-Glucoside compound, although the relationship between these two is not clear, due to the complexity of the existing genetic system. According to research Ham et al. (2015), there was a significant positive correlation on C3G content towards the brightness and yellow color of rice bran. However, the genetic system responsible for rice-bran pigmentation and Cyanidin-3-Glucoside content is not same, but is related through a specific pathway with anthocyanin metabolism. Mackon 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.

When viewed from the aspect of rice-bran size, based on the analysis carried out, the average rice-bran size of the 5 (five) lines studied (PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18 , 482-9-134) differ from the PHMW 487-24-8. Meanwhile, the rice-bran size of all lines was higher than that of the comparison varieties. The length of the rice bran of the lines was above the length of the rice-bran of the parent, Mentik Wangi, and had a size that was not significantly different from the length of the rice bran of the Black Rice variety. This condition could be caused by transgressive segregation of alleles responsible for the expression of rice bran length characters. Based on the analysis of Skewness and Kurtosis, it was known that this character is controlled by complementary epistasis gene action and controlled by a large number of genes (polygenic). According to IRRI (2013), the length of the rice bran in all these lines was grouped into the medium classification, except for the 482-17-7 line, which was grouped under the short criteria. The length of the rice-bran is part of determining the shape of the rice grain. The shape of rice grain is one of the determinants of the quality of rice grain. Grain quality is one of the selection parameters in plant breeding program (Kush and Cruz, 2000; Kartahadimaja et al., 2021). When compared with previous studies (Oktaviani et al., 2021), F7 rice bran size did not have a significant difference with F6 rice-bran size. All the lines studied in the F6 generation had higher rice-bran size compared to the rice-bran size of the Padi Hitam and Mentik Wangi varieties. All F6 and F7 lines were also categorized into the moderate, based on the standards of the International Rise Research Institute (2013). Based on the research of Kartahadimaja et al. (2021), rice-bran size of the 12 genotypes studied varied in length, width, and thickness. B3 line is a new line with the shortest length (8.3 mm) but the widest among all lines. Meanwhile, the other 9 lines varied between 9.04 – 10.31 mm, and were included in the long seed criteria. Other lines, such as B2, B4, B7, F2, F3, F4, H1, H4, L2, and two varieties IR 64 and Ciherang, were included in the criteria for length with a narrower width. The IR64 variety is the variety with the smallest width (2.55 mm).

Amylose content is one of the criteria that determines grain quality (IRRI, 2012). In addition, amylose content is also one of the parameters used to predict the quality of processed rice (Juliano et al., 1965; Bhattacharaya and Juliano, 1985). The amylose content of the studied lines was between the amylose content of the comparison varieties. In addition, each line showed a significant difference in amylose content. Based on the criteria of Khush & Cruz (2000), the amylose content of grain could be grouped into 5 (five) criteria, waxy rice (0% to 2%), very low amylose rice (3% to 9%), low amylose rice (10% to 19 %), medium amylose rice (20 % to 25 %), and rice with high amylose (> 25 %). The amylose content of the studied lines was at a low criteria. The results of this study are a continuation of previous studies that examined the amylose content in the F6 line. The F6 generation PHMW lines were in various criteria. The classification includes very low amylose (lines 482-1-14), low amylose (lines 487-24-8, 482-9-134, 482-1-4, and 482-17-7) and medium amylose (lines 487-24-8, 482-9-134, 482-1-4, and 482-17-7) and medium amylose (lines 482-17-18) (Oktaviani et al., 2021).

It is important to measure the antioxidant profile of the lines to map the antioxidant profile of the lines compared to checked varieties. The results showed that there was 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 a greater reducing power than the long white rice bran extract. The main antioxidant compounds detected by High Performance Liquid Chromatography (HPLC) were oryzanol (39-63%) and phenolic acids (33-43%). In addition, Laokuldilok et al. (2011) also found that black rice had 18-26% anthocyanin content. Ferulic acid was the dominant phenolic acid in the rice samples studied. Black rice contained higher levels of gallic acid, hydroxybenzoic acid, and protocatechoic acid compared to red rice and white rice. In addition, the research of Jun et al. (2011) reported that antioxidant activity of 40% pigmented rice-bran acetone extract, at an antioxidant concentration of 500 g/mL, red rice with the highest total phenolic and total flavonoids showed highest antioxidant activity (83.6% based on the radical DPPH test). In addition, there was an interesting study by Setyaningsih et al. (2015) who studied the positive correlation between amylose content and levels of antioxidant compounds, such as melatonin and phenolics.

Conclusions

All agronomic parameters studied were thought to be controlled by many genes (polygenic) and additive gene action, except for rice-bran length. The data from the analysis showed that there were various broad-sense heritability for each agronomic character. The characters of dry weight, plant height, weight of 1000 grain, and grain weight per panicle had moderate broad-sense heritability. Characters with high broad-sense heritability were number of tillers, number of panicles, and days to flowering. Based on the results of the study, all the characters observed in each line showed a different effect in each line studied. The value of broad-sense heritability this study varied for each character. Path analysis showed that the number of tillers had an effect on 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 seeds of the F6 and F7 lines showed that there were 4 (four) lines with completely the same color as the black rice, but the other 2 (two) lines still showed the color combination between the two checked varieties. When viewed from the aspect of rice-bran size, the rice-bran size mean of the 5 (five) lines studied (PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18 , 482-9-134) differ from the PHMW 487-24-8. Meanwhile, the rice-bran size of all studied lines was higher than the rice-bran size of the comparison varieties. The amylose content of lines was between the amylose content of the comparison 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 sticky rice traits (low amylose content) with multi-location and multi-season field trial, selection based on molecular markers related to genes associated with low amylose content and high antioxidant content, as well as organoleptic testing of processed rice from these lines.

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Bogor Agricultural University,

IPB Campus Darmaga, Bogor 16680, Indonesia.

E-mail: hayati.jbiosci@apps.ipb.ac.id

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[HJB] Editor Decision: Revisions Required

2022-02-15 01:41 PM

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hayati.jbiosci@apps.ipb.ac.id

HAYATI Journal of Biosciences

Department of Biology,

HAYATI Journal of Biosciences

Department of Biology,

Faculty of Mathematics and Natural Sciences

Bogor Agricultural University,

IPB Campus Darmaga, Bogor 16680, Indonesia.

E-mail: hayati.jbiosci@apps.ipb.ac.id

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Genetic Parameters and Inter-relationship Between Agronomic Traits of F6 Lines, and Rice Bran Morpho-Biochemical Profile of F7 Lines Derived from a Crossing of Black Rice and Mentik Wangi

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ABSTRACT

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The development of sticky pigmented rice with high antioxidant content as superior varieties can be carried out by crossing the Black Rice with Mentik Wangi varieties. Rice breeding program to obtain rice lines with low amylose content and high antioxidants has reached the F6 lines. The purpose of this study was to determine genetic parameters and the relationship between agronomic traits of the F6 lines. Another objective of this study was to determine the morpho-biochemical profile of F7 grain, including length, cumulative color, amylose content and antioxidant activity. The results showed that all agronomic parameters had a coefficient of variance less than 20%, which indicates that phenotypic differences were caused by genotypic factors, rather than environmental ones. All the values of the Genetic Diversity Coefficient are in the low range, as well as the values of the Phenotypic Diversity Coefficient. The broad sense heritability of dry weight character was in the low range. The characters of plant height, weight of 1000 grain and weight of grain per panicle were categorized as having moderate broad sense heritability, while the characters of number of tillers, number of panicles and age of flowering had high broad sense heritability. The phenotypic variability of weight of 1000 grain and age of flowering were included in the narrow criteria, while the other characters were broad one. Path analysis showed that the number of tillers had an effect on the dry weight and grain weight per panicle, but the dry weight did not directly affect the weight of 1000 grain. Directly, the weight of 1000 grain was only significantly affected by the grain weight per panicle trait. In terms of grain length profile and amylose content, the F7 lines had difference in grain length (2 subsets) and amylose content (6 subsets) traits. The cumulative grain color of the PHMW482-17-7 and 482-17-18 lines showed the color combination of the two parents. Based on the T test conducted on F6 and F7 grain samples, 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.

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Keywords: antioxidant, black rice, mentik wangi, morpho-biochemical, sticky

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1. Introduction

Black rice is one type of pigmented rice, in addition to red rice and brown rice. This rice is often consumed as functional food, not as the main food ingredient (Purwanto et al., 2019). This is due to the various nutritional content of this type of rice. Black rice contains various micro and macronutrients that are important for human health (Apridamayanti et al., 2017). Protein, fat, fiber, vitamins, and minerals important for the human body are the various nutrients that this rice has (Nurhidajah, 2018; Apridamayanti et al., 2017; Kristamtini et al., 2012). Important nutrients reported in black rice are vitamin B, vitamin E, Fe ions, thaimin,

Commented [NC12]: Please consider the order, newest to oldest magnesium, niacin, phosphorus, dietary fiber (Kristamtini et al., 2021; Murali & Kumar, 2020), Zn ions, and Mn (Kristamtini et al. et al., 2021; Murali & Kumar, 2020), Zn, and Mn ions (Kristamtini et al., 2021; Murali & Kumar, 2020), et al., 2012). Murali & Kumar (2020) also reported that black rice is free from gluten and cholesterol, low in sugar, salt and fat.

Black rice, is one of the pigmented rice classified on the basis of the color of the pericarp, aleurone, and endosperm of the rice grains (Kristamtini et al., 2012). One of the important compounds that contribute to black rice aleurone color is anthocyanin (Yoshimura et al., 2012; Palupi et al., 2020). Anthocyanins are responsible for the appearance of blue, purple, red, and orange colors in many fruits and vegetables (Miguel, 2011). Anthocyanins have high antioxidant activity and play an important role in human health (Prastiwi & 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 & Kumar, 2020; Tena et al., 2020), support health eye, anti-microbial, and prevention of neuro-degenerative diseases (Tena et al., 2020).

Anthocyanins, one of the most important types of plant flavonoid compounds, are pigments with a flavylium cation structure (AH+) that act as acids (Tena et al., 2020). This structure is directly related to antioxidant activity, because it is able to prevent or inhibit oxidation reactions by scavenging free radicals and reduce levels of oxidative stress in cells (Tena et al., 2020). Based on basic chemical reactions, anthocyanins act as donors of H atoms or as single electron transferors (Tena et al., 2020).

Although black rice is known as a functional food with the above benefits, consumer acceptance of the texture of rice prepared from this rice is low, due to the non-stickiness texture of the cooked rice (Adi et al, 2020). Non-tender/non-sticky/non-glutinous rice has a dry, hard, and separate texture, even though it has been through the cooking process. Rice texture is determined by amylose amylopectin ratio (Cameron & Wang, 2005; Adi et al., 2020; Li et al., 2016a), post-harvest processing, and cooking method (Li et al., 2016b). In addition, Cameron & Wang (2005) also found that the texture/stickiness/hardness of rice was associated with protein and crude lipid content. The higher the amylose content of rice, the more tender the texture of the rice will be, and vice versa (Khumar & Khush, 1986; Bhattacharaya et al., 1999; Luna et al, 2015; Panesar & Kaur, 2016). Crude protein and lipid content were negatively correlated with the hardness of pasta flour and processed rice, but positively correlated with the level of rice stickiness (Cameron & Wang, 2005).

A rice plant breeding program to produce rice plants with a quality texture of soft/soft/fluffy processed rice has been carried out in Indonesia. Indonesia, through the Ministry of Agriculture, has released a rice variety with an amylose content of 19.6% in 2019, the result of a cross between Black Sticky Rice and Pandan Wangi cv Cianjur. In addition, research for the development of fluffier pigmented rice has also been carried out by researchers (Kim et al., 2010; Zhang et al., 2018; Roy & Shil, 2020). The development of pigmented rice with a fluffier rice texture in Indonesia is expected to increase the source of rice germplasm with superior characters in Indonesia.

The development of superior varieties of pigmented rice that is fluffier and has a high antioxidant content can be done by crossing the Black Rice variety with Mentik Wangi. Rice breeding research to obtain rice lines with high antioxidants and a fluffier texture of rice has reached the F6 offspring. The purpose of this study was to determine various genetic parameters and the relationship between characters based on the agronomic character of the F6 line. Another objective of this study was to determine the morpho-biochemical profile of F7 seeds, including length, cumulative color, amylose content and antioxidant activity.

2. Matrials and Methods

The genetic material used consisted of 6 (six) potential F6 lines from crosses of Black Rice and Mentik Wangi varieties and 2 (two) comparison varieties, namely Black Rice cv Cilacap and Mentik Wangi. These six lines are the results of 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, 482-17-18. Black rice and Mentik Wangi were used as comparison varieties. The F6 lines and comparison varieties were planted until harvest to obtain the F7 line. Seeds of the F7 line obtained were analyzed for bran size (seed), cumulative bran color (seed), amylose content, and antioxidant activity. Meanwhile, the seeds of the F6 line were also subjected to the same analysis.

2.1. Field Trial of F6 Lines

Field testing of the F6 line was carried out in the Experimental Farm greenhouse, Faculty of Agriculture, Universitas Jenderal Soedirman. The study was conducted from April to October 2020. The design used was a completely randomized block design, with 3 (three) blocks and 5 (five) replications in each block. The soil used is ultisol. The lines were planted in polybags, with a total of 115 polybags. Each polybag is filled with one individual rice plant. The fertilizer used consists of NPK fertilizer and manure. The planting media used consisted of ultisol soil,

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What is the difference with other studies? In which part this study is different with others?
What is the novelty and originality?
What is the contribution to science and technology or rice genetic improvement?

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roasted rice husks, and manure. Weeds, pests, and diseases are controlled by conventional means and the use of chemical pesticides. The agronomic parameters observed included flowering age, plant height, dry weight, number of tillers, weight of 1000 grain, number of panicles, and weight of grain per panicle.

2.2. Morphological Characterization of F7 Rice Bran

Rice-bran morphology observed in the form of seed length, seed shape, and seed cumulative color of each line. The determination of seed size classification was determined based on ~~parameters from~~ the International Rice Research Institute (IRRI) (2012). The basis used is the length and the shape of the rice-bran. Cumulative rice-bran color is used to determine the segregation phenomenon.

2.3. Amylose Quantification

The amylose content of the seeds of the F7 strain 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 in the sample was then measured based on the linear regression equation in the standard curve.

2.4. Determination of Antioxidant Activity

Measurement of antioxidant activity was carried out on seeds of the F6 and F7 strains, using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method (Blois, 1958).

2.5. Data analysis

Data on agronomic parameters of the F6 line, F7 rice-bran length, and F7 amylose content of the six lines were analyzed using SAS 9.4 software. If the results of the analysis of variance indicate that there is an influence of the planted genotype on the various agronomic parameters studied, then a different test is carried out with the Least Significant Difference Test (BNT) at the 95% confidence level ($\alpha = 0.05$). The results of this analysis are also used as the basis for determining the value of genetic diversity (Coefficient of Variance/CV), phenotypic diversity, and heritability values of broad meaning, as well as phenotypic variability. The difference in antioxidant activity of the samples of the F7 strain and the F6 strain was identified by the T test. Path analysis was performed using the LISREL 8.2 software. This analysis was used to determine the direct and indirect factors determining the productivity character, namely weight of 1000 grain and weight of grain per panicle.

The estimation of gene action and number of gene control were analyzed based on Skewness and Kurtosis values, respectively, for each trait observed in the F6 generation. Skewness is the slope of the graph. Skewness shows epistasis effected expression of a trait (Lestari et al., 2015).

Commented [NC21]: variables

Commented [NC22]: what kind of dry weight?

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Commented [NC24]: water content?

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Commented [NC27]: Assessing what kind of antioxidant? hydrogen atom transfer (HAT), single electron transfer (ET), reducing power, and metal chelation? Which one?

Commented [NC28]: Please use standard English. What is the meaning of planted genotype?

Commented [NC29]: What is the formula for assessing these parameters?

Commented [NC30]: Student t-test

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If Skewness equals to zero, it means there is no epistasis. Skewness > 0 means there is a complementary epistasis gene action, and Skewness < 0 means there is a duplicate epistasis gene action. Kurtosis describes the shape of the distribution curve and shows several genes controlling a trait (Herawati et al., 2019). Kurtosis is the value of the taperedness of the graph. When Kurtosis > 3, has a positive value, it shows the leptokurtic graph indicates a few gene controls trait. If Kurtosis < 3, has a negative value, it shows a platykurtic diagram and its trait is controlled by many genes. Interpretation Skewness and Kurtosis value refer to scheme in Jambormias research (Jambormias, 2014). The value of the skewness ratio and the value of the kurtosis ratio can also be used to determine whether the data distribution is normal or not. If the value of the skewness ratio and the value of the kurtosis ratio are between -2 and 2, the data distribution is normal. The value of the skewness and kurtosis ratio can be obtained by dividing the skewness and kurtosis values by their respective standard errors.

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The data obtained from the observations and analysis of variance (F test) were used as the basis for calculating the coefficient of variance (CV), coefficient of genetic diversity, coefficient of phenotypic diversity, and heritability values of broad meaning, as well as phenotypic variability. Based on the analysis of variance, the genotypic variance (σ^2g), phenotypic variance (σ^2p), coefficient of genotypic diversity (KKG) and heritability (h^2), can be estimated using the following formula (Singh & Chaudary, 1977).

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$$KTe = \sigma^2 e$$

$$\sigma^2 g = KTg - KTeb$$

$$\sigma^2 p = \sigma^2 g + \sigma^2 e$$

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Note :

σ^2g = genotip of variance

Commented [NC37]: genotype

σ^2p = phenotip of variance

Commented [NC38]: phenotype

σ^2e = error of variance

b = replication

KTg = kuadrat tengah genotip

KTe = kuadrat tengah error

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The value of the coefficient of genetic diversity can be determined by the following formula:

$$CGV = \sqrt{\sigma^2 g}\bar{X} \times 100\%$$

Note :

CGV= Coefficient of Genetic Variance

$\sigma^2 g$ = genotip of variance

$\bar{X}$ = mean of population

According to Moedjiono & Mejaya (1994) in Handayani (2018) the criteria for the coefficient of genetic diversity are determined based on absolute and relative values. The criteria for coefficients of genetic and phenotypic diversity are presented in Table 1.

Coefficient of Genetic Variance	Criteria
0 - 25% of the highest	Low
25 - 50% of the highest	Quite low
50 - 75% of the highest	Quite high
75% - 100% of the highest	High

Commented [NC40]: Please use appropriate Table

The estimated value of broad sense heritability is determined by the following formula:

$$h^2 = \sigma^2 g \sigma^2 p \text{ x } 100\%$$

Note:

h^2 = broad sense heritability

$\sigma^2 g$ = variance of genotype

$\sigma^2 p$ = Variance of phenotype

The determination of heritability criteria follows the following criteria (McWhirter, 1979) (Table 2):

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Value of broad sense heritability	Criteria
$h^2 \leq 20\%$	Low
$20\% < h^2 < 50\%$	Medium
$h^2 > 50\%$	High

Furthermore, the value of phenotypic variability is calculated by the formula:

Variability of Phenotipic = 2 x SD

Note : SD = Standar Deviation

Determination of the criteria for phenotypic variability is calculated by comparing the value between the phenotypic variance with a value of 2 (two) times the standard deviation of the data. If the value of variability is greater than the value of SD, then it is categorized into broad criteria. And vice versa.

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3. Results

3.1. Agronomic Traits of The Lines

Commented [NC43]: What is your finding regarding evaluation of agronomic traits?

The results of the Least Significant Difference (LSD) test to determine the difference in the response of each studied line based on agronomic characters can be seen in Table 3. Black rice and Mentik Wangi varieties were used as comparison varieties, which were excluded from the LSD test.

Table 3. Differences agronomic traits of six lines studied

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Genotype	Dry weight	Plant length	Number of tillers	Number of panicles	Weight of 1000 grain	Grain weight per panicle	Days to flowering
Line 482-1-14	46.34±3.70 ab	62.43±1.38a	36.40±6.01b	16.75±1.38c	13.67±0.39ab	2.15±0.28ab	60.92±1.80a
Line 482-17-7	40.03±1.20a	70.94±3.29b	26.80±3.13a	13.95±1.31ab	12.10±1.85a	1.97±0.36ab	60.67±3.10a
Line 482-1-4	51.91±5.53b	69.93±3.97b	36.70±5.43b	16.50±2.27bc	14.21±1.63ab	1.95±0.33a	69.00±1.52c
Line 482-17-18	45.98±5.30ab	81.08±8.90c	25.10±2.27a	11.55±1.55a	15.12±1.75b	2.23±0.52ab	65.08±2.67b
Line 482-9-134	48.29±6.32b	82.83±2.61c	25.95±1.89a	16.20±2.24bc	15.65±1.31b	2.40±0.30ab	68.08±3.27bc
Line 487-24-8	46.99±6.01ab	71.53±1.86b	28.50±6.43a	14.60±2.03bc	15.77±1.48b	2.61±0.41b	61.67±1.31a
Black Rice	37.47±2.95	60.56±2.62	28.25±1.95	15.42±0.63	15.1±0.65	2.24±0.47	64.00±1.41
Mentik wangi	66.90±13.78	101.80±6.60	25.59±4.48	14.08±2.76	15.22±2.34	2.77±0.71	82.83±4.09

Note : Blue color represents the highest value and red color represents the lowest value

3.2. Gene Action and Number of Controlling Genes

Commented [NC45]: To predict how many genes are involved for specific traits, it is not advised to use F6 or F7 progenies since they have been selected in previous generation, become 6 progenies which are used in this experiment. For this purpose, F2 are recommended.

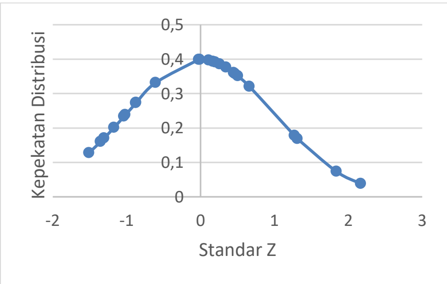
Gene action and the number of controlling genes were determined based on Skewness and Kurtosis analysis. The results of Skewness and Kurtosis analysis for dry weight, days to flowering, plant height, number of tillers, weight of 1000 grain, panicle number, and grain weight per panicle, rice-bran length, amylose content, and antioxidant activity can be seen in Table 4.

Table 4. Gene action and the number of genes controlling the characters studied

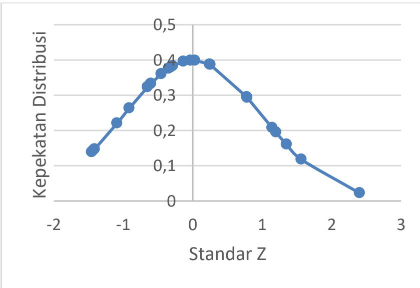
Traits	Skewness	Kurtosis	Gene action	Number of Controlling Gene
Dry weight of F6	0.358	-0.506	Additive	Many
Plant length of F6	0.558	-0.194	Additive	Many
Number of tillers F6	0.914	0.161	Additive	Many
Number of panicle F6	-0.212	-0.965	Additive	Many
Weight of 1000 grain F6	-0.560	0.077	Additive	Many
Grain weight per panicle F6	0.327	-0.694	Additive	Many
Days to flowering F6	0.246	-1.141	Additive	Many
Length of grain F7	1.192	2.213	Complementary epistasis	Many

Amylose content F7	-0.016	-0.046	Additive	Many
Antioxidant activity F7	0.809	-1.329	Additive	Many

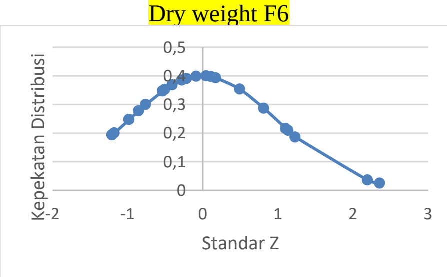
Furthermore, the normal distribution curve of the studied characters can be seen in Figure 1. All the analyzed characters show that the residual data are normally distributed.



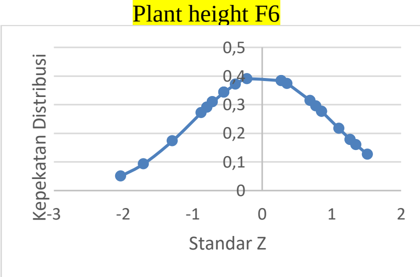
Dry weight F6



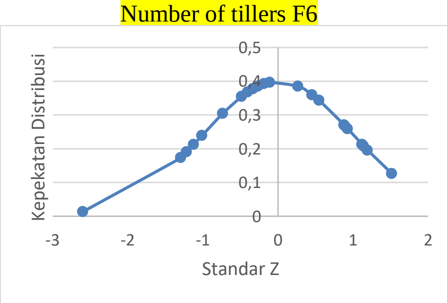
Plant height F6



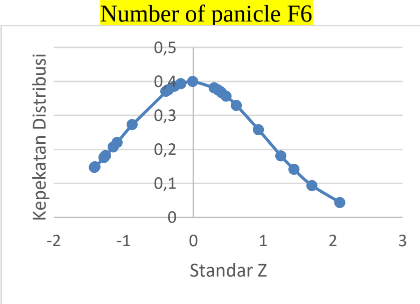
Number of tillers F6



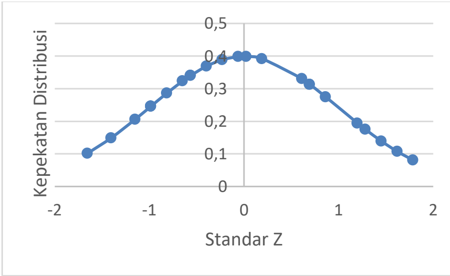
Number of panicle F6



Weight of 1000 grain F6



Grain weight per panicle F6



Days to flowering F6

Figure 1. Residual distribution curve of the data of various observed characters

3.3. Broad Sense Heritability and Phenotypic Variability

It is important to determine the value of the coefficient of diversity first to determine the cause of the differences in the appearance of the observed characters. The results of the calculation of the coefficient of diversity are presented in Table 5. All of the observed characters show a coefficient of diversity that is less than 20%.

Commented [NC46]: Coefficient of variation?

Table 5. Coefficient of diversity of various characters studied

Traits	Mean	Max	Min	CV (%)
Dry weight	46.59	84.61	28.63	11,48
Number of tillers	29.91	54	14	15,22
Plant height	73.12	96.8	53.2	6,03
Weight of 1000 grain	14.42	22.12	7.04	9,74
Number of panicle	14.93	25	9	11,44
Grain Weight per panicle	2.22	3.64	1.1	14,63
Days to flowering	64.24	72	57	3,20

Next, the estimated value of heritability in the broad sense describes the level of similarity of the traits of the offspring to the parental varieties. The results of the analysis of heritability estimates, the coefficient of genotypic diversity and the coefficient of phenotypic diversity can be seen in Table 6. The data from the analysis show that there are various heritability estimates for each agronomic character studied. The characters of dry weight, plant height, weight of 1000 grain, and grain weight per panicle had moderate heritability estimates. Characters with high heritability estimates were number of tillers, number of panicles, and age of flowering.

The character that has the highest coefficient of genetic variation (CGV) and coefficient of phenotypic variation (CPV) is the number of tillers. Based on the data obtained, using the criteria from Hayati (2018), the character categorized as having a high CGV value is the number of tillers. Characters with high CGV values were plant height, weight of 1000 grain, number of panicles, and weight of grain per panicle. Characters with a rather low CGV were dry weight and flowering age. Meanwhile, the characters with a high CPV value were the number of tillers, the number of panicles, and the weight of seeds per panicle. Characters with a fairly high CPV value included dry weight, plant height, and weight of 1000 grain. The CGV value for the number of tillers is the same as the CPV value. The CGV value on the character of the number of panicles and the age of flowering is greater than the CPV value. In addition to these characters, the CGV has a lower value than the CPV.

Table 6. Estimated value of heritability (h2), Coefficient of Genetic Variation, and Coefficient of Phenotypic Variation of the characters studied

Commented [NC47]: Use standard English!

Traits	Mean	Phenotypic Variation	Middle Square of Error	Phenotypic Variation	h2 (%)	h2 criteria	CGV (%)	CGV criteria	CPV (%)	CPV criteria
Dry weight	46.59	7.86	28.44	36.30	21.65	Moderate	6.02	37.91% (quite low)	12.93	58.8 (quite high)
Number of tillers	29.91	22.56	20.73	43.29	52.11	High	15.88	100% (high)	21.99	100 (high)
Plant height	73.12	36.90	83.97	120.87	30.53	Moderate	8.31	52.33% (quite high)	15.04	68.4 (quite high)
Weight of 1000 grain	14.42	1.47	1.97	3.44	42.79	Moderate	8.42	53.02 (quite high)	12.87	58.53 (quite high)
Number of panicle	14.93	3.25	2.92	6.16	52.68	High	12.07	76.01 (quite high)	16.64	75.67 (high)
Grain weight per panicle	2.22	0.04	0.11	0.14	26.57	Moderate	8.80	55.42 (quite high)	17.06	77.58 (high)
Days to flowering	64.24	12.66	4.23	16.89	74.95	High	5.54	34.89 (quite low)	6.40	29.1 (quite low)

Variability is calculated based on the variance formula. The value of phenotypic variability along with the criteria for each character studied can be seen in Table 7. Based on the data below, the characters categorized as having narrow phenotypic variability are weight of 1000 grain and grain weight per panicle. Characters of dry weight, number of tillers, plant height, and number of panicles had wide phenotypic variability. Based on the calculation of the value of genetic variability, all the characters studied showed a narrow genetic variability.

Table 7. Value of phenotypic variability (PV) and genotypic variability (GV) of the traits

Traits	Mean	Variance	SD	2 X SD	PV Criteria	GV	SD GV	2 x SD	GV Criteria
Dry weight	46.59	31.21	5.59	11.17	broad	7.86	8.36	16.73	narrow
Number of tillers	29.91	38.81	6.23	12.46	broad	22.56	41.94	83.88	narrow
Plant height	73.12	62.99	7.94	15.87	broad	36.90	87.53	175.06	narrow
Weight of 1000 grain	14.42	3.29	1.81	3.63	narrow	1.47	2.97	5.95	narrow
Number of panicles	14.93	5.85	2.42	4.84	narrow	3.25	6.01	12.04	narrow
Grain weight per panicle	2.22	0.16	0.40	0.80	narrow	0.04	0.09	0.19	narrow
Days to flowering	64.24	15.76	3.97	7.94	broad	12.66	20.73	41.47	Narrow

3.4. Inter-Relationship Traits

Path analysis results with LISREL 8.2 software. The dependent variable was the weight of 1000 seeds and the weight of seeds per panicle. Another agronomic character as an influencing variable (independent variable). The path analysis diagram can be seen in Figure 2. Path analysis showed that the number of tillers had an effect on the dry weight and grain weight per panicle, but the dry weight did not directly affect the weight of 1000 seeds.

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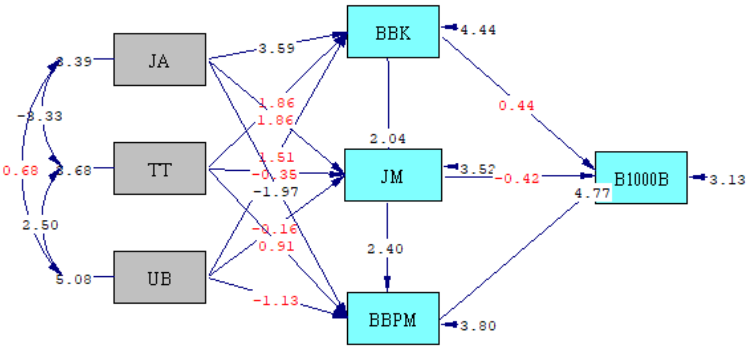

Figure 2. Path diagram of inter-relationship traits studied (P-value = 0.19579)

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3.5. Morpho-Biochemical Profile of Bran Rice

The results of the analysis of the grain size of the F6 and F7 lines studied can be seen in Figure 3. This figure shows that the cumulative color of each F6 and F7 intergenerational line has similarities. There were 4 (four) with completely the same color as the black rice parents, but the other 2 (two) lines still showed the color combination between the two crossed parents.

Figure 3. Cumulative color comparison of F6 and F7 rice bran, and also the checked varieties

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When viewed from the aspect of rice-bran size, based on the analysis carried out, the average rice-bran size of the 5 (five) lines studied (PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18 , 482-9-134) differed from the PHMW line 487-24-8 (Figure 4; Table 8). Meanwhile, the size of rice-bran of all lines was higher than the rice bran size of the comparison varieties used.

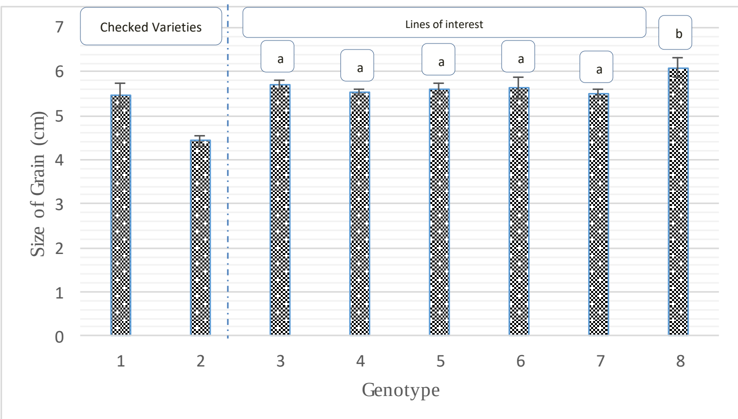

Figure 4. Size of F7 Rice Bran

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The amylose profile of the rice-bran samples of the F7 lines showed that the average amylose content of each line studied was different from one another (Figure 5). Line 482-1-14 showed the lowest amylose content value 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 amylose content value compared to all lines (16.81±0.05), but it was still below the amylose content of the Black Rice comparison variety (22.37±0.04). The amylose content of all lines was in the range of amylose content of the two comparison varieties studied (Black Rice and Mentik Wangi).

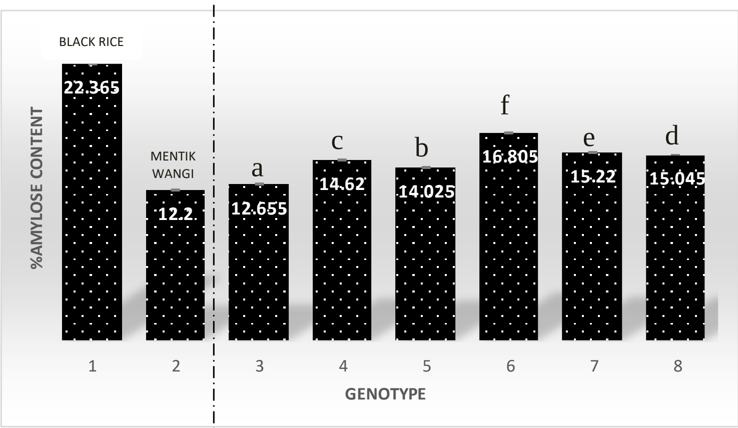

Figure 5. Amylose profile of F7 and checked varieties

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For the character of antioxidant activity, independent sample T-test was conducted on 2 groups of seeds of the F6 and F7 strains. This test aims 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.

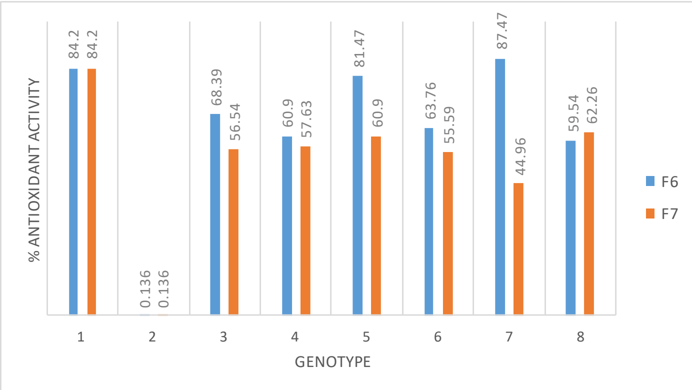

Figure 6. Antioxidant activity of F6 and F7 rice bran samples

4. Discussion

The purpose of this study was to determine genetic parameters based on agronomic data of F6 lines. Another objective of this research is to determine the agronomic character that determines the character of agricultural productivity, through path analysis. These genetic parameters can be used as the basis for determining selection criteria in plant breeding programs, so that the selection is more effective and efficient (Yudilastari et al., 2018). In addition, a morpho-biochemical profile that is suitable for the purpose of developing low amylose pigmented rice is also carried out as a basis for the selection process for potential lines.

4.1. Agronomic Traits

All the traits observed in each line showed a different effect in each line studied. The dry weight of all lines was between the dry weight of the two comparison varieties. There were two subsets of dry weight trait for the 6 (six) lines. The same analysis was applied to the trait of plant height. The plant height of all lines was between the plant heights of the two comparison varieties. There were 3 (three) subsets of plant height characters for the 6 (six) lines studied. Line 482-1-4 has differences with other strains. Each line 482-1-7, 482-1-4, and 487-24-8 had no difference in plant height. Meanwhile, lines 482-17-18 and 482-9-134 also did not have differences in plant height, because they were in one subset (group). For the number of tillers, there were lines that had a higher number of tillers than the comparison varieties, namely lines 482-1-14 and 482-1-4. The number of tillers of the other lines was the same as in the comparison variety. The grouping of panicle numbers is more varied. There are 3 (three) subsets that group the lines based on the character of the number of panicles. The number of panicles of the 482-1-14 line

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was higher and statistically different compared to the checked varieties. Meanwhile, lines 482-1-4, 482-9-134, 487-24-8 and black rice varieties did not have statistical differences in the number of panicles. The number of panicles of the Mentik Wangi variety did not differ from that of the 482-17-7 strain. For weight of 1000 grain, there were only 2 (two) subsets that grouped each line. The weight of 1000 grain of the comparison varieties did not differ from those of 482-17-18, 482-9-134, and 487-24-8. The other three lines had 1000 seed weights lower than the two comparison varieties. For the character of grain weight per panicle, there are 2 (two) subsets formed from the statistical analysis. Lines 482-1-14, 482-17-7, 482-17-18, and 4829-134 had no difference in grain weight per panicle of black rice varieties. Meanwhile, the grain weight per panicle of the Mentik Wangi variety was not different from that of the 487-24-8 line, which indicated the highest value of grain weight per panicle among all other lines and the Black Rice variety. Flowering age characters resulted in 3 (three) subsets of the genotypes studied. The flowering age of the Mentik Wangi variety was different from all the studied lines, because the flowering age was the longest compared to the Black Rice lines and varieties. Flowering age of lines 482-1-14, 482-17-7, and 487-24-8 were not significantly different. For the Black Rice variety, it has the same flowering age as the 482-17-18 line.

Research by Kartahadimaja et al. (2021) reported that statistical analysis of agronomic characters 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 variations. Meanwhile, research by Kasim et al. (2020) showed that the rice plant height of the 17 rice varieties studied ranged from 95-160 cm. The plant heights 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 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 heights are preferred by farmers and researchers. While the parameters of flowering age, varied between 104-151 DAS. This indicates that these varieties have a longer flowering life than the lines used in this study. The earlier flowering age of rice varieties is preferred by farmers and researchers, because varieties with an earlier flowering age have a shorter life cycle, thus allowing a higher frequency of harvesting per year than varieties with a longer flowering age. This can have an impact on farmers' annual income. Meanwhile, the number of panicles varied between 12-24. The range of genotypic panicles in this study was in the range of the number of panicles studied by Kasim

et al. (2020). Meanwhile, the total number of grain per plant also varied, between 128 to 305. The weight of 100 seeds of the spadi variety also varied, between 1.4 – 2.56 grams.

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Based on the agronomic data of the cross lines studied, characters still appear that are outside the range of character values of the two comparison varieties used. This is in accordance with the opinion of Welsh (1981) which states that the action of duplicate genes and additive genes can cause transgressive segregation. Transgressive segregation is segregation that causes offspring to have characters with measurement ranges that are below or even above their parents, so that it can provide opportunities for breeders to get the desired segregate (Nugraha & Suwarno, 2007).

4.2. Action and Number of Gene Controllers

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Agronomic character is a phenotype, whose expression is determined by the interaction between genotypic factors and environmental factors. The characters studied in this study are quantitative characters, or some call them complex characters (Mackay, 2009). According to Ikram & Chardon (2010), quantitative characters are controlled by a complex genetic system, because it involves several genes (polygenic), with each gene having a minor influence, can be in the form of a small additive effect, dominant or epistatic, and sensitive to environmental conditions. (Mackay, 2009; Ikram & Chardon, 2010). Genetic complexity arises from alleles that experience segregation at multiple loci (Mackay, 2009).

The genetic variation of quantitative characters is assumed to be controlled by the collective influence (together) of the Quantitative Trait Loci (QTL), epistasis (interaction between QTL-QTL), environment, and interactions between QTL and environment (Semagn et al., 2010). Because of this complexity, many genotype characters can produce the same phenotype, and the same genotype can express different phenotypes (Mackay, 2009). Therefore, there is no clear relationship between genotype and phenotype in the expression of this trait. Semagn et al. (2010) wrote that unlike monogenic controlled characters, these polygenic characters do not follow the Mendelian inheritance pattern as in qualitative characters.

The analysis of skewness and kurtosis can provide information about the nature of gene action and the number of genes that control a character (Samak et al., 2011). The analysis of skewness and kurtosis plays an important role in determining the presence or absence of epistasis in the cross zuriat (Ramadhan et al., 2018). The results showed that most of the agronomic characters of the studied lines were controlled by additive gene action, with only seed length being controlled by the complementary gene action of epistasis. Yudilastari et al. (2018) wrote that

gene action in controlling a character can be divided into two, namely additive and non-additive (dominant gene action and epistasis). Allard (1964) and Crowder (1981) wrote that the term additive gene action is used in relation to genes affecting trait expression, where each allele contributes to the trait's phenotype. These contributions are known as additive effects, because the phenotype is determined by the sum of the effects of each allele of the gene loci involved. Changes caused by allelic substitution at each locus are not affected by alleles at other loci. The effect of additive genes from each allele can be passed from parents to offspring, because the contribution of each allele does not depend on allelic interactions (Yudilastari et al., 2018; Nugraha & Suwarno, 2007). Characters controlled by additive gene action indicate that selection can take place in the early generations because these characters can be inherited in the next generation (Ramadhan et al., 2018). On the other hand, for characters controlled by dominant or epistatic gene action, selection is carried out in the next generation (Mahalingam et al., 2011; Sulistyowati et al., 2015).

Based on this research, the characters affected by the action of additive genes, which can be passed on to the lines, were dry weight, plant height, number of tillers, number of panicles, weight of 1000 grain, and grain weight per panicle, amylose content, and antioxidant activity. On the other hand, the character of the length of the rice-bran is controlled by the action of non-additive genes, in this case it is epistasis, so that there is a tendency that it cannot be passed on to the lines. The same thing was reported by Anis et al. (2016), that there is a tendency for the character of plant height and harvest time to be influenced by the action of additive genes in their inheritance. In addition, the research of Ramadhan et al (2018) reported that there was additive gene action that affected the character of the number of primary branches in the zuriat population of IPB 3S/IPB160-F-36 rice crosses and almost all of the characters whose panicle architecture studied were controlled by additive gene action on zuriat population of IPB160-F-36/IPB 5R crosses, except for the length of the primary branches.

The results showed that all the characters studied were controlled by many controlling genes. This supports the etymology of the quantitative character itself, which is a polygenic (many gene) controlled character. Compared with the research of Ramadhan et al. (2018), the characters controlled by multiple genes in all cross populations studied were panicle length, number of primary branches, and grain density. The length of the primary branch and the number of branches/primary branches in the IPB160-F-36/IPB 5R cross zuriat were also controlled by many genes. Riyanto et al (2021) also reported that the characters of plant height,

flowering age, harvest age, panicle length, and number of grain per panicle were controlled by many genes.

4.3. Heritability, Coefficient of Variation, and Variability

Yudilastari et al. (2018) wrote that heritability is a genetic parameter that can be used to determine the role of genetics in the inheritance of a character from parents to offspring/lines. Heritability is used as the basis for estimating the relative contribution of differences in the magnitude of genetic and non-genetic factors to the total phenotypic diversity in a population (Ene et al., 2015; Konate et al., 2016). Information about heritability can be used by breeders to determine the extent to which the intensity of selection is carried out to distinguish environmental influences on the phenotype of a plant (Zehra et al., 2017). Heritability is an important concept in quantitative genetics, especially in selection in plant breeding programs (Konate et al., 2018).

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The value of heritability in the broad sense of this study is in the range that varies for each character. The data from the analysis showed that there were various heritability estimates for each agronomic character studied. The characters of dry weight, plant height, weight of 1000 grain, and grain weight per panicle had moderate broad-sense heritability. Characters with high heritability estimates were number of tillers, number of panicles, and age of flowering. Adhikari et al. (2018) wrote that the low heritability value indicates that the character appears due to variations in environmental factors involved in the expression of the character, and vice versa. A high broad sense heritability indicates a high selection response for a particular character. A good character to be used as a selection character is a character that has a high heritability value (Begum et al., 2015). A small heritability value will have an impact on a small selection progress value (Mursito, 2003).

The results of this study are in line with the research of Adhikari et al. (2018), that the flowering age also has a high broad-sense heritability. Meanwhile, the character of weight of 1000 grain and grain weight per panicle, which can be used as yield component parameters, also had moderate borad-sense heritability, compared to research by Adhikari et al. (2018). Research by Ogunbayo et al. (2014) also showed results in accordance with this study. The characters of flowering age and number of tillers also had high broad-sense heritability. This suggests that these characters are primarily under genetic control, rather than the environment. Similar results were confirmed in the study of Konate et al. (2016), that flowering age, number of tillers, and

number of panicles are also categorized as characters with high broad-sense heritability. The weight of 1000 grain character also had a moderate broad-sense heritability.

The results showed that the entire value of the coefficient of phenotypic variation of all characters was higher than the coefficient of genotypic variation. Adhikari et al. (2018) wrote that this indicates the influence of the environment on these characters. The magnitude of the influence of the plant growth environment on the observed characters is explained by the level of difference value between those two parameters. Furthermore, Adhikari et al. (2018) wrote that the large difference in the values of these parameters indicates a large environmental influence on the expression of certain characters. The CPV value for all characters in this study showed a higher tendency than the GPV. This is in line with the research results of Bagati et al. (2016), that the value of the coefficient of phenotypic diversity is higher than the coefficient of genotype diversity in all the characters studied.

Based on the data above, the characters categorized as having narrow phenotypic variability were weight of 1000 grain and grain weight per panicle. Characters of dry weight, number of tillers, plant height, and number of panicles had broad phenotypic variability. Based on the calculation of the value of genotypic variability, all the characters studied showed a narrow genotypic variability. Hayati (2018) wrote that characters with narrow phenotypic variability are not effective for selection. Therefore, characters with dry weight, number of tillers, plant height, and number of panicles can be used as selection criteria. A high coefficient of variability indicated a favorable selection range for the desired character, while a low coefficient of variability indicated a need to create variability and conduct selection (Adhikari et al., 2018).

4.4. Inter-Relationship Between Characters

Path analysis showed that the number of tillers had an effect on 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. This analysis is used to determine the magnitude of the direct or indirect effect on the yiled component towards the yiled (Akhmadi et al., 2017). A good selection character is one that has a large real correlation value and a high direct or indirect effect value on the yield (Boer, 2011). Yield is a complex character and depends on a number of related characters. Therefore, crop yields usually depend on the actions and interactions of a number of important characters. Knowledge of the various characters that determine crop yields is important for examining various yield components (growth parameters) and paying more attention to yield

components that have the greatest influence on crop yields (Kinfe et al, 2015). A good selection character is one that has a large real correlation value and a high direct or indirect effect value on the results (Boer, 2011).

The results of this study have differences when compared with the research of Akhmadi et al. (2017). The results of the research Akhmadi et al. (2017) showed that characters that had a direct effect on high yields were length of panicle, weight of 1000 filled grain, number of filled grains per panicle and grain filling period. The character of the generative plant height and the total number of grain per panicle had a high negative direct effect on yield, but the indirect effect through panicle length was quite high. Several studies that have been carried out using this analysis showed the characteristics of flowering age, 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 100 grains which have a direct effect on high yield on some rice plant populations (Aryana et al., 2011; Rachmawati et al., 2014; Safitri et al. (2011).

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4.5. Morpho-Biochemical Profile

The rice-bran cumulative color of the F6 and F7 lines showed that there were 4 (four) lines with completely the same color as the black rice parent, but the other 2 (two) lines still showed the color combination between the two parents. The research of Laokuldilak et al. (2011) reported that pigmented rice contains high Cyanidin-3-Glucoside (58-95%). C3G is part of the anthocyanin group of compounds. The appearance of rice-bran color in pigmented rice is thought to be related to the content of Cyanidin-3-Glucoside compound, although the relationship between these two is not clear, due to the complexity of the existing genetic system. According to research Ham et al. (2015), there was a significant positive correlation on C3G content towards the brightness and yellow color of rice bran. However, the genetic system responsible for rice-bran pigmentation and Cyanidin-3-Glucoside content is not same, but is related through a specific pathway with anthocyanin metabolism. Mackon 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.

When viewed from the aspect of rice-bran size, based on the analysis carried out, the average rice-bran size of the 5 (five) lines studied (PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18 , 482-9-134) differ from the PHMW 487-24-8. Meanwhile, the rice-bran size of all lines was higher than that of the comparison varieties. The length of the rice bran of the lines was above

the length of the rice-bran of the parent, Mentik Wangi, and had a size that was not significantly different from the length of the rice bran of the Black Rice variety. This condition could be caused by transgressive segregation of alleles responsible for the expression of rice bran length characters. Based on the analysis of Skewness and Kurtosis, it was known that this character is controlled by complementary epistasis gene action and controlled by a large number of genes (polygenic). According to IRRI (2013), the length of the rice bran in all these lines was grouped into the medium classification, except for the 482-17-7 line, which was grouped under the short criteria. The length of the rice-bran is part of determining the shape of the rice grain. The shape of rice grain is one of the determinants of the quality of rice grain. Grain quality is one of the selection parameters in plant breeding program (Kush and Cruz, 2000; Kartahadimaja et al., 2021). When compared with previous studies (Oktaviani et al., 2021), F7 rice bran size did not have a significant difference with F6 rice-bran size. All the lines studied in the F6 generation had higher rice-bran size compared to the rice-bran size of the Padi Hitam and Mentik Wangi varieties. All F6 and F7 lines were also categorized into the moderate, based on the standards of the International Rise Research Institute (2013). Based on the research of Kartahadimaja et al. (2021), rice-bran size of the 12 genotypes studied varied in length, width, and thickness. B3 line is a new line with the shortest length (8.3 mm) but the widest among all lines. Meanwhile, the other 9 lines varied between 9.04 – 10.31 mm, and were included in the long seed criteria. Other lines, such as B2, B4, B7, F2, F3, F4, H1, H4, L2, and two varieties IR 64 and Ciherang, were included in the criteria for length with a narrower width. The IR64 variety is the variety with the smallest width (2.55 mm).

Amylose content is one of the criteria that determines grain quality (IRRI, 2012). In addition, amylose content is also one of the parameters used to predict the quality of processed rice (Juliano et al., 1965; Bhattacharaya and Juliano, 1985). The amylose content of the studied lines was between the amylose content of the comparison varieties. In addition, each line showed a significant difference in amylose content. Based on the criteria of Khush & Cruz (2000), the amylose content of grain could be grouped into 5 (five) criteria, waxy rice (0% to 2%), very low amylose rice (3% to 9%), low amylose rice (10% to 19 %), medium amylose rice (20 % to 25 %), and rice with high amylose (> 25 %). The amylose content of the studied lines was at a low criteria. The results of this study are a continuation of previous studies that examined the amylose content in the F6 line. The F6 generation PHMW lines were in various criteria. The classification includes very low amylose (lines 482-1-14), low amylose (lines 487-24-8, 482-

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9-134, 482-1-4, and 482-17-7) and medium amylose (lines 487-24-8, 482-9-134, 482-1-4, and 482-17-7) and medium amylose (lines 482-17-18) (Oktaviani et al., 2021).

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It is important to measure the antioxidant profile of the lines to map the antioxidant profile of the lines compared to checked varieties. The results showed that there was 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 a greater reducing power than the long white rice bran extract. The main antioxidant compounds detected by High Performance Liquid Chromatography (HPLC) were oryzanol (39-63%) and phenolic acids (33-43%). In addition, Laokuldilok et al. (2011) also found that black rice had 18-26% anthocyanin content. Ferulic acid was the dominant phenolic acid in the rice samples studied. Black rice contained higher levels of gallic acid, hydroxybenzoic acid, and protocatechoic acid compared to red rice and white rice. In addition, the research of Jun et al. (2011) reported that antioxidant activity of 40% pigmented rice-bran acetone extract, at an antioxidant concentration of 500 g/mL, red rice with the highest total phenolic and total flavonoids showed highest antioxidant activity (83.6% based on the radical DPPH test). In addition, there was an interesting study by Setyaningsih et al. (2015) who studied the positive correlation between amylose content and levels of antioxidant compounds, such as melatonin and phenolics.

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Conclusions

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All agronomic parameters studied were thought to be controlled by many genes (polygenic) and additive gene action, except for rice-bran length. The data from the analysis showed that there were various broad-sense heritability for each agronomic character. The characters of dry weight, plant height, weight of 1000 grain, and grain weight per panicle had moderate broad-sense heritability. Characters with high broad-sense heritability were number of tillers, number of panicles, and days to flowering. Based on the results of the study, all the characters observed in each line showed a different effect in each line studied. The value of broad-sense heritability this study varied for each character. Path analysis showed that the number of tillers had an effect on 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 seeds of the F6 and F7 lines showed that there were 4 (four) lines with completely the same color as the black rice, but the

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other 2 (two) lines still showed the color combination between the two checked varieties. When viewed from the aspect of rice-bran size, the rice-bran size mean of the 5 (five) lines studied (PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18 , 482-9-134) differ from the PHMW 487-24-8. Meanwhile, the rice-bran size of all studied lines was higher than the rice-bran size of the comparison varieties. The amylose content of lines was between the amylose content of the comparison 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 sticky rice traits (low amylose content) with multi-location and multi-season field trial, selection based on molecular markers related to genes associated with low amylose content and high antioxidant content, as well as organoleptic testing of processed rice from these lines.

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Acknowledgements

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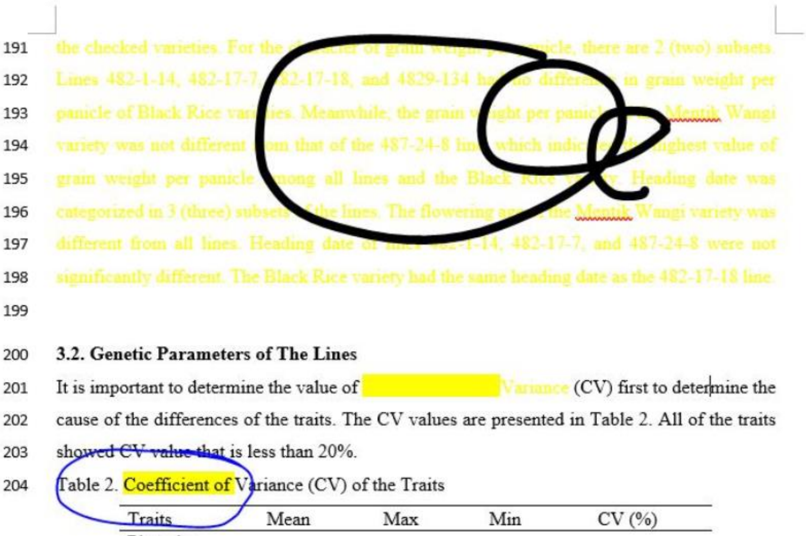
SUBMISSION OF REVISION DRAFT

Submission of Revision Draft

Participants Edit

Messages

Note	From
Dear Mr. Mafrikhul Muttaqin, Originally, I have sent the revision draft at 5th Jan, this night. However, some improvements at Title and the order of References must be made, so, I've re-sent the revision. Thank you. Regards Oktaviani	oktaviani 2022-01-06 04:20 AM
Dear Eka Oktaviani Good morning, Thank you for your revised manuscript. However, I am afraid that you are better to resubmit your revision. As you can see in the picture below the font should be yellow highlighted (blue mark), not yellow-colored font (black mark). Using yellow-colored font will be difficult to be read as well as comment.	aguspurwokousu 2022-01-06 07:46 AM



the checked varieties. For the [illegible] of grain weight per panicle, there are 2 (two) subsets. Lines 482-1-14, 482-17-7, 482-17-18, and 4829-134 had no difference in grain weight per panicle of Black Rice varieties. Meanwhile, the grain weight per panicle [illegible] Mentik Wangi variety was not different from that of the 487-24-8 line, which indic[illegible] highest value of grain weight per panicle among all lines and the Black Rice variety. Heading date was categorized in 3 (three) subsets of the lines. The flowering age of the Mentik Wangi variety was different from all lines. Heading date of lines 482-1-14, 482-17-7, and 487-24-8 were not significantly different. The Black Rice variety had the same heading date as the 482-17-18 line.

3.2. Genetic Parameters of The Lines

It is important to determine the value of [illegible] Variance (CV) first to determine the cause of the differences of the traits. The CV values are presented in Table 2. All of the traits showed CV value that is less than 20%.

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

Traits	Mean	Max	Min	CV (%)

Plant dry

Kindly delete your uploaded revised manuscript, make corrections, and resubmit as soon as possible.

Thank you!

Thank you, Mr.

I've resubmitted the draft correction just now.

(Oktaviani)

oktaviani
2022-01-06 10:52 AM

Dear Reviewer and Mr. Mafrikhul Muttaqin,

I'm sorry for the late response. I need an explanation about the comment from the reviewer in the title section at 25th January 2022 :

"Please response by adding what you have done, not just do revision without informing us"

What does that mean, Mr?

Thank you

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oktaviani
2022-03-01 01:16 PM

Dear Eka Oktaviani

Good afternoon,

Regarding your question, you may better to
1. highlight what you edit / change in the manuscript (done)
2. response / answer questions from reviewer in the 'comments' directly by using 'reply' function in the Ms.O. Word
3. explain what you change using 'insert a comment' function, especially for some big edit / change; a small change corresponded to reviewer suggestion is also better to be explained similar way
4. recheck the language (English)

aguspurwokousu
2022-03-01 02:13 PM

If there are further assistance are needed, feel free to reach me using this discussion board.

Thank you, Mr. I"ll try to revise according to the suggestion from reviewer.	oktaviani 2022-03-01 03:06 PM
Because I need to compare between the first round revision and the second one, may I send the revision about next two days? Thank's for your consideration, Mr.	oktaviani 2022-03-01 03:08 PM
Dear Eka Oktaviani Good morning, Yes, you can do that. Make sure you check the whole manuscript and address the issue; one way is, using my guidance. Lastly, if further assistance is needed, feel free to reach me using this discussion board.	aguspurwokousu 2022-03-02 06:47 AM

Small note: In this kind of correspondence, kindly refrain from calling someone using *only* 'mr./mrs./ms.'; it is weird and uncommon in the English-speaking country and or scientific community. You may better call directly by the name of a person you talk to or simply by 'title + name'.

Thank you for the guidance, Dr. Mafrikhul Muttaqin. I've submitted the revision by following the 2nd and 4th instructions. I highlighted yellow in several big changes and added comments to that change. Thank you	oktaviani 2022-03-04 06:46 AM

Add Message

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

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The development of low amylose pigmented rice with high antioxidant as superior varieties can be carried out by crossing the Black Rice with var. Mentik Wangi. Rice breeding program to obtain rice lines with low amylose content and high antioxidants has reached the F6 lines. The purpose of this study was to determine the agronomic traits, to figure up the genetic parameters, to describe the relationship among agronomic traits of the F6 lines, and to determine the morpho-biochemical profile of F7 de-hulled rice. Agronomic traits showed a different in each line. Genetic parameters in each trait showed a various category. Path analysis showed that the number of tillers had an effect on the dry weight and grain weight per panicle, but the plant dry weight did not directly affect the weight of 1000 grain. Directly, the weight of 1000 grain was only significantly affected by the grain weight per panicle trait. The F7 lines had difference in grain length and amylose content. The cumulative color of the PHMW482-17-7 and 482-17-18 lines showed the color 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. The lines have the potency to be developed as low amylose pigmented rice, although further research is still needed.

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Keywords: Black Rice, Mentik Wangi, morpho-biochemical, low amylose

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1. Introduction

Black rice is one type of pigmented rice, in addition to red rice and brown rice. This rice is often consumed as functional food, not as the main food ingredient (Purwanto et al., 2019). This is due to the various nutritional content of this type of rice. Black rice contains various micro and macronutrients that are important for human health (Apridamayanti et al., 2017). Protein, fat, fiber, vitamins, and minerals important for the human body are the various nutrients that this rice has (Kristamtini et al., 2012; Apridamayanti et al., 2017; Nurhidajah et al., 2018). Nutrients reported in black rice are vitamin B, vitamin E, Fe ion, thiamin, magnesium, niacin, phosphorus, dietary fiber (Kristamtini et al., 2012; Murali & Kumar, 2020), Zn, and Mn ion (Kristamtini et al., 2012). Murali & Kumar (2020) also reported that black rice is free from gluten and cholesterol, low in sugar, salt and fat.

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Black rice, is one of the pigmented rice classified on the basis of the color of the pericarp, aleurone, and endosperm of the rice grain (Kristamtini et al., 2012). One of the important compounds that contribute to black rice aleurone color is anthocyanin (Yoshimura et al., 2012; Palupi et al., 2020). Anthocyanins are responsible for the appearance of blue, purple, red, and orange colors in many fruits and vegetables (Miguel, 2011). Anthocyanins have high antioxidant activity and play an important role in human health (Prastiwi & 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 & Kumar, 2020; Tena et al., 2020), support health eye, anti-microbial, and prevention of neuro-degenerative diseases (Tena et al., 2020). The anthocyanins chemical structure is directly related to antioxidant activity, because it is able to prevent or inhibit oxidation reactions by scavenging free radicals and reduce levels of oxidative stress in cells (Tena et al., 2020).

Although black rice is known as a functional food with the above benefits, consumer acceptance of the texture of rice prepared from this rice is low, due to the non-stickiness texture of the cooked rice (Adi et al, 2020). Non-tender/non-sticky/non-glutinous rice has a dry, hard, and separate texture, even though it has been through the cooking process. Rice texture is determined by amylose amylopectin ratio (Cameron & Wang, 2005; Li et al., 2016a; Adi et al., 2020), post-harvest processing, and cooking method (Li et al., 2016b). In addition, Cameron & Wang (2005) also found that the texture/stickiness/hardness of rice was associated with protein and crude lipid content. The higher the amylose content of rice, the more tender the texture of the rice will be, and vice versa (Khumar & Khush, 1986; Bhattacharaya et al., 1999; Luna et al, 2015; Panesar & Kaur, 2016). Crude protein and lipid content were negatively correlated with the hardness of pasta flour and processed rice, but positively correlated with the level of rice stickiness (Cameron & Wang, 2005).

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. Researches to develop black rice with superior traits had been carried out by researchers from various countries. A research team from Korea, Kim et al. (2010) had created black rice C3GHi variety, with antioxidant compounds and cyanidin-3-glucoside content that was higher than that of local Korean black rice. Meanwhile, Wickert et al. (2014) reported the new varieties SCS120 Onix (black pericarp) and SCS119 Rubi (red pericarp) were released in 2013, and were recommended as new pigmented rice for the specialty rice market. SCS119 Rubi was a variety that resulted from mass selection towards accessions collected between 1993 and 1999, for the character of the long grain and had red pericarp. Meanwhile, the SCS120 Onix variety was the result of a conventional crossing between Epagri 107 and Riso Nero in 1996, with black pericarp. However, both varieties were recommended for their better nutritional content than white rice and for high productivity in the field. Zhu et al. (2017) had also 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. Sadimantara et al. (2021) have also carried out red rice plant breeding, but to get varieties with high productivity in the field.

This research has similarities and differences with previous studies, in aspects of germplasm sources, methods and objectives of the pigmented rice plant breeding program. The research method is the same as the pigmented rice development method reported by Wickert et al. (2014), but with different germplasm sources and plant breeding objectives. Meanwhile, the method of developing pigmented rice in the research of Zhu et al. (2017) and Zhang et al (2018) were carried out using modern breeding methods, namely genetic transformation and genome editing (CRISPR-Cas9). This study differs from the research of Roy and Shil (2020) in terms of the genetic material used for the germplasm source. In addition, this study also uses the same plant breeding method as the research of Kim et al. (2010) and Sadimantara et al. (2021), although with different germplasm sources. The purpose of this plant breeding program also has similarities with the research of Kim et al. (2010), but with the addition of another superior character, i.e low amylose pigmented rice. Although the low amylose pigmented rice variety named Jeliteng was released by the Ministry of Agriculture in 2019, through a conventional breeding between var. Ketan Hitam and Pandan Wangi cv Cianjur, the development of pigmented rice from other germplasm collections plays an important role in increasing the diversity of rice germplasm. The development of pigmented rice with a fluffier rice texture in Indonesia is expected to increase the source of rice germplasm with superior characters in Indonesia. This research contributes to give scientific information about the method of developing low amylose pigmented rice and related studies in it.

The development of superior varieties of pigmented rice that is fluffier and has a high antioxidant can be done by crossing the Black Rice variety with Mentik Wangi. Rice breeding to obtain rice lines with high antioxidants and a fluffier texture of rice has reached the 6^{th} line. The purpose of this study was to determine the agronomic traits, to figure up the genetic parameters, to describe the relationship among agronomic traits of the F6 lines, and to determine the morpho-biochemical profile of F7 dehulled rice. Analysis of agronomic traits played an important role in comparing the agronomic traits of lines with checked varieties. Determination of genetic parameters aimed to define the influence of environmental and genetic factors on the 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.

2. Materials and Methods

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The genetic materials consisted of 6 (six) potential F6 lines (from a conventional breeding of Black Rice cv Purworejo and var. Mentik Wangi) and 2 (two) checked varieties. These six lines were the result of 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 6th lines and checked varieties were planted until harvest to obtain the 7th lines. Dehulled rice of the 7th lines were analyzed for size, cumulative color, amylose content, and antioxidant activity. Meanwhile, the dehulled rice of the 6th lines were also subjected to the same analysis.

2.1. Field Trial of F6 Lines

Field trial of the 6th line 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) blocks and 5 (five) replications in each block, so the total of genotypes were 120 polybags. The soil used was ultisols. . The fertilizer consisted of NPK and manure. The media consisted of ultisols soil, roasted rice husks, and manure. Weeds, pests, and diseases were controlled by conventional method and by chemical pesticides. The agronomic traits observed were heading date, plant height, plant dry weight, number of tillers, weight of 1000 grain, number of panicle, and weight of grain per panicle.

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2.2. Morphology Characterization of F7 Dehulled Rice

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

2.3. Amylose Quantification of The Lines

The amylose content of the dehulled rice of the 7^{th} line 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

Measurement of antioxidant activity was carried out on de-hulled rice of the 6^{th} and 7^{th} 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 & Kitts, 2014).

Commented [NC14]: Assessing what kind of antioxidant? hydrogen atom transfer (HAT), single electron transfer (ET), reducing power, and metal chelation? Which one?

2.5. Data analysis

Data on agronomic traits of the 6^{th} lines, de-hulled rice length of 7^{th} lines, and amylose content of the 7^{th} lines were analyzed using SAS 9.4 software. Least Significant Difference (LSD) Test at the 95% confidence level ($\alpha = 0.05$) was carried out there was an indication of the influence of the lines on agronomic traits. The results of analysis of variance (F test) were used to quantify the value of Coefficient of Variance/CV, broad sense heritability, Coefficient of Genotypic and Phenotypic Variance. Broad sense heritability was calculated using formula suggested by Allard (1960). The determination of heritability criteria follows the following criteria of McWhirter (1979). Coefficient of Genotypic and Phenotypic Variance were calculated using Singh & Chaudhary (1977) formula. Coefficient of genotypic and phenotypic variation were categorized as proposed by Sivasubramanian & Madhavamenon (1973). The difference in antioxidant activity of the 7^{th} and the 6^{th} lines were identified by the student T-test. Path analysis was performed using the LISREL 8.2 software. Path analysis was used to determine the direct and indirect factors determining the yield component, namely weight of 1000 grain and weight of grain per panicle.

Commented [NC15]: Student t-test

3. Results

3.1. Agronomic Traits of The Lines

Commented [NC16]: What is your finding regarding evaluation of agronomic traits?

The results of the Least Significant Difference (LSD) test to determine the difference in the response of each line based on agronomic traits can be seen in Table 1.

Table 1. Differences agronomic traits of six lines

Genotype	Plant Dry weight (g)	Plant length (cm)	Number of tillers	Number of panicles	Weight of 1000 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±6.01b	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±3.13a	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±3.97 b	36.70±5.43b	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±2.27a	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±1.89a	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±6.43a	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±1.95a	15.42±0.63 bc	15.1±0.65b	2.24±0.47 ab	64.00±1.41 b
Mentik wangi	66.90±13.78c	101.80±6.60d	25.59±4.48a	14.08±2.76 b	15.22±2.34 b	2.77±0.71 b	82.83±4.09 d

Note : Blue color represents the highest value and red color represents the lowest value

The traits observed in each line showed a different effect in each line. The plant dry weight of all lines was in the range of the two checked varieties. There were two subsets of plant dry weight trait. The same analysis was applied to the trait of plant height. The plant height of all lines was in the range of the two checked varieties. There were 3 (three) subsets of plant height traits. Line 482-1-4 had difference with other lines. Lines 482-1-7, 482-1-4, and 487-24-8 had no difference in plant height trait. Meanwhile, lines 482-17-18 and 482-9-134 also did not have difference in plant height trait, because they were in one subset (group). For the number of tillers, there were lines that had a higher number of tillers than the checked varieties, namely lines 482-1-14 and 482-1-4. The number of tillers of the other lines was the same as in the checked varieties. The grouping of panicle numbers is more varied. There are 3 (three) subsets that group the lines based on the character of the number of panicles. The number of panicles of the 482-1-14 line was higher and statistically different compared to the checked varieties. Meanwhile, lines 482-1-4, 482-9-134, 487-24-8 and Black Rice variety did not have differences statistically in the number of panicle trait. The number of panicles of the Mentik Wangi variety did not differ from that of the 482-17-7 line. For weight of 1000 grain trait, there were only 2 (two) subsets. The weight of 1000 grain of the checked varieties did not differ from those of 482-17-18, 482-9-134, and 487-24-8. The other three lines had weight of 1000 grain lower than the checked varieties. For the character of grain weight per panicle, there are 2 (two) subsets. Lines 482-1-14, 482-17-7, 482-17-18, and 4829-134 had no difference in grain weight per panicle of Black Rice varieties. Meanwhile, the grain weight per panicle of the Mentik Wangi variety was not different from that of the 487-24-8 line, which indicated the highest value of grain weight per panicle among all lines and the Black Rice variety. Heading date was categorized in 3 (three) subsets of the lines. The flowering age of the Mentik Wangi variety was different from all lines. Heading date of lines 482-1-14, 482-17-7, and 487-24-8 were not significantly different. The Black Rice variety had the same heading date as the 482-17-18 line.

3.2. Genetic Parameters of The Lines

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

Commented [NC17]: Coefficient of variation?

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	14	15,22
Plant height (cm)	73.12	96.8	53.2	6,03
Weight of 1000 grain (g)	14.42	22.12	7.04	9,74
Number of panicle	14.93	25	9	11,44
Grain weight per panicle (g)	2.22	3.64	1.1	14,63
Heading date (days)	64.24	72	57	3,20

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, weight of 1000 grain, and grain weight per panicle were in the moderate range of broad sense heritability. Traits with high value of broad sense heritability were number of tiller, number of panicle, and heading date.

The traits with the highest CGV and CPV value was the number of tillers. Furthermore, traits with high CGV value were plant height, weight of 1000 grain, number of panicle, and weight of grain per panicle. Traits with a rather low CGV value were plant dry weight and heading date. Meanwhile, the traits with a high CPV value were the number of tillers, the number of panicle, and the weight of grain per panicle. Traits with a fairly high CPV value included plant dry weight, plant height, and weight of 1000 grain. The CGV value on the number of panicle and the heading date traits was greater than the CPV value. In addition, the CGV of these traits had a lower value than the CPV.

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 1000 grain (g)	14.42	1.47	1.97	3.44	42.79	Moderate	8.42	quite high	12.87	quite high
Number of panicle	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

3.3. Inter-Relationship Among Traits

The dependent variables as the yield component were the weight of 1000 grain and the weight of grain per panicle. Another agronomic traits as an independent variable. The path analysis diagram could be seen in Figure 1. Path analysis showed that the number of tillers had an direct effect on the plant dry weight and grain weight per panicle, but the plant dry weight did not directly affect the weight of 1000 grain.

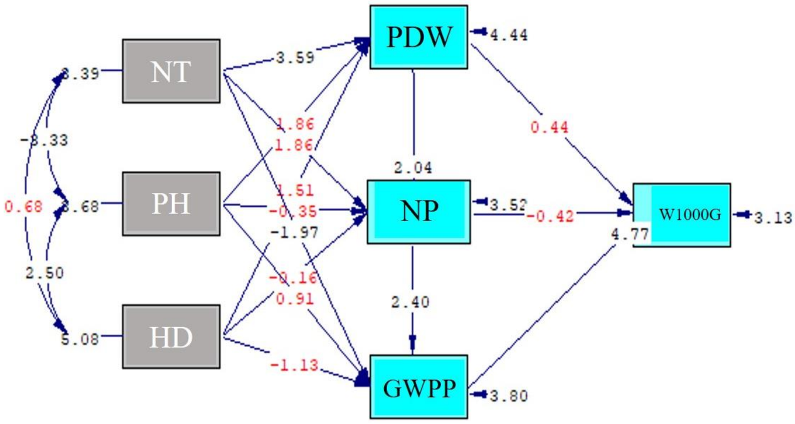

Figure 1. Path diagram of inter-relationship traits (P-value = 0.19579) (Notes: NT: Number of tillers; PH: Plant height; HD: Heading date; PDW: Plant dry weight; NP: Number of panicle; GWPP: Grain weight per panicle: W1000G: Weight of 1000 Grain).

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3.4. Morpho-Biochemical Profile of Dehulled Rice

Figure 2 showed that the cumulative color of each F6 and F7 line has similarities. Four lines completely had the same color as the Black Rice variety, but the other lines still showed the color combination between the checked varieties.

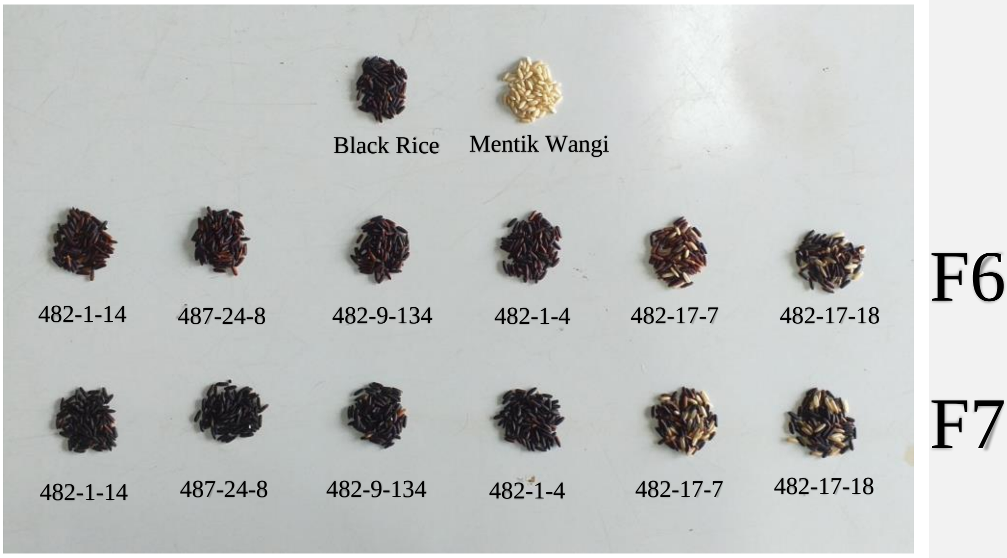

Figure 2. Color of the de-hulled rice of lines and checked varieties

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Based on the aspect of de-hulled 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, the size of de-hulled rice of all lines was higher than these of the checked varieties.

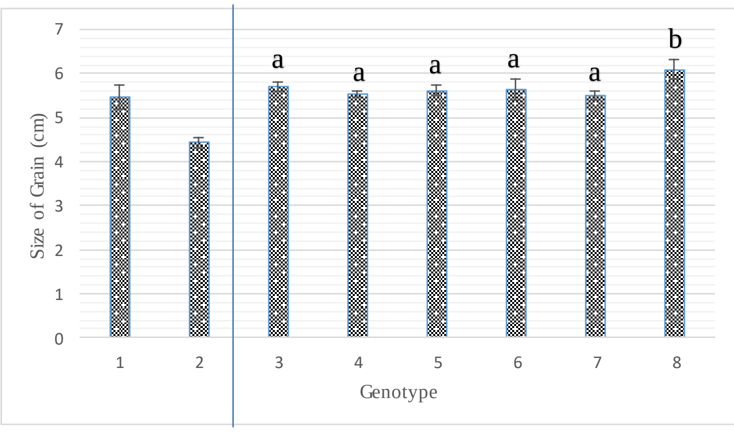

Figure 3. Size of F7 Dehulled Rice (Note : No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; while no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18 , 482-9-134, 487-24-8 lines, respectively)

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 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 of 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).

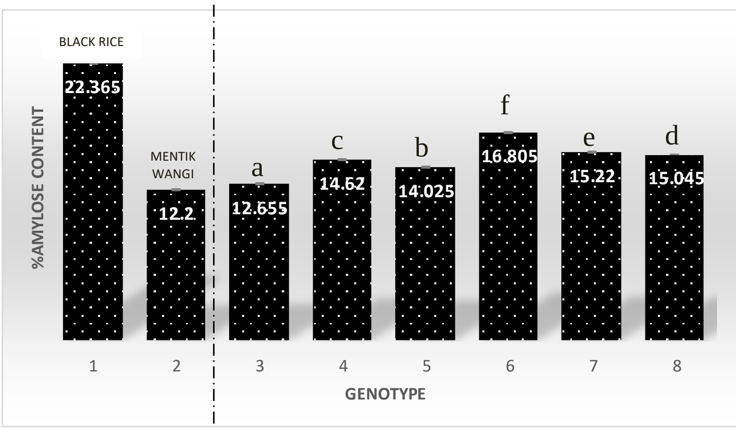

Figure 4. Amylose profile of F7 lines and checked varieties (Note : No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; while no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18 , 482-9-134, 487-24-8 lines, respectively)

Commented [NC20]: Remark 1, 2, a, b, etc ?

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

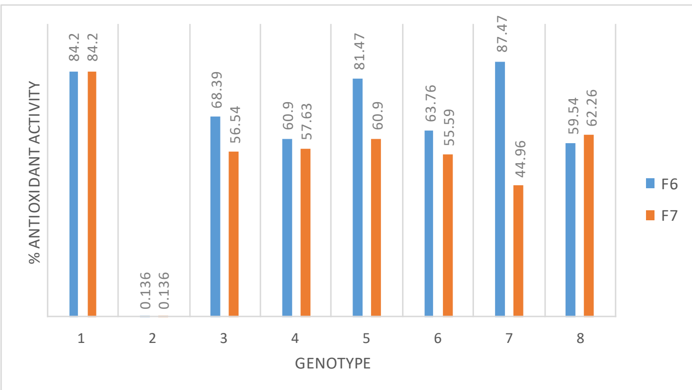

Figure 6. Antioxidant activity of F6 and F7 dehulled rice. (Note : No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; while no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18 , 482-9-134, 487-24-8 lines, respectively)

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, research by Kasim et al. (2020) showed that the rice plant height of the 17 rice varieties studied ranged from 95-160 cm. The plant heights 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 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 heights are preferred by farmers and researchers. While the parameters of heading date, varied between 104-151 DAS (Days After Sowing). This indicates that these varieties have a longer flowering life than the lines used in this study. The earlier flowering age of rice varieties is preferred by farmers and researchers, because varieties with an earlier flowering age have a shorter life cycle, thus allowing a higher frequency of harvesting per year than varieties with a longer flowering age. This can have an impact on farmers' annual income. Meanwhile, the number of panicles varied between 12-24. The range of genotypic panicles in this study was in the range of the number of panicles studied by Kasim et al. (2020). Meanwhile, the total number of grain per plant also varied, between 128 to 305. The weight of 100 grain of rice also varied, between 1.4 – 2.56 grams.

Commented [NC21]: ???

Based on the agronomic data of the lines, there were outside the range of traits values of the checked varieties. This corresponds with the opinion of Welsh (1981), which stated that the action of duplicate genes and additive genes could cause transgressive segregation. Transgressive segregation was segregation that causes offspring to have characters with measurement ranges that are below or even above their parents, so that it can provide opportunities for breeders to get the desired segregate (Nugraha & Suwarno, 2007).

4.2. Genetic Parameters of The Lines

The value of broad sense heritability of this study is in the range that varies for each character. Heritability was used as the basis to estimate the relative contribution effect of genetic and non-genetic factors to the total phenotypic variance in a population (Ene et al., 2015; Konate et al., 2016). The data from the analysis showed that there were various value of broad sense heritability for each traits. The characters of dry weight, plant height, weight of 1000 grain, and grain weight per panicle had moderate broad-sense heritability. Traits with high heritability estimates were number of tillers, number of panicles, and heading date. Adhikari et al. (2018) wrote that the low heritability value indicates that the traits appears 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 which would be used as a basis of selection was a character that has a high broad-sense heritability value (Begum et al., 2015). A low broad-sense heritability value would have an impact on a low 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 weight of 1000 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). Research by Ogunbayo et al. (2014) also showed results in accordance with this study. 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 the environment one. Similar results were confirmed in the study of Konate et al. (2016), that heading date, number of tillers, and number of panicles are also categorized as traits with high broad-sense heritability. The weight of 1000 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. This is in line with the research of Bagati et al. (2016), 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 influence of the plant growth environment on the observed traits is explained by the level of difference value between those two parameters. Furthermore, Adhikari et al. (2018) wrote that the large difference in the values of these parameters indicated a large environmental influence on the expression of certain traits.

4.3. Inter-Relationship Among Traits

Path analysis showed that the number of tillers had an effect on 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 a number of related traits. Therefore, crop yields usually depend on the actions and interactions of a number of important traits. Knowledge of the various traits that determine crop yields is important for examining various yield components (growth parameters) and paying more attention to yield components that have the greatest influence on crop yields (Kinfe et al, 2015). A good selection trait was one that has a large real correlation value and a high direct or indirect effect value on the yield (Boer, 2011).

The results of this study have differences when compared with the research of Akhmadi et al. (2017). The research by Akhmadi et al. (2017) showed that traits that had a direct effect on high yields were length of panicle, weight of 1000 filled grain, number of filled grains per panicle and grain filling period. The character of the generative plant height and the total number of grain per panicle had a high negative direct effect on yield, but the indirect effect through panicle length was quite high. Several studies that have been carried out using this analysis showed the characteristics of 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 100 grains which have a direct effect on high yield on some rice populations (Safitri et al., 2011; Rachmawati et al., 2014). If a certain trait is known to have a direct effect on the yield component, then the determination of yield can be known by looking at the profile of the trait.

Commented [NC22]: What are the consequences for the research? What is the contribution of these finding to our knowledge, science and technology advancement!

4.4. Morpho-Biochemical Profile of The Lines

The cumulative color of the dehulled rice of F6 and F7 lines showed that there were 4 (four) lines with completely have the same color as the Black Rice variety, but the other 2 (two) lines still showed the color combination between the parents. The research of Laokuldilak et al. (2011) reported that pigmented rice contains high Cyanidin-3-Glucoside (58-95%). C3G is part of the anthocyanin group of compounds. The appearance of rice-bran color in pigmented rice is thought to be related to the content of Cyanidin-3-Glucoside compound, although the relationship between these two is not clear, due to the complexity of the existing genetic system. According to research Ham et al. (2015), there was a significant positive correlation on C3G content towards the brightness and yellow color of rice-bran. However, the genetic system responsible for rice-bran pigmentation and Cyanidin-3-Glucoside content is not same, but is related through a specific pathway with anthocyanin metabolism. Mackon 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 aspect of dehulled rice size, the average size of the 5 (five) lines studied (PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18 , 482-9-134) differ from the PHMW 487-24-8. Meanwhile, the dehulled rice size of all lines was higher than that of the checked varieties. The length of the dehulled rice of the lines was above the these 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 transgressive segregation of alleles responsible for the expression of 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 to determine the shape of the rice. The shape of rice grain is one of the determinants of the quality of rice grain. Grain quality is one of the selection parameters in plant breeding program (Kush and Cruz, 2000; Kartahadimaja et al., 2021). When compared with previous studies (Oktaviani et al., 2021), dehulled rice size of F7 lines did not have a significant difference with F6 ones. All the lines studied in the F6 generation had higher dehulled rice size compared to those of the Black Rice and Mentik Wangi varieties. All F6 and F7 lines were also categorized into the moderate, based on the standard of the International Rise Research Institute (2013). Based on the research of Kartahadimaja et al. (2021), grain size of the 12 genotypes studied varied in length, width, and thickness. B3 line is a new line with the shortest length (8.3 mm) but the widest among all lines. Meanwhile, the other 9 lines varied between 9.04 – 10.31 mm, and were included in the long seed criteria. Other lines, such as B2, B4, B7, F2, F3, F4, H1, H4, L2, and two varieties IR 64 and Ciherang, were included in the criteria for length with a narrower width. The IR64 was the variety with the smallest width (2.55 mm).

Amylose content was one of the criteria that determines grain quality (IRRI, 2013). In addition, amylose content was also used to predict the quality of processed rice (Juliano et al., 1965; Bhattacharaya and Juliano, 1985). 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 a low criteria. The results of this study are a continuation of previous studies that examined the amylose content in the F6 line (Oktaviani et al., 2021).

It is important to measure the antioxidant activity of the lines to map the antioxidant profile of the lines compared to checked varieties. The results showed that there was 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 a greater reducing power than the long white rice bran extract. In addition, Laokuldilok et al. (2011) also found that black rice had 18-26% anthocyanin content, and ferulic acid was the dominant phenolic acid in the rice samples studied. In the same research, Black Rice variety contained higher levels of gallic acid, hydroxybenzoic acid, and protocatechoic acid compared to red rice and white rice. In addition, the research of Jun et al. (2012) reported that antioxidant activity of 40% pigmented rice-bran acetone extract, at an antioxidant concentration of 500 g/mL, red rice with the highest total phenolic and total flavonoids showed highest antioxidant activity (83.6% based on the radical DPPH test). In addition, there was an interesting study by Setyaningsih et al. (2015) who studied the positive correlation between amylose content and levels of antioxidant compounds, such as melatonin and phenolics. This finding can provide an important basis of information if one of the biochemical compound known, although the case in each variety will be specific.

Commented [NC23]: Then. What is the implication?

Conclusion

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The data from the analysis showed that there were various broad-sense heritability for each agronomic character. The characters of dry weight, plant height, weight of 1000 grain, and grain weight per panicle had moderate broad-sense heritability. Characters with high broad-sense heritability were number of tillers, number of panicles, and days to flowering. Based on the results of the study, all the characters observed in each line showed a different effect in each line studied. The value of broad-sense heritability this study varied for each character. Path analysis showed that the number of tillers had an effect on 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 seeds of the F6 and F7 lines showed that there were 4 (four) lines with completely the same color as the black rice, but the other 2 (two) lines still showed the color combination between the two checked varieties. Based on the aspect of 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) differ from the PHMW 487-24-8. Meanwhile, the dehulled rice size of all studied lines was higher than the 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 trial, selection based on molecular markers related to genes associated with low amylose content and high antioxidant content, as well as organoleptic testing of processed rice from these lines.

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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 antioxidants can be used as candidates for rice varieties with superior characters. This is able to enrich to the collection of superior rice germplasm in Indonesia.

Acknowledgements

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NOTIFICATION OF ACCEPTANCE

Notifications

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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

ABSTRACT

A comprehensive understanding of the genetic parameters and the inter-relationship among characters in the breeding population is crucial for selecting low amylose black rice varieties. Meanwhile, dehulled rice morpho-biochemistry can be used to determine the grain profile of F6 and F7 lines. This study aimed 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. 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 1000 grains. Directly, the weight of 1000 grains was only significantly affected by the grain weight per panicle trait. The F7 lines had a difference in grain length and amylose content. The color of the PHMW482-17-7 and 482-17-18 dehulled rice 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.

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Keywords: Black Rice, Mentik Wangi, morpho-biochemical, low amylose

1. Introduction

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.

Black rice is one type of pigmented rice instead of red rice and brown rice. It is often consumed as functional food, not the main food ingredient (Purwanto et al., 2019), because of the different nutritional content of this type of rice. Black rice contains various micro and macronutrients important for human health (Apridamayanti et al., 2017). Protein, fat, fiber, vitamins, and minerals essential for the human body are the various nutrients this rice has (Kristamtini et al., 2012; Apridamayanti et al., 2017; Nurhidajah et al., 2018). Nutrients reported in black rice are vitamin B, vitamin E, Fe iron, thiamin, magnesium, niacin, phosphorus, and dietary fiber (Kristamtini et al., 2012; Murali & Kumar, 2020), Zn, and Mn ion (Kristamtini et al., 2012). Murali & Kumar (2020) also reported that black rice is free from gluten and cholesterol low in sugar, salt, and fat.

Black rice is a pigmented rice classified based on the color of the rice grain's pericarp, aleurone, and endosperm (Kristamtini et al., 2012). One of the important compounds contributing to black rice aleurone is anthocyanin (Yoshimura et al., 2012; Palupi et al., 2020). Anthocyanins are responsible for blue, purple, red, and orange colors in many fruits and vegetables (Miguel, 2011). Anthocyanins have high antioxidant activity and play an essential role in human health (Prastiwi & 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 & Kumar, 2020; Tena et al., 2020), support health eye, anti-microbial, and prevention of neurodegenerative diseases (Tena et al., 2020). The anthocyanins' chemical structure is directly related to antioxidant activity because it can prevent or inhibit oxidation reactions by scavenging free radicals and reducing levels of oxidative stress in cells (Tena et al., 2020).

Although black rice is known as a functional food with the above benefits, consumer acceptance of the texture of rice prepared from this rice is low due to the non-stickiness texture of the cooked rice (Adi et al., 2020). Non-tender/non-sticky/non-glutinous rice has a dry, hard, and separate texture, even though it has been through the cooking process. Rice texture is determined by amylose amylopectin ratio (Cameron & Wang, 2005; Li et al., 2016a; Adi et al., 2020), post-harvest processing, and cooking method (Li et al., 2016b). In addition, Cameron & Wang (2005) also found that rice's texture, stickiness, and hardness were associated with protein and crude lipid content. The higher the amylose content of rice, the more tender the texture of the rice will be, and vice versa (Khumar & Khush, 1986; Bhattacharaya et al., 1999; Luna et al., 2015; Panesar & Kaur, 2016). Crude protein and lipid content were negatively correlated with the hardness of pasta flour and processed rice but positively correlated with the level of rice stickiness (Cameron & Wang, 2005).

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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. Researchers from various countries have carried out research to develop black rice with superior traits. 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) reported the new varieties SCS120 Onix (black pericarp) and SCS119 Rubi (red pericarp) were released in 2013 and were recommended as new pigmented rice for the specialty rice market. SCS119 Rubi was a variety that resulted from mass selection towards accessions collected between 1993 and 1999 for the character of the long grain and had red pericarp.

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Meanwhile, the SCS120 Onix variety resulted from a conventional crossing between Epagri 107 and Riso Nero in 1996, with black pericarp. However, 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. Sadimantara et al. (2021) have also carried out red rice plant breeding to get varieties with high productivity in the field.

This research has similarities and differences with previous studies regarding germplasm sources, methods and objectives of the pigmented rice plant breeding program. The research method is the same as the pigmented rice development method reported by Wickert et al. (2014), but with different germplasm sources and plant breeding objectives. Meanwhile, the method of developing pigmented rice in the research of Zhu et al. (2017) and Zhang et al. (2018) was carried out using modern breeding methods, namely genetic transformation and genome editing (CRISPR-Cas9). This study differs from Roy and Shil's (2020) research regarding the genetic material used for the germplasm source. In addition, this study also uses the same plant breeding method as the research of Kim et al. (2010) and Sadimantara et al. (2021), although with different germplasm sources. The purpose of this plant breeding program also has similarities with the research of Kim et al. (2010), but with another superior character, i.e., low amylose pigmented rice. However, the Ministry of Agriculture released the low amylose pigmented rice variety named Jeliteng in 2019 through conventional breeding. Ketan Hitam and Pandan Wangi cv Cianjur, the development of pigmented rice from other germplasm collections play an important role in increasing the diversity of rice germplasm. The development of pigmented rice with a fluffier rice texture in Indonesia is expected to increase the source of rice germplasm with superior characters in Indonesia. This research gives scientific information about developing low amylose pigmented rice and related studies.

The development of superior varieties of pigmented rice that are fluffier and have a high antioxidants can be done by crossing the Black Rice variety with Mentik Wangi. Rice breeding to obtain rice lines with high antioxidants and a fluffier texture of rice has reached the 6^{th} line. The purpose of this study was 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 played an important role in comparing the agronomic traits of lines with checked varieties. Determination of genetic parameters aimed 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.

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 six 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 6^{th} lines and checked varieties were cultivated until harvest to obtain the 7^{th} lines. Dehulled rice of the 7^{th} line was analyzed for size, cumulative color, amylose content, and antioxidant activity. Meanwhile, the dehulled rice of the 6^{th} line was also subjected to the same analysis.

2.1. Field Trial of F6 Lines

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Field trial of the 6^{th} line 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) blocks and 5 (five) replications in each block, so the total of genotypes was 120 polybags. The growth media contains ultisols soil, roasted rice husks, and manure. The growth media was fertilized with NPK and manure. Conventional techniques and chemical pesticides are employed for controlling weeds, pests, and diseases. The agronomic traits observed were heading date, plant height, plant dry weight, number of tillers, the weight of 1000 grain, number of panicles, and grain weight per panicle.

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2.2. Morphology Characterization of F7 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 segregation pattern.

2.3. Amylose Quantification of The Lines

The amylose content of the dehulled rice of the 7^{th} line 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

Measurement of antioxidant activity was measured on dehulled rice of the 6th and 7th 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 & Kitts, 2014).

2.5. Data analysis

Data on agronomic traits of the 6th lines, dehulled rice length of the 7th lines, and amylose content of the 7th lines was analyzed using SAS 9.4 software. The Least Significant Difference (LSD) test at the 95% confidence level ($\alpha = 0.05$) 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 following criteria of McWhirter (1979). The Coefficient of Genotypic and Phenotypic Variance was calculated using Singh & Chaudhary's (1977) formula. The genotypic and phenotypic variation coefficient was categorized as proposed by Sivasubramanian & Madhavamenon (1973). The student T-test identified the difference in antioxidant activity of the 7th and the 6th 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 1000 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.

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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 1000 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±6.01b	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±3.13a	13.95±1.31 ab	12.10±1.85 a	1.97±0.36 ab	60.67±3.10 a

Genotype	Plant Dry Weight (g)	Plant length (cm)	Number of tillers	Number of panicles	Weight of 1000 grain (g)	Grain weight per panicle (g)	Heading date (days)
Line 482-1-4	51.91±5.53 b	69.93±3.97 b	36.70±5.43b	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±2.27a	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±1.89a	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±6.43a	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±1.95a	15.42±0.63 bc	15.1±0.65b	2.24±0.47 ab	64.00±1.41 b
Mentik Wangi	66.90±13.78c	101.80±6.60d	25.59±4.48a	14.08±2.76 b	15.22±2.34 b	2.77±0.71 b	82.83±4.09 d

Note: Blue color represents the highest value, and the red color represents the lowest value

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The traits observed in each line showed a different effect in each line. The plant dry weight of all lines was in the range of the two checked varieties. There were two subsets of plant dry weight trait. The same analysis was applied to the trait of plant height. The plant height of all lines was in the range of the two checked varieties. There were 3 (three) subsets of plant height traits. Line 482-1-4 had a difference from other lines. Lines 482-1-7, 482-1-4, and 487-24-8 had no difference in plant height traits.

Meanwhile, lines 482-17-18 and 482-9-134 did not differ in plant height because they were in one subset (group). For the number of tillers, some lines had a higher number of tillers than the checked varieties, namely lines 482-1-14 and 482-1-4. The number of tillers of the other lines was the same as in the checked varieties. The grouping of panicle numbers is more varied. There are 3 (three) subsets that group the lines based on the character of the number of panicles. The number of panicles of the 482-1-14 line was higher and statistically different than the checked varieties.

Meanwhile, lines 482-1-4, 482-9-134, 487-24-8, and the Black Rice variety did not have differences statistically in the number of panicle traits. The number of panicles of the Mentik Wangi variety did not differ from that of the 482-17-7 line. For the weight of 1000 grain trait, there were only 2 (two) subsets. The weight of 1000 grains of the checked varieties did not differ from those of 482-17-18, 482-9-134, and 487-24-8. The other three lines weighted 1000 grains lower than the checked varieties. For the character of grain weight per panicle, there are

2 (two) subsets. Lines 482-1-14, 482-17-7, 482-17-18, and 4829-134 had no difference in grain weight per panicle of Black Rice varieties.

Meanwhile, the grain weight per panicle of the Mentik Wangi variety was not different from that of the 487-24-8 line, which indicated the highest value of grain weight per panicle among all lines and the Black Rice variety. The heading date was categorized into 3 (three) subsets. The flowering age of the Mentik Wangi variety was different from all lines. The heading dates of lines 482-1-14, 482-17-7, and 487-24-8 were not significantly different. The Black Rice variety had the same heading date as the 482-17-18 line.

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3.2. Genetic Parameters of The Lines

It is 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%.

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

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Traits	Mean	Max	Min	CV (%)
Plant dry weight (g)	46.59	84.61	28.63	11,48
Number of tillers	29.91	54	14	15,22
Plant height (cm)	73.12	96.8	53.2	6,03
Weight of 1000 grain (g)	14.42	22.12	7.04	9,74
Number of panicles	14.93	25	9	11,44
Grain weight per panicle (g)	2.22	3.64	1.1	14,63
Heading date (days)	64.24	72	57	3,20

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 1000 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.

The trait with the highest CGV and CPV value was the number of tillers. Furthermore, traits with high CGV value were plant height, the weight of 1000 grains, number of panicles, and weight of grain per panicle. Traits with a low CGV value were total dry weight and heading date. Meanwhile, the traits with a high CPV value were the number of tillers, the number of panicles, and the weight of grain per panicle. Traits with a fairly high CPV value included plant dry weight, plant height, and weight of 1000 grain. The CGV value on the number of panicles and the heading date traits was greater than the CPV value. In addition, the CGV of these traits had a lower value than the CPV.

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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 1000 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

3.3. Inter-Relationship Among Traits

The dependent variables as the yield component were the weight of 1000 grains and the weight of grain per panicle. Other agronomic traits as an independent variable. The path analysis diagram can 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 grains.

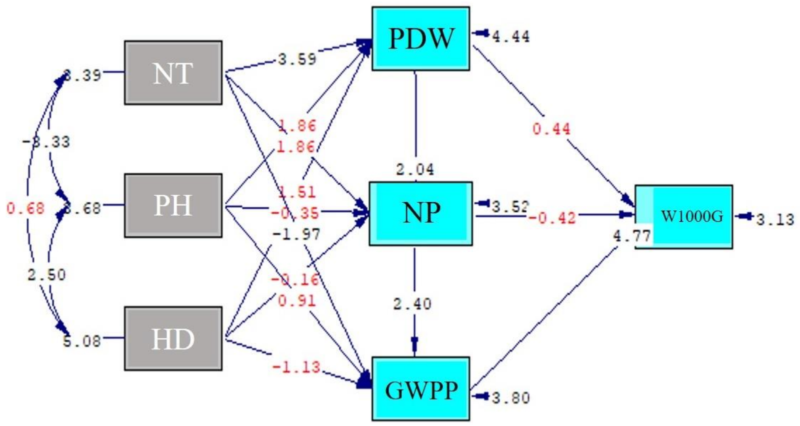

Figure 1. Path diagram of inter-relationship traits (P-value = 0.19579) (Notes: NT: Number of tillers; PH: Plant height; HD: Heading date; PDW: Plant dry weight; NP: Number of panicles; GWPP: Grain weight per panicle: W1000G: Weight of 1000 Grain).

3.4. Morpho-Biochemical Profile of Dehulled Rice

Figure 2 shows that the cumulative color of each F6 and F7 line has similarities. Four lines had the same color as the Black Rice variety, but the other lines showed the color combination between the checked varieties.

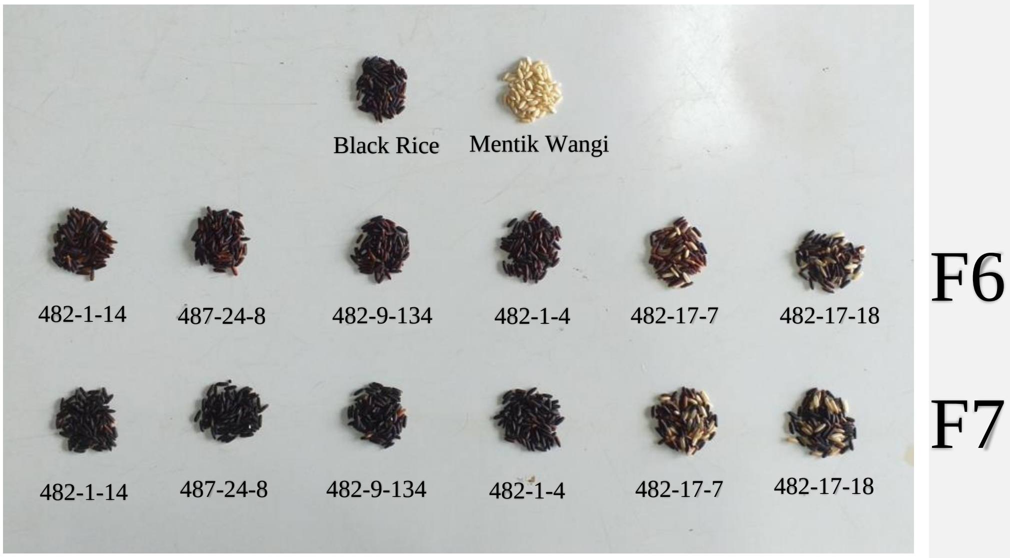

Figure 2. Color of the dehulled rice of lines and checked varieties

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.

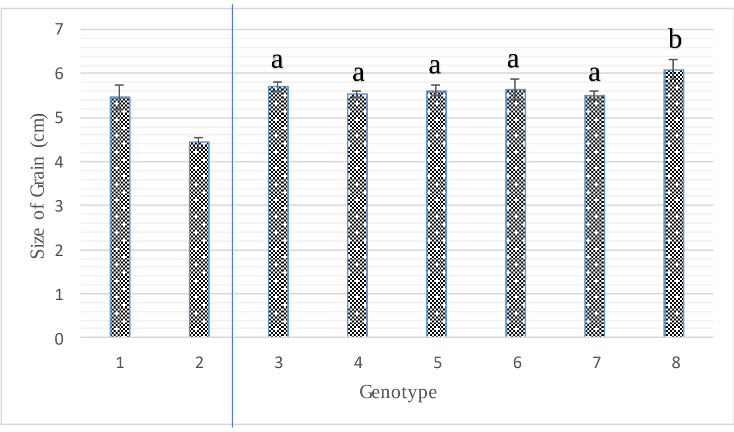

Figure 3. Length Size of F7 Dehulled Rice (Note: No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; while no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134, 487-24-8 lines, respectively)

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 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).

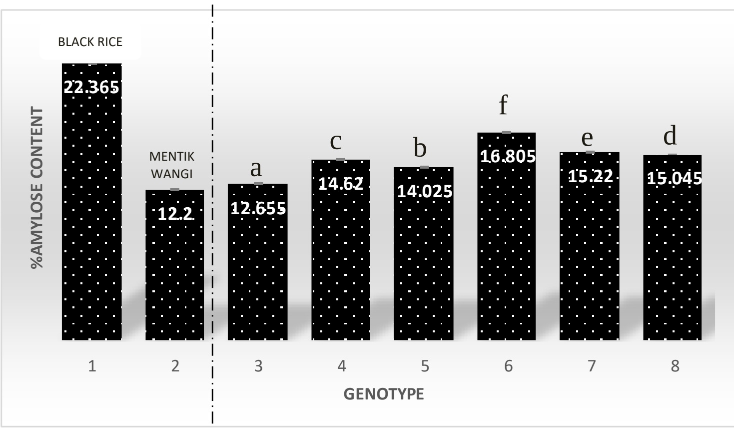

Figure 4. Amylose profile of F7 lines and checked varieties (Note: No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; while no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134, 487-24-8 lines, respectively)

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

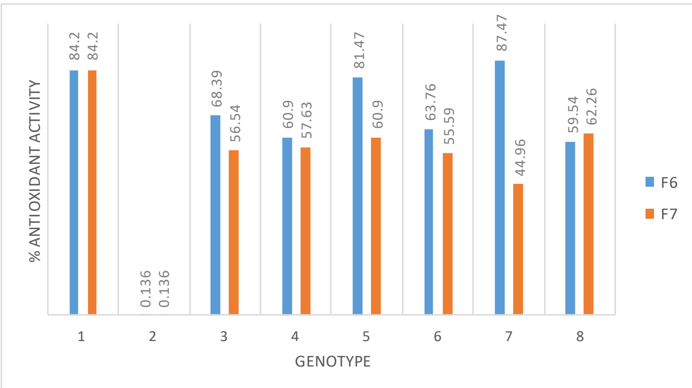

Figure 6. Antioxidant activity of F6 and F7 dehulled rice. (Note: No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134, 487-24-8 lines, respectively)

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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 studied ranged from 95-160 cm. The plant heights 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 heights are preferred by farmers and researchers.

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In comparison, the parameters of heading date varied between 104-151 DAS (Days After Sowing). It indicates that these varieties have a longer flowering life than the lines used in this study. Farmers and researchers prefer the earlier flowering age allowing a higher frequency of harvesting per year than varieties with a longer flowering age. It can have an impact on farmers' annual income. Meanwhile, the number of panicles varied between 12-24. The range of genotypic panicles in this study was in the range of the number of panicles studied by Kasim et al. (2020). Meanwhile, the total number of grains per plant also varied, between 128 to 305. The weight of 100 grains also varied, between 1.4 – 2.56 grams.

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Based on the agronomic data of the lines, there were outside the range of traits values of the checked varieties. It corresponds with the opinion of Welsh (1981), which stated that the action of duplicate genes and additive genes could cause transgressive segregation. Transgressive segregation was segregation that causes offspring to have characters with measurement ranges below or even above their parents to provide opportunities for breeders to get the desired segregation (Nugraha & Suwarno, 2007).

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4.2. Genetic Parameters of The Lines

The value of the broad-sense heritability of this study is in the range that varies for each character. Heritability was used to estimate the relative contribution effect of genetic and non-genetic factors to the total phenotypic variance in a population (Ene et al., 2015; Konate et al., 2016). 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 1000 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 a low 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 1000 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). Research by Ogunbayo et al. (2014) also showed results following this study. 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. Similar results were confirmed in Konate et al.'s (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 1000 grain traits also had a moderate broad-sense heritability.

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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.'s (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 influence of the plant growth environment on the observed traits is explained by the level of a difference value between those two parameters. Furthermore, Adhikari et al. (2018) wrote that the large difference in the values of these parameters indicated a sizeable environmental influence on the expression of certain traits.

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 grains. Directly, the weight of 1000 grains 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 important 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 have differences when compared with 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 grains, 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 that have been carried out using this analysis showed the characteristics of 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 100 grains which have a direct effect on the high yield on some rice populations (Safitri et al., 2011; Rachmawati et al., 2014). If a specific trait is known to affect the yield component directly, then the yield determination can be known by looking at the profile of the trait. The results of this study can give information related to characters that correlate with the yield. Therefore, knowledge related to these can be used as initial information before yield or productivity are known.

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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%). C3G is part of the anthocyanin group of compounds. 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. Mackon 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 dehulled rice size, the average size of the 5 (five) lines studied (PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134) differ from the PHMW 487-24-8. Meanwhile, the dehulled rice size of all lines was larger than that of the checked varieties. 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 of alleles responsible for the expression of 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. 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 previous studies (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. B3 line is a new line with the shortest length (8.3 mm) but the widest among all lines.

Meanwhile, the other nine lines varied between 9.04 – 10.31 mm and were included in the long seed criteria. Other lines, such as B2, B4, B7, F2, F3, F4, H1, H4, L2, and two varieties IR 64 and Ciherang, were included in the criteria for length with a narrower width. The IR64 was the variety with the smallest width (2.55 mm).

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Amylose content was one of the criteria that determined grain quality (IRRI, 2013). In addition, amylose content was also used to predict the quality of processed rice (Juliano et al., 1965; Bhattacharaya and Juliano, 1985). 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. The results of this study are a continuation of previous studies that examined the amylose content in the F6 line (Oktaviani et al., 2021).

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It is 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. In addition, Laokuldilok et al. (2011) also found that black rice had 18-26% anthocyanin content, and ferulic acid was the dominant phenolic acid in the rice samples studied. In the same research, the Black Rice variety contained higher levels of gallic acid, hydroxybenzoic acid, and protocatechuic acid than red rice and white rice. In addition, the research of Jun et al. (2012) reported that antioxidant activity of 40% pigmented rice-bran acetone extract, at an antioxidant concentration of 500 g/mL, red rice with the highest total phenolic and total flavonoids showed the highest antioxidant activity (83.6% based on the radical DPPH test). In addition, there was an interesting study by Setyaningsih et al. (2015), who studied the positive correlation between amylose content and levels of antioxidant compounds, such as melatonin and phenolics. This finding can provide an important basis of information if one of the biochemical compounds is known, although the case in each variety will be specific.

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 1000 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 grains. Directly, the weight of 1000 grains was only significantly affected by the character of grain weight per panicle. The cumulative color of the seeds of the F6 and F7 lines showed 4 (four) lines with completely the same color as the black rice, but the other 2 (two) lines still showed the color combination between the two checked varieties. Based on 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) differ from the PHMW 487-24-8.

Meanwhile, the dehulled rice size of all studied lines was larger than those 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 antioxidants can be used as candidates for rice varieties with superior characters. It can enrich the collection of superior rice germplasm in Indonesia.

Acknowledgments

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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

ABSTRACT

A comprehensive understanding of the genetic parameters and the inter-relationship among characters in the breeding population is crucial for selecting low amylose and high antioxidants black rice varieties. Meanwhile, dehulled rice morpho-biochemistry can be used to determine the grain profile of F6 and F7 lines of Black Rice x Mentik Wangi var. This study aimed 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 1000 grains. Directly, the weight of 1000 grains 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.

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Keywords: Black Rice, Mentik Wangi, morpho-biochemical, low amylose

1. Introduction

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The Black Rice breeding program is directed to improve the quality and productivity of black rice in order to fulfill the 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) reported the new varieties resulting from conventional breeding, SCS120 Onix (black pericarp) and SCS119 Rubi (red pericarp), were released in 2013 and 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 for the black rice breeding program in Indonesia is to acquire black rice with low amylose content and high antioxidant. In that country, the development of black rice to obtain superior traits with low amylose content and high antioxidants has been carried out. The Indonesian Ministry of Agriculture released the low amylose pigmented rice variety (19,6% 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 determined 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 (Juliano et al., 1965; Bhattacharaya and Juliano, 1985; Avaro et al., 2011), in addition to other criteria such as post-harvest processing and cooking method (Li et al., 2016). The lower the amylose content of rice, the more tender the texture of the rice, and vice versa (Panesar and Kaur, 2016; Luna et al., 2015; Bhattacharaya et al., 1999; Khumar and Khush, 1986). Meanwhile, rice with high antioxidant activity plays an essential role in human health (Prastiwi & 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 & 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 content and high antioxidants have the potential as a superior variety with a fluffier texture of rice and high health benefits.

However, to obtain this superior variety, black rice breeding program requires testing of various characters, both those 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 antioxidants can be done by crossing the Black Rice with Mentik Wangi variety. Rice breeding to obtain lines with high antioxidants and a fluffier texture of rice has reached the 6th line, which was started in 2014.

The purpose of this study was 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 played an important role in comparing the agronomic traits of lines with checked varieties. Determination of genetic parameters aimed 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 six 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 6th lines and checked varieties were cultivated until harvest to obtain the 7th lines. Dehulled rice of the 7th line was analyzed for size, cumulative color, amylose content, and antioxidant activity. Meanwhile, the dehulled rice of the 6th line was also subjected to the same analysis.

2.1. Field Trial of F6 Lines

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Field trial of the 6th 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) blocks and 5 (five) replications in each block, so the total of genotypes was 120 polybags. The growth media contained ultisols soil, roasted rice husks, and manure (4: 1:1, respectively). The growth media was fertilized with NPK (15 grams per polybag). Conventional techniques and chemical pesticides are employed for controlling weeds, pests, and diseases. The agronomic traits observed were heading date, plant height, plant dry weight, number of tillers, the weight of 1000 grain, number of panicles, and grain weight per panicle.

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2.2. Morphology Characterization of F7 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 segregation pattern.

2.3. Amylose Quantification of The Lines

The amylose content of the dehulled rice of the 7th line 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

Measurement of antioxidant activity was measured on dehulled rice of the 6th and 7th 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 & Kitts, 2014).

2.5. Data analysis

Data on agronomic traits of the 6th lines, dehulled rice length of the 7th lines, and amylose content of the 7th lines was analyzed using SAS 9.4 software. The Least Significant Difference (LSD) test at the 95% confidence level ($\alpha = 0.05$) 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 following criteria of McWhirter (1979). The Coefficient of Genotypic and Phenotypic Variance was calculated using Singh & Chaudhary's (1977) formula. The genotypic and phenotypic variation coefficient was categorized as proposed by Sivasubramanian & Madhavamenon (1973). The student T-test identified the difference in antioxidant activity of the 7th and the 6th 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 1000 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.).

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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 1000 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±6.0 1b	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±3.1 3a	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±3.97 b	36.70±5.4 3b	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±2.2 7a	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±1.8 9a	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±6.4 3a	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±1.9 5a	15.42±0.63 bc	15.1±0.65b	2.24±0.47 ab	64.00±1.41 b
Mentik Wangi	66.90±13.7 8c	101.80±6.6 0d	25.59±4.4 8a	14.08±2.76 b	15.22±2.34 b	2.77±0.71 b	82.83±4.09 d

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

The traits observed in each line showed a different effect in each line. 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 1000 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%.

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

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Number of tillers	29.91	54	14	15.22
Plant height (cm)	73.12	96.8	53.2	6.03
Weight of 1000 grain (g)	14.42	22.12	7.04	9.74
Number of panicles	14.93	25	9	11.44
Grain weight per panicle (g)	2.22	3.64	1.1	14.63
Heading date (days)	64.24	72	57	3.20

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 1000 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.

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 1000 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

3.3. Inter-Relationship Among Traits

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

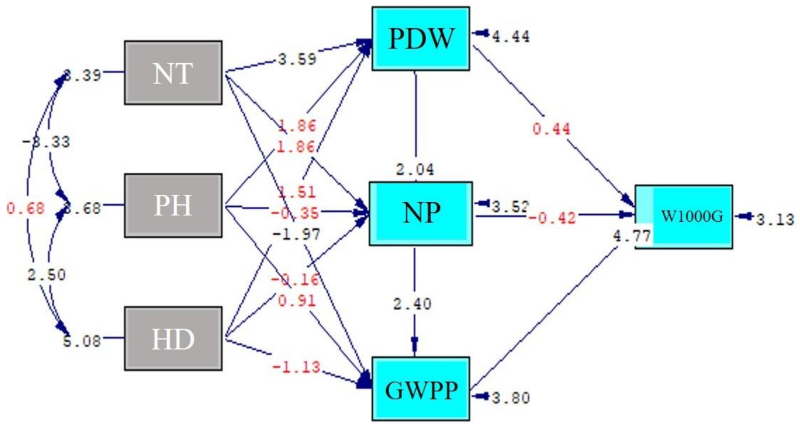

Figure 1. Path diagram of inter-relationship traits (P-value = 0.19579) (Notes: NT: Number of tillers; PH: Plant height; HD: Heading date; PDW: Plant dry weight; NP: Number of panicles; GWPP: Grain weight per panicle: W1000G: Weight of 1000 Grain).

3.4. Morpho-Biochemical Profile of Dehulled Rice

Figure 2 shows that the cumulative color of each F6 and F7 line has similarities. Four lines had the same color as the Black Rice variety, but the other lines showed the color combination between the checked varieties.

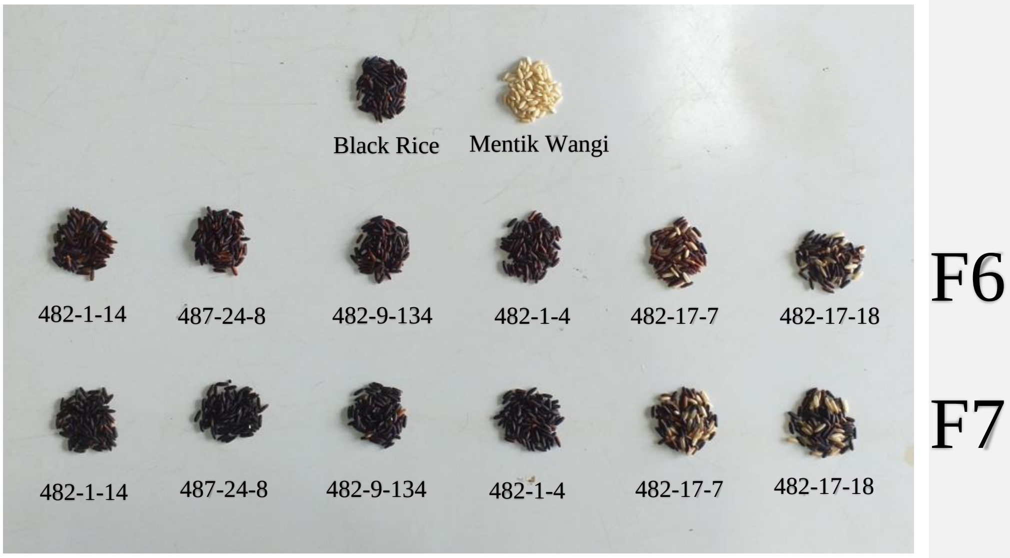

Figure 2. Color of the dehulled rice of lines and checked varieties

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.

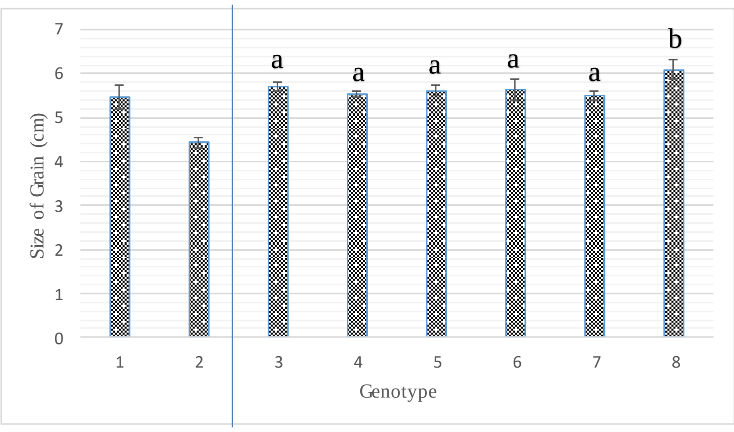

Figure 3. Length Size of F7 Dehulled Rice (Note: No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; while no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134, 487-24-8 lines, respectively)

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 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).

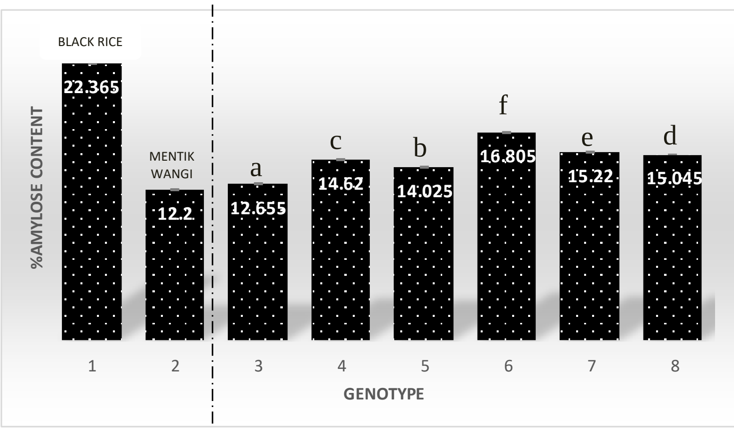

Figure 4. Amylose profile of F7 lines and checked varieties (Note: No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; while no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134, 487-24-8 lines, respectively)

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

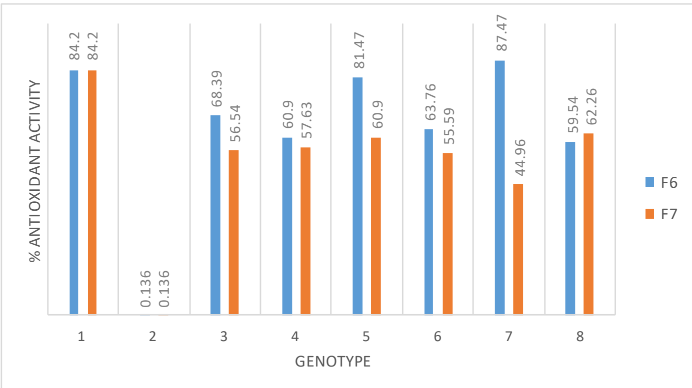

Figure 5. Antioxidant activity of F6 and F7 dehulled rice. (Note: No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134, 487-24-8 lines, respectively)

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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 heights 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 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 the research of Kasim et al. (2020), the lines had a shorter heading date. Farmers prefer the earlier heading date allowing a higher frequency of harvesting 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.

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Meanwhile, the number of panicles varied between 12-24. The range of 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 grains also varied, between 1.4 – 2.56 grams. The number of panicles, grain weight, and the number of grains per panicle determined grain yield in rice (Xing & 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 yield (Khush, 2000).

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4.2. Genetic Parameters of The Lines

The value of the broad-sense heritability of this study is in the range that varies for each character. Heritability was used to estimate the relative contribution effect of genetic and non-genetic factors to the total phenotypic variance in a population (Ene et al., 2015; Konate et al., 2016). 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 1000 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 1000 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 follows the result 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.'s (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 1000 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.'s (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 grains. Directly, the weight of 1000 grains 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

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The results of this study have differences when compared with 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 grains, 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 that have been carried out using this analysis showed the characteristics of 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 100 grains which have a direct effect on the high yield on some rice populations (Safitri et al., 2011; Rachmawati et al., 2014). 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 pertaining to these can be used as initial information before yield or productivity are known.

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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%). C3G is part of the anthocyanin group of compounds. 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.

The average size of the 5 (five) lines studied (PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134) is based on dehulled rice size differs from the PHMW 487-24-8. Meanwhile, the dehulled rice size of all lines was more significant than that of the checked varieties. 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 & 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. 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.

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Amylose content was one of the criteria that determined 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 it will produce a fluffier/glutinous texture of rice. The low amylose content of the studied lines follows the objectives of the black rice breeding program.

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It is 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.

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 1000 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 grains. Directly, the weight of 1000 grains 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 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) differ from the PHMW 487-24-8.

Meanwhile, the dehulled rice size of all studied lines was more significant than those 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 antioxidants can be used as candidates for rice varieties with superior characters. It can enrich the collection of superior rice germplasm in Indonesia.

Acknowledgments

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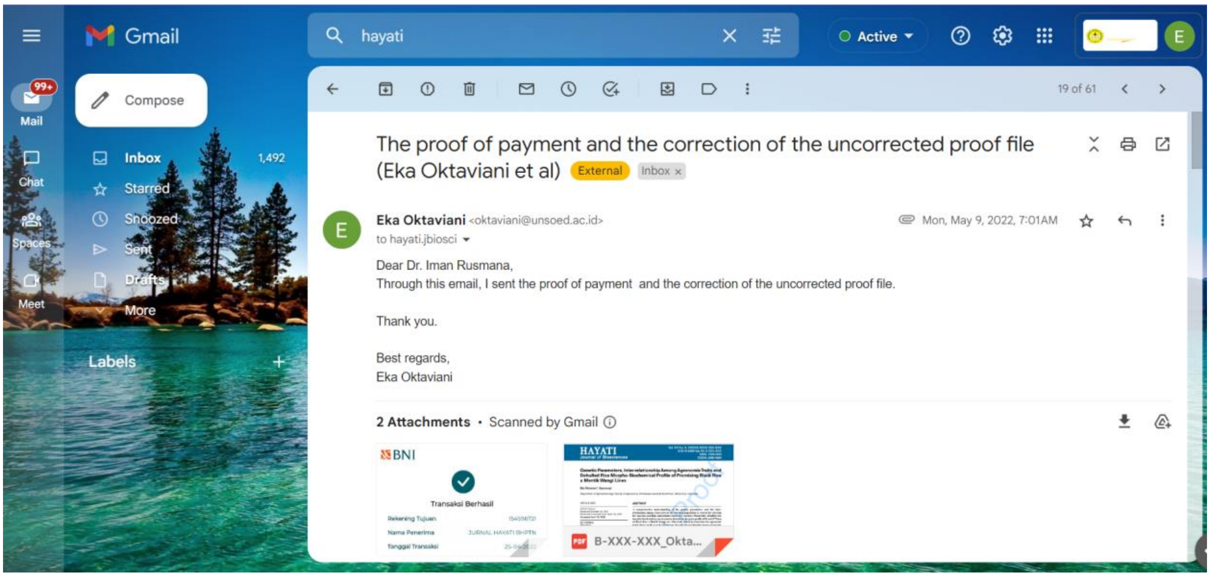

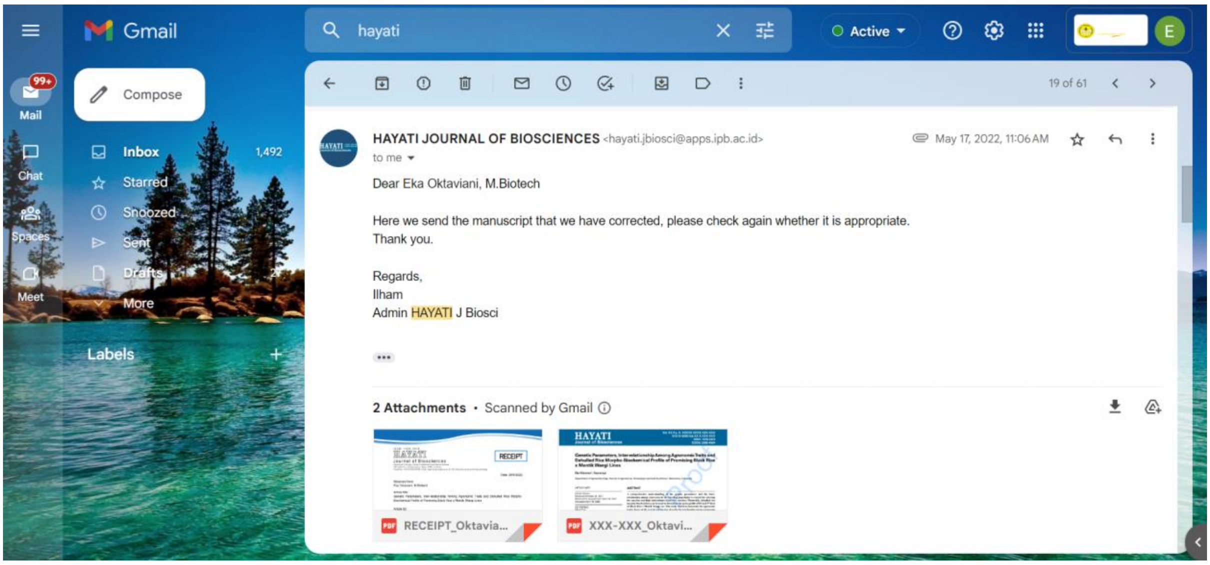

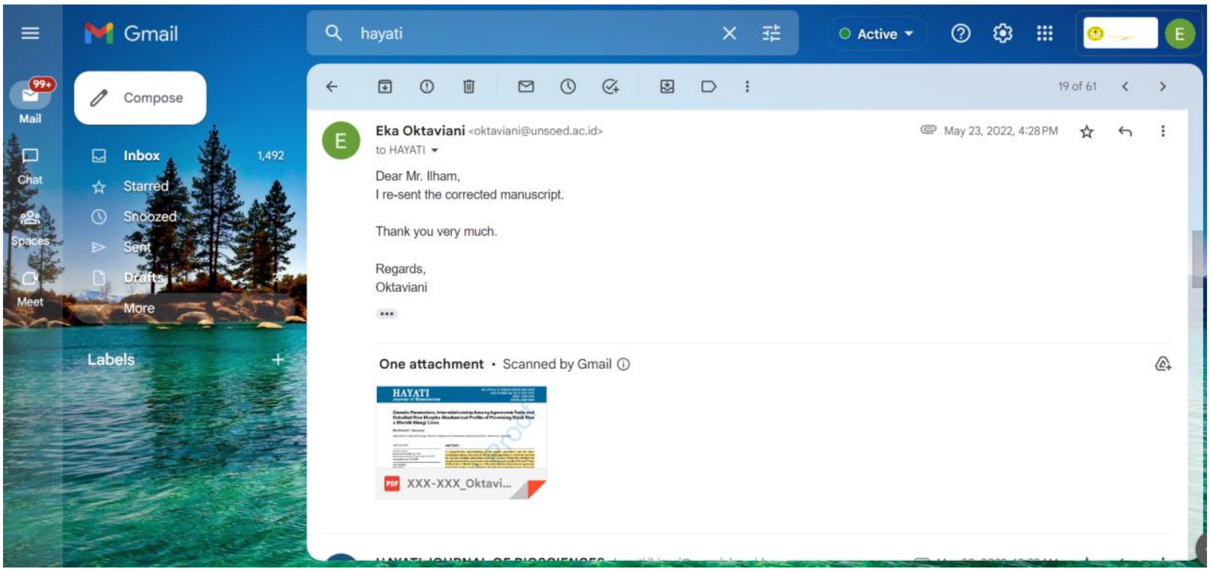

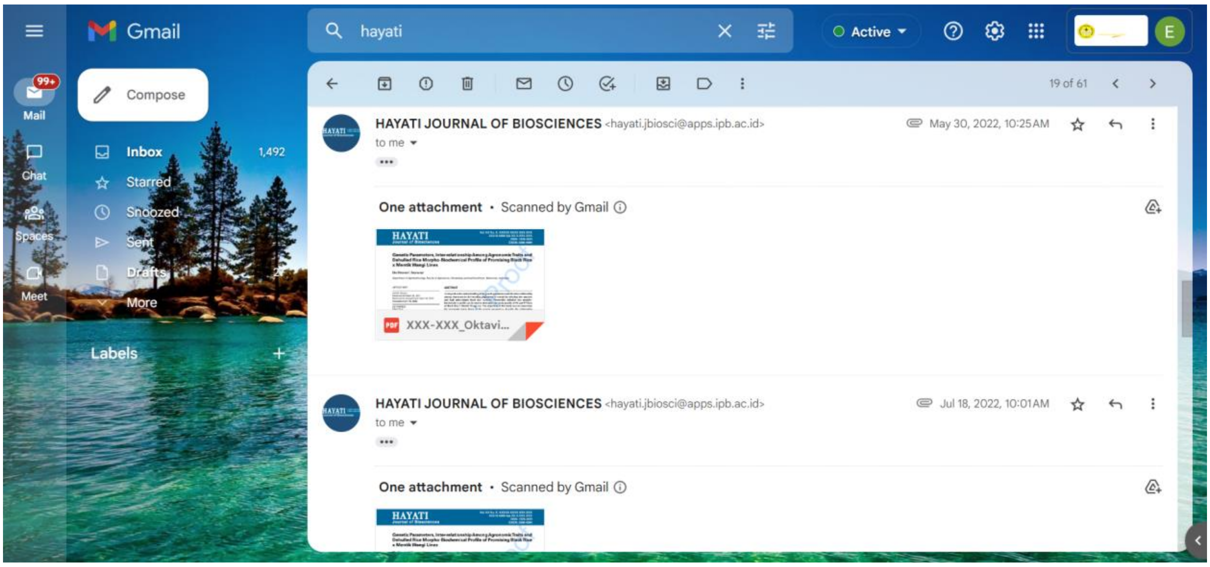
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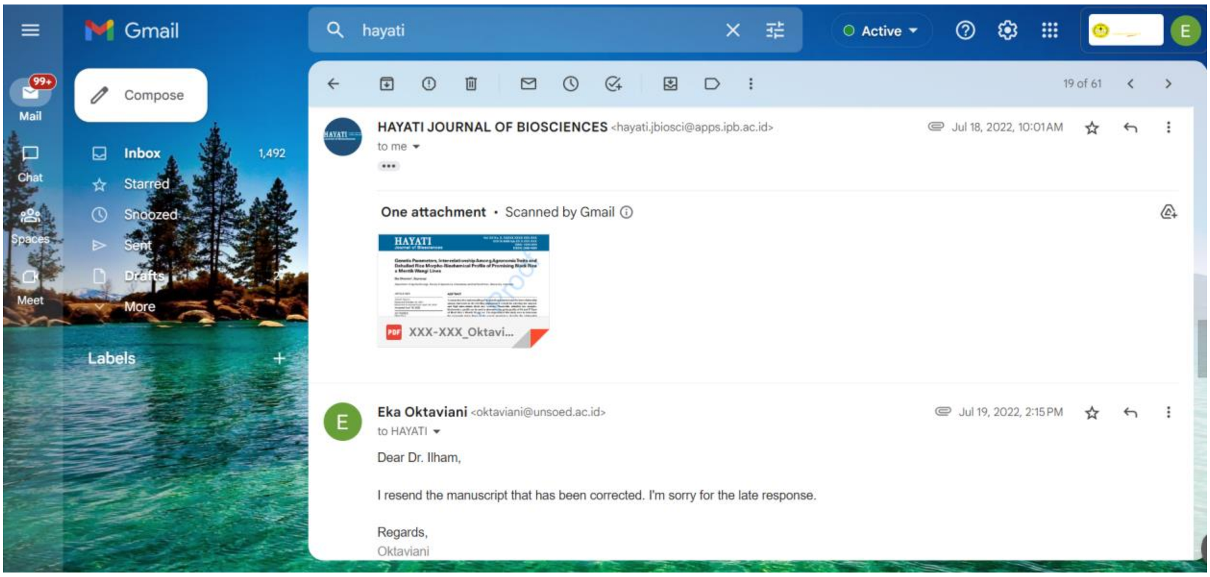
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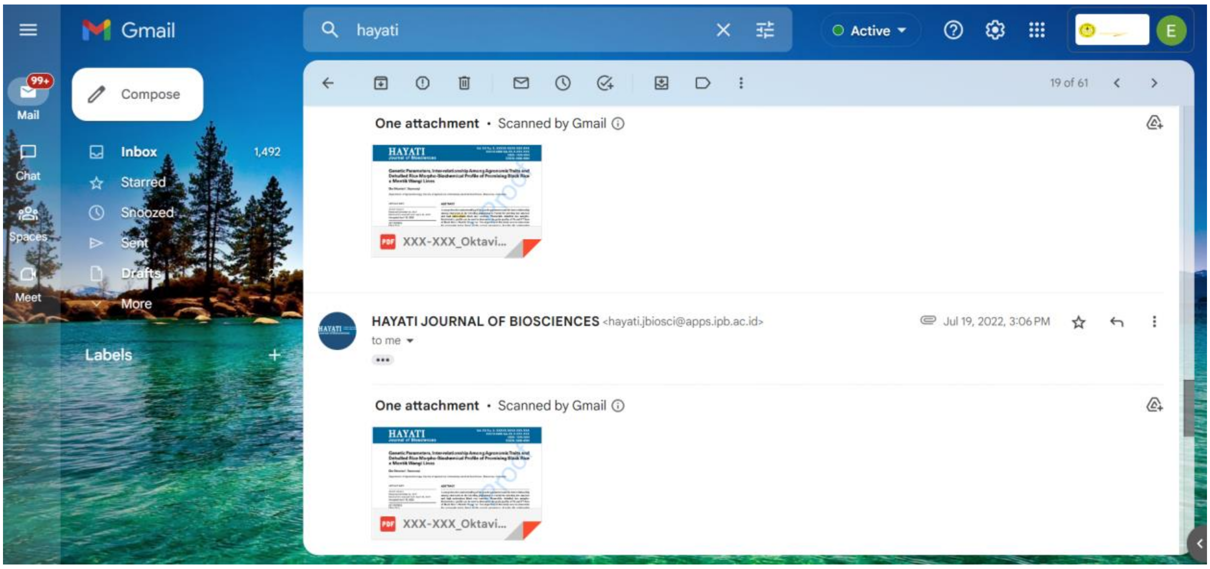
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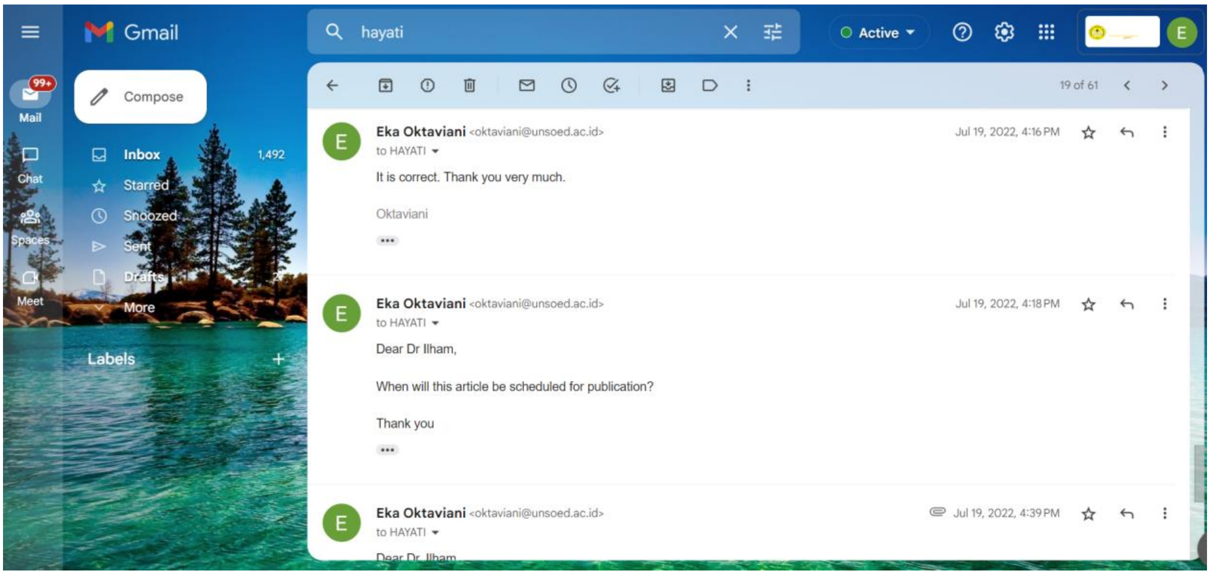
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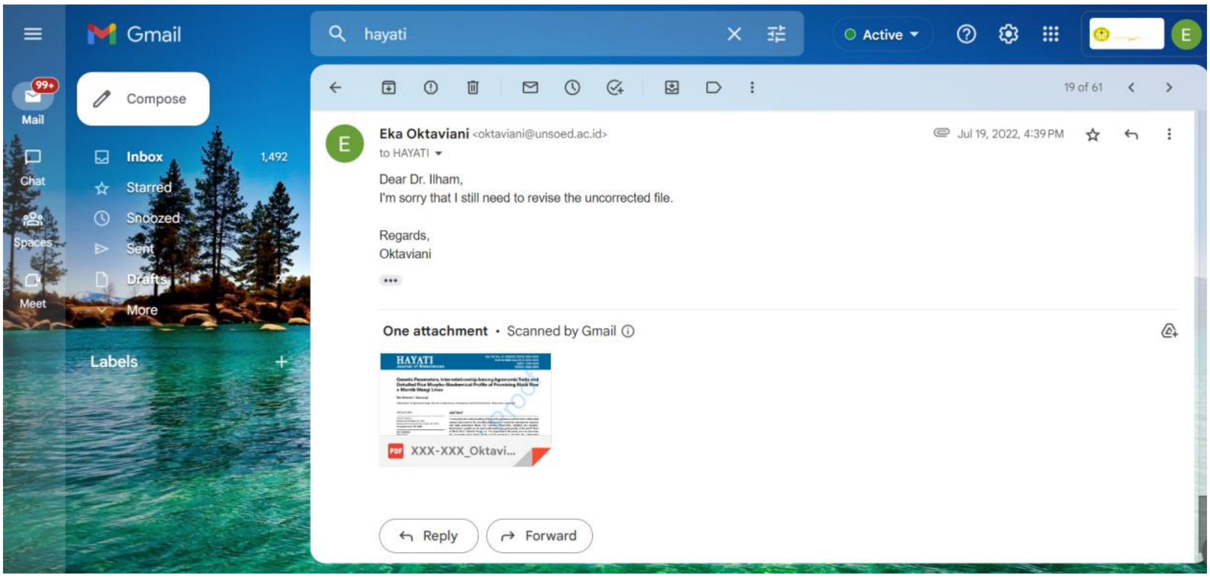
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Genetic Parameters, Inter-relationship Among Agronomic Traits and Dehulled Rice Morpho-Biochemical Profile of Promising Black Rice x Mentik Wangi Lines

Eka Oktaviani*, Suprayogi

Department of Agrotechnology, Faculty of Agriculture, Universitas Jenderal Soedirman, Banyumas, Indonesia

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ABSTRACT

A comprehensive understanding of the genetic parameters and the inter-relationship among characters in the breeding population is crucial for selecting low amylose and high antioxidants black rice varieties. Meanwhile, dehulled rice morpho-biochemistry can be used to determine the grain profile of F6 and F7 lines of Black Rice x Mentik Wangi var. This study aimed 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 grains. Directly, the weight of 1,000 grains 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

The 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) reported the new varieties resulting from conventional breeding, SCS120 Onix (black pericarp) and SCS119 Rubi (red pericarp), were released in 2013 and 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. In that country, the development of black rice to obtain superior traits with low amylose content and high antioxidants has been carried out. The Indonesian Ministry of

* Corresponding Author
E-mail Address: oktaviani@unsoed.ac.id

Agriculture released the low amylose pigmented rice variety (19.6% 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 method (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 content and high antioxidants has the potential 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 antioxidants can be done by crossing the Black Rice with the Mentik Wangi variety. Rice breeding to obtain lines with high antioxidants and a fluffier texture of rice has reached the 6th line, which was started in 2014.

This study aimed 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 played an important role in comparing the agronomic traits of lines with checked varieties. Determination of genetic parameters aimed 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 six 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 6^{th} lines and checked varieties were cultivated until harvest to obtain the 7^{th} lines. Dehulled rice of the 7^{th} line was analyzed for size, cumulative color, amylose content, and antioxidant activity. Meanwhile, the dehulled rice of the 6th line was also subjected to the same analysis.

2.1. Field Trial of F6 Lines

Field trial of the 6^{th} 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) blocks and 5 (five) replications in each block, so the total of genotypes was 120 polybags. The growth media contained ultisols soil, roasted rice husks, and manure (4:1:1, respectively). The growth media was fertilized with NPK (15 grams per polybag). Conventional techniques and chemical pesticides are employed for controlling 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 F7 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 segregation pattern.

2.3. Amylose Quantification of The Lines

The amylose content of the dehulled rice of the 7th line 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

Measurement of antioxidant activity was measured on dehulled rice of the 6th and 7th 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 6th lines, dehulled rice length of the 7th lines, and amylose content of the 7th lines was analyzed using SAS 9.4 software. The Least Significant Difference (LSD) test at the 95% confidence level ($\alpha = 0.05$) 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 following criteria 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 7th and the 6th 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 different effect in each line. 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.

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

3.3. Inter-Relationship Among Traits

The dependent variables as the yield component were the weight of 1,000 grains and the weight of 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 1,000 grains.

3.4. Morpho-Biochemical Profile of Dehulled Rice

Figure 2 shows that the cumulative color of each F6 and F7 line has similarities. 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-

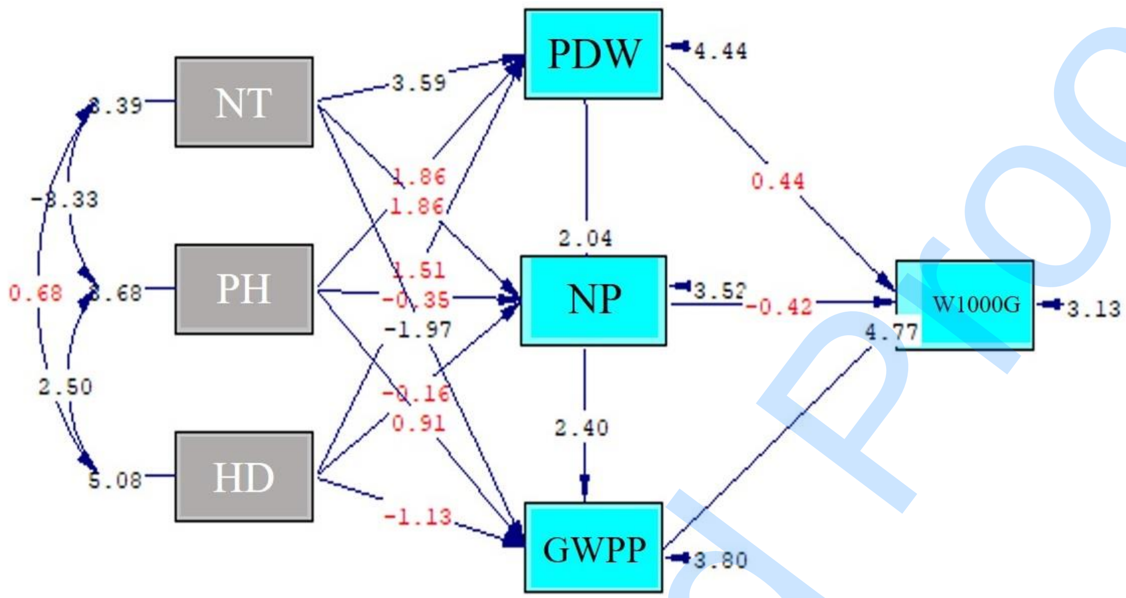

Figure 1. Path diagram of inter-relationship traits (p-value = 0.19579). (Notes: NT: number of tillers, PH: plant height, HD: heading date, PDW: plant dry weight, NP: number of panicles, GWPP: grain weight per panicle, W1000G: weight of 1,000 grain)

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

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 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

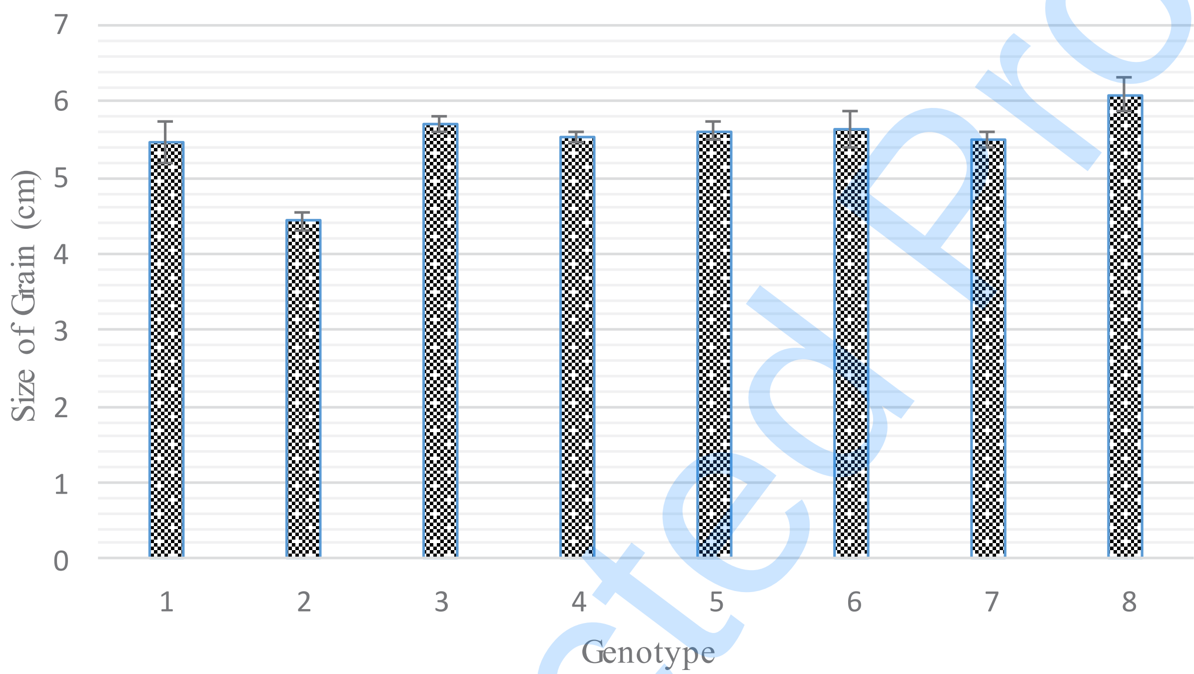

Figure 3. Length Size of F7 Dehulled Rice (Note: No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; while no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134, 487-24-8 lines, respectively)

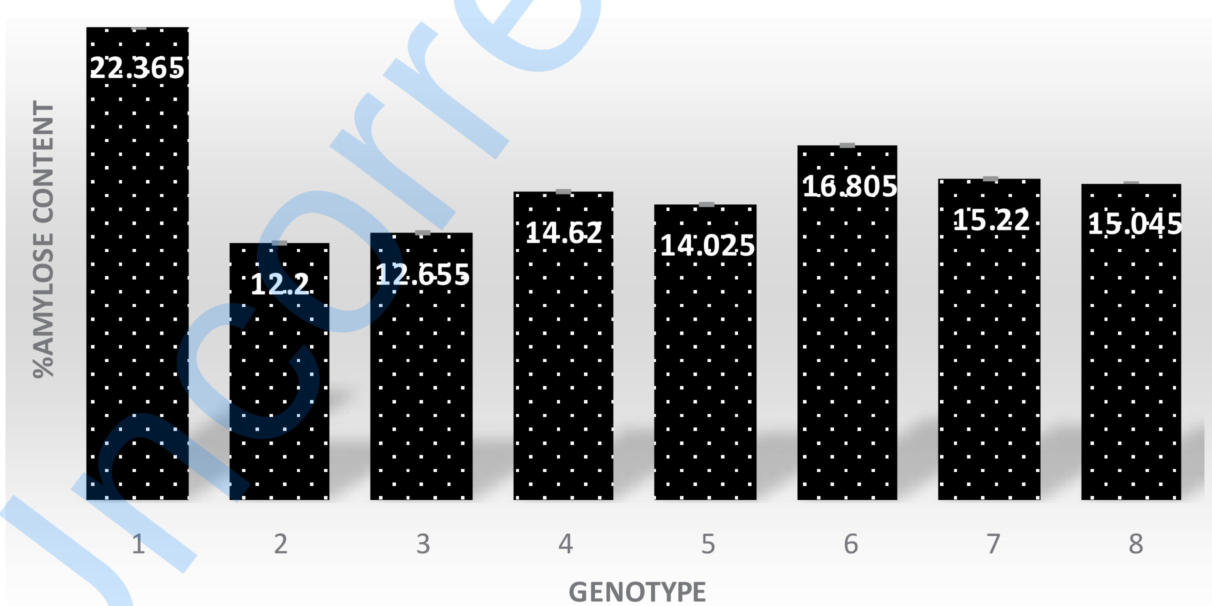

Figure 4. Amylose profile of F7 lines and checked varieties (Note: No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; while no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134, 487-24-8 lines, respectively)

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) 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 heights 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 heights are preferred by 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.

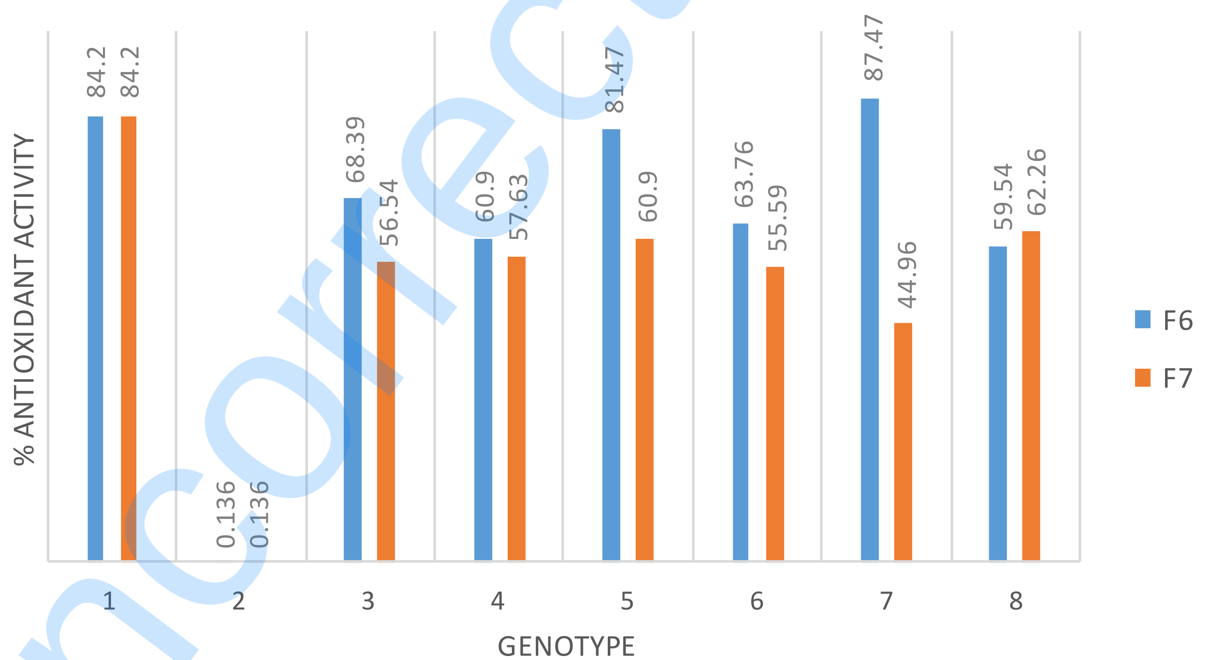

Figure 5. Antioxidant activity of F6 and F7 dehulled rice. (Note: No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134, 487-24-8 lines, respectively)

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 1,000 grains also varied, between 1.4–2.56 grams. The number of panicles, grain weight, and the number 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 is in the range that varies for each character. Heritability was used to estimate the relative contribution effect of genetic and non-genetic factors to the total phenotypic variance in a population (Ene *et al.* 2015; Konate *et al.* 2016). 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 follows the result 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 1,000 grains. Directly, the weight of 1,000 grains 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 have differences when compared with 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 1,000 filled grains, 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 that have been carried out using this analysis showed the characteristics of 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 100 grains which 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%). C3G is part of the anthocyanin group of compounds. 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.

The average size of the 5 (five) lines studied (PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134) is based on dehulled rice size differs from the PHMW 487-24-8. Meanwhile, the dehulled rice size of all lines was more significant than that of the checked varieties. 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. 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 was one of the criteria that determined 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 to produce a fluffier/glutinous texture of rice. The low amylose content of the studied lines follows the objectives of the black rice breeding program.

It is 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 1,000 grains. Directly, the weight of 1,000 grains 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 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) differ from the PHMW 487-24-8. Meanwhile, the dehulled rice size of all studied lines was more significant than those 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 antioxidants 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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Genetic Parameters, Inter-relationship Among Agronomic Traits and Dehulled Rice Morpho-Biochemical Profile of Promising Black Rice x Mentik Wangi Lines

Eka Oktaviani*, **Suprayogi**

Department of Agrotechnology, Faculty of Agriculture, Universitas Jenderal Soedirman, Banyumas, Indonesia

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ABSTRACT

A comprehensive understanding of the genetic parameters and the inter-relationship among characters in the breeding population is crucial for selecting low amylose and high antioxidants black rice varieties. Meanwhile, dehulled rice morpho-biochemistry can be used to determine the grain profile of F6 and F7 lines of Black Rice x Mentik Wangi var. This study aimed 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 grains. Directly, the weight of 1,000 grains 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

The 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 antioxidants. In that country, the development of black rice to obtain superior traits with low amylose content and high antioxidants has been carried out. The Indonesian Ministry of Agriculture released the low amylose pigmented rice

* Corresponding Author
E-mail Address: oktaviani@unsoed.ac.id

variety (19.6% 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 antioxidants 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 antioxidants can be done by crossing the Black Rice with the Mentik Wangi variety. Rice breeding to obtain lines with high antioxidants and a fluffier texture of rice has reached the 6th line, which was started in 2014.

This study aimed 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 6th lines and checked varieties were cultivated until harvest to obtain the 7th lines. Dehulled rice of the 7th line was analyzed for size, cumulative color, amylose content, and antioxidant activity. Meanwhile, the dehulled rice of the 6th line was also subjected to the same analysis.

2.1. Field Trial of F6 Lines

Field trial of the 6th 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) blocks and 5 (five) replications in each block, so the total of genotypes was 120 polybags. The growth media contained ultisols soil, roasted rice husks, and manure (4:1:1, respectively). The growth media was fertilized 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 F7 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 7th line 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 6th and 7th 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 6th lines, dehulled rice length of the 7th lines, and amylose content of the 7th 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 7th and the 6th 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 different effect in each line. 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 1,000 grains 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

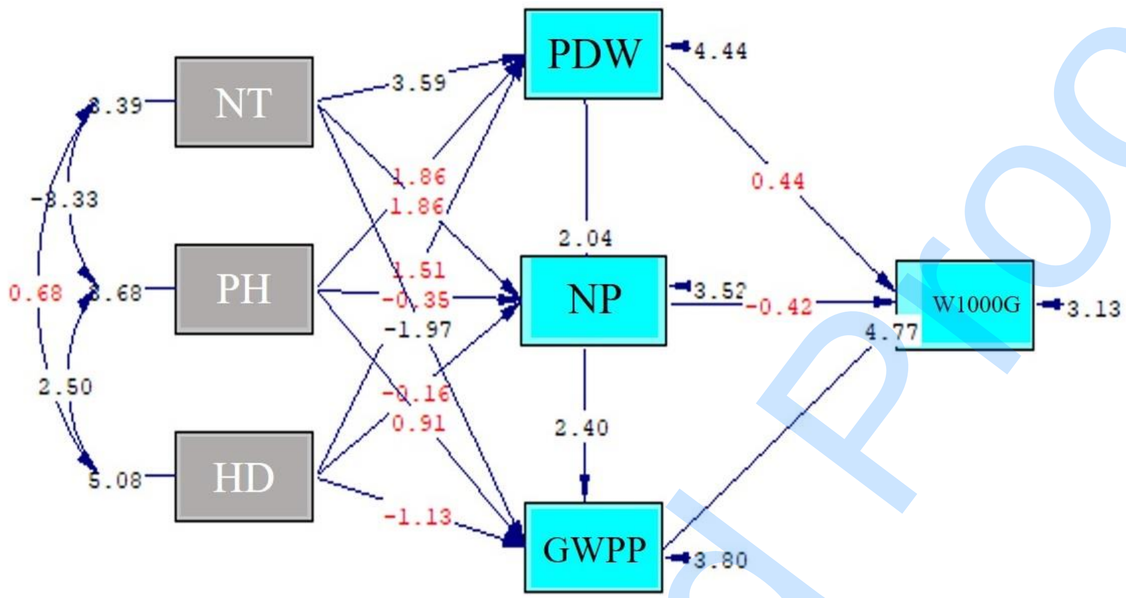

Figure 1. Path diagram of inter-relationship traits (p-value = 0.19579). (Notes: NT: number of tillers, PH: plant height, HD: heading date, PDW: plant dry weight, NP: number of panicles, GWPP: grain weight per panicle, W1000G: weight of 1,000 grain)

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)

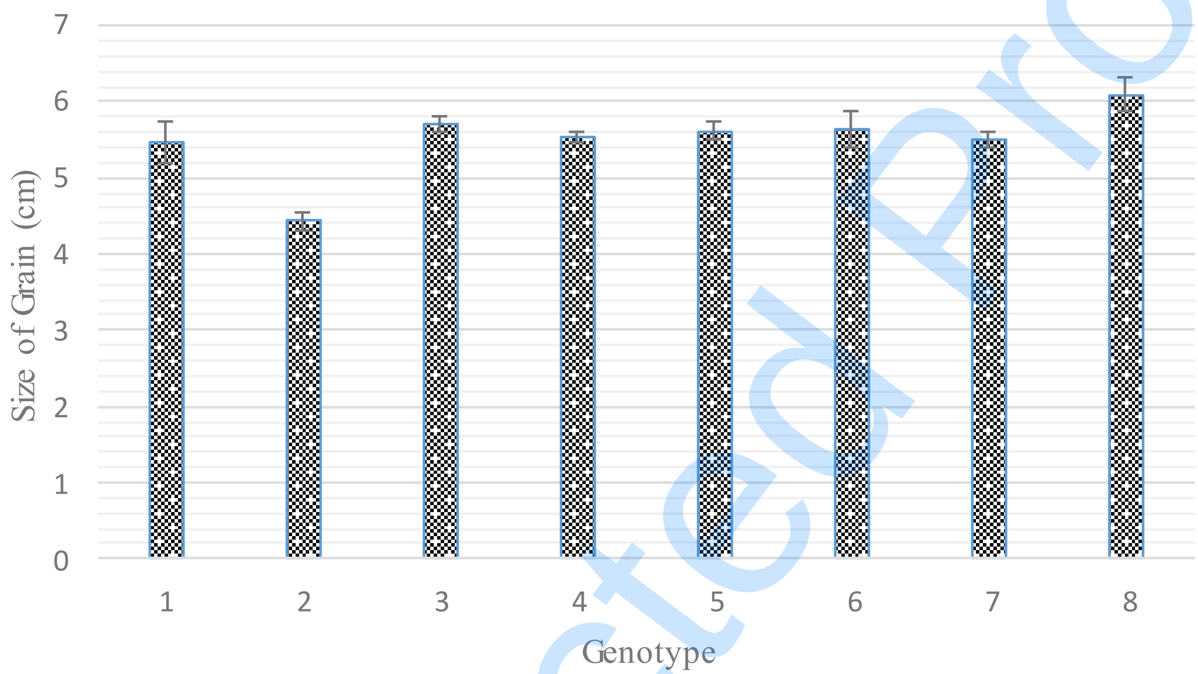

Figure 3. Length Size of F7 Dehulled Rice (Note: No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; while no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134, 487-24-8 lines, respectively)

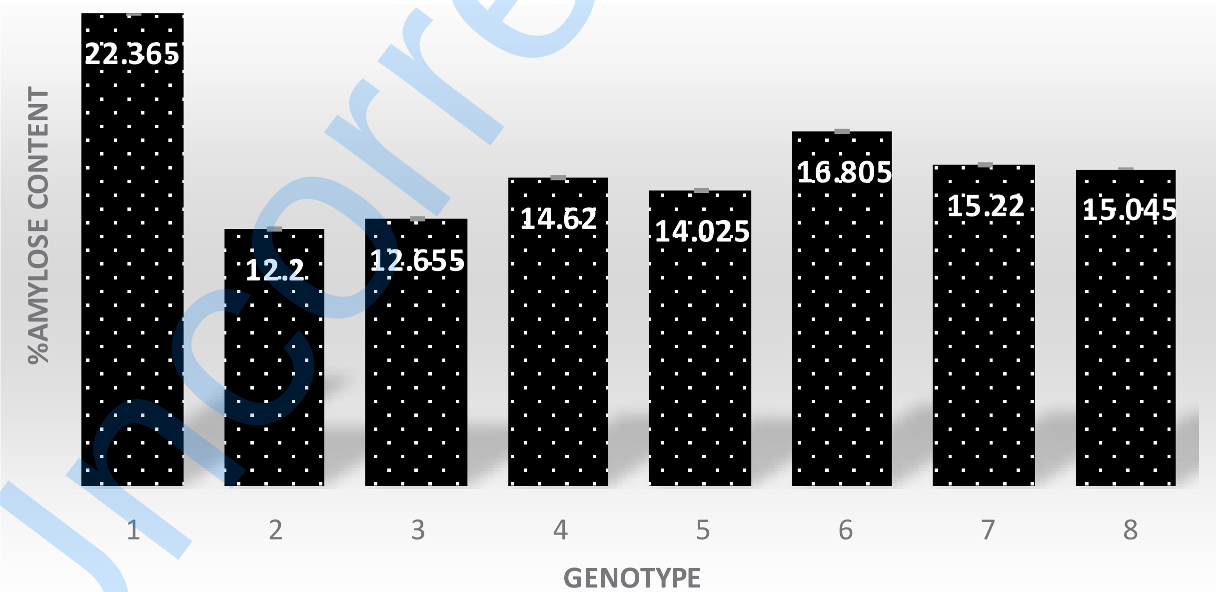

Figure 4. Amylose profile of F7 lines and checked varieties (Note: No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; while no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134, 487-24-8 lines, respectively)

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

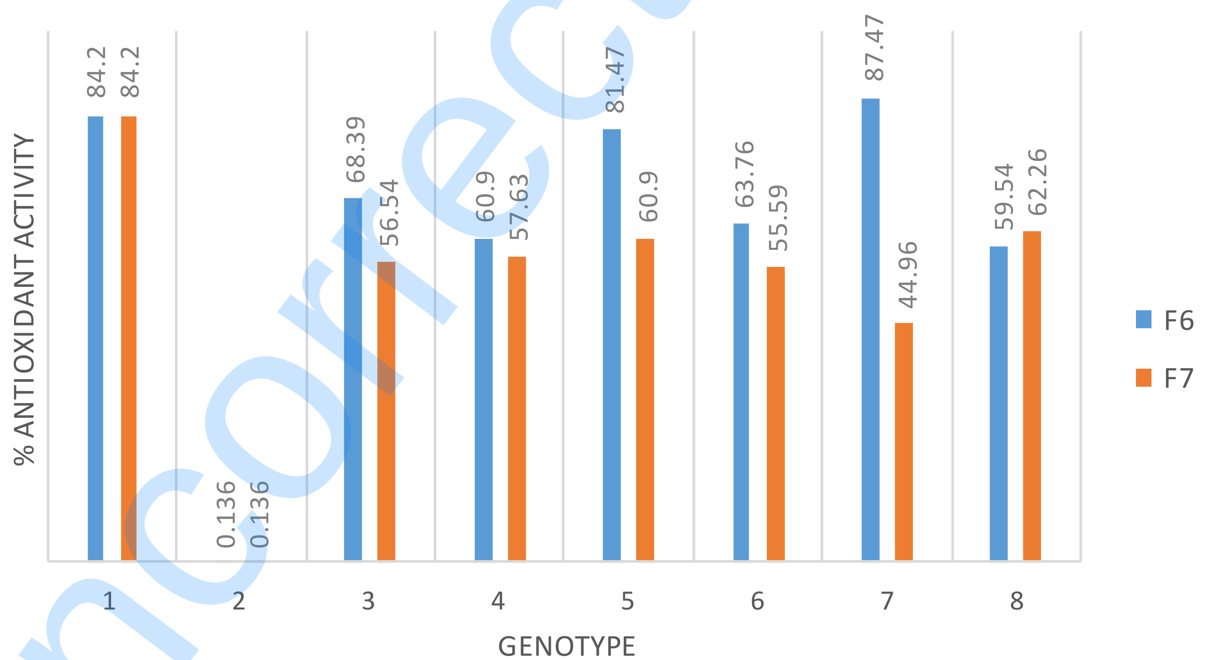

Figure 5. Antioxidant activity of F6 and F7 dehulled rice. (Note: No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134, 487-24-8 lines, respectively)

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 1,000 grains. 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 antioxidants 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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Genetic Parameters, Inter-relationship Among Agronomic Traits and Dehulled Rice Morpho-Biochemical Profile of Promising Black Rice x Mentik Wangi Lines

Eka Oktaviani*, **Suprayogi**

Department of Agrotechnology, Faculty of Agriculture, Universitas Jenderal Soedirman, Banyumas, Indonesia

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ABSTRACT

A comprehensive understanding of the genetic parameters and the inter-relationship among characters in the breeding population is crucial for selecting low amylose and high antioxidants black rice varieties. Meanwhile, dehulled rice morpho-biochemistry can be used to determine the grain profile of F6 and F7 lines of Black Rice x Mentik Wangi var. This study aimed 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 grains. Directly, the weight of 1,000 grains 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

The 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 antioxidants. In that country, the development of black rice to obtain superior traits with low amylose content and high antioxidants has been carried out. The Indonesian Ministry of Agriculture released the low amylose pigmented rice

* Corresponding Author
E-mail Address: oktaviani@unsoed.ac.id

variety (19.6% 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 antioxidants 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 antioxidants can be done by crossing the Black Rice with the Mentik Wangi variety. Rice breeding to obtain lines with high antioxidants and a fluffier texture of rice has reached the 6th line, which was started in 2014.

This study aimed 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 6th lines and checked varieties were cultivated until harvest to obtain the 7th lines. Dehulled rice of the 7th line was analyzed for size, cumulative color, amylose content, and antioxidant activity. Meanwhile, the dehulled rice of the 6th line was also subjected to the same analysis.

2.1. Field Trial of F6 Lines

Field trial of the 6th 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) blocks and 5 (five) replications in each block, so the total of genotypes was 120 polybags. The growth media contained ultisols soil, roasted rice husks, and manure (4:1:1, respectively). The growth media was fertilized 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 F7 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 7th line 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 6th and 7th 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 6th lines, dehulled rice length of the 7th lines, and amylose content of the 7th 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 7th and the 6th 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 different effect in each line. 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 1,000 grains 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

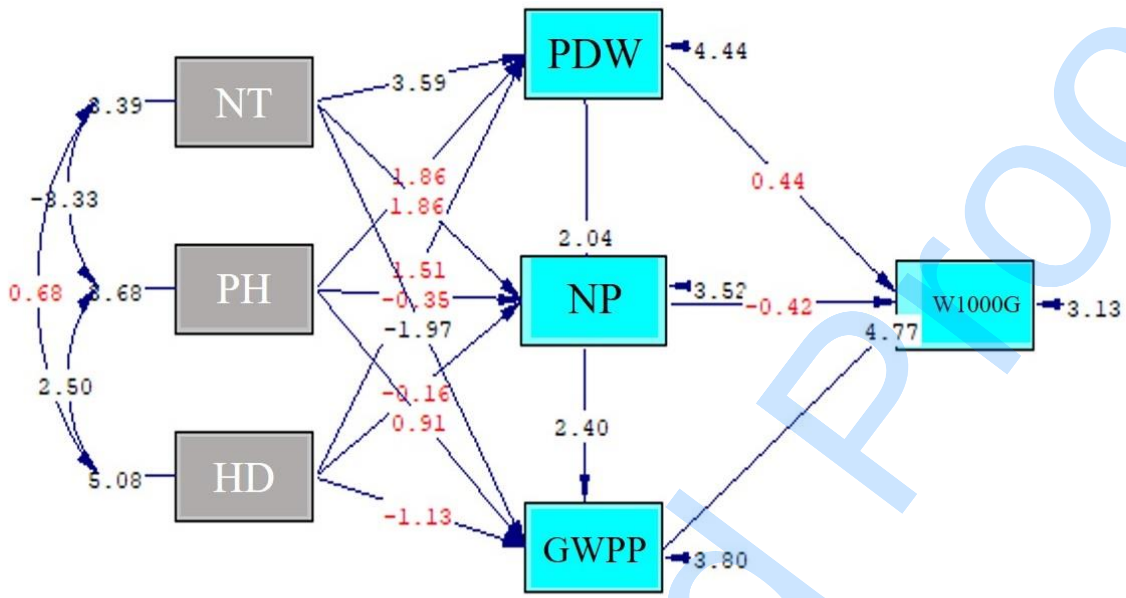

Figure 1. Path diagram of inter-relationship traits (p-value = 0.19579). (Notes: NT: number of tillers, PH: plant height, HD: heading date, PDW: plant dry weight, NP: number of panicles, GWPP: grain weight per panicle, W1000G: weight of 1,000 grain)

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)

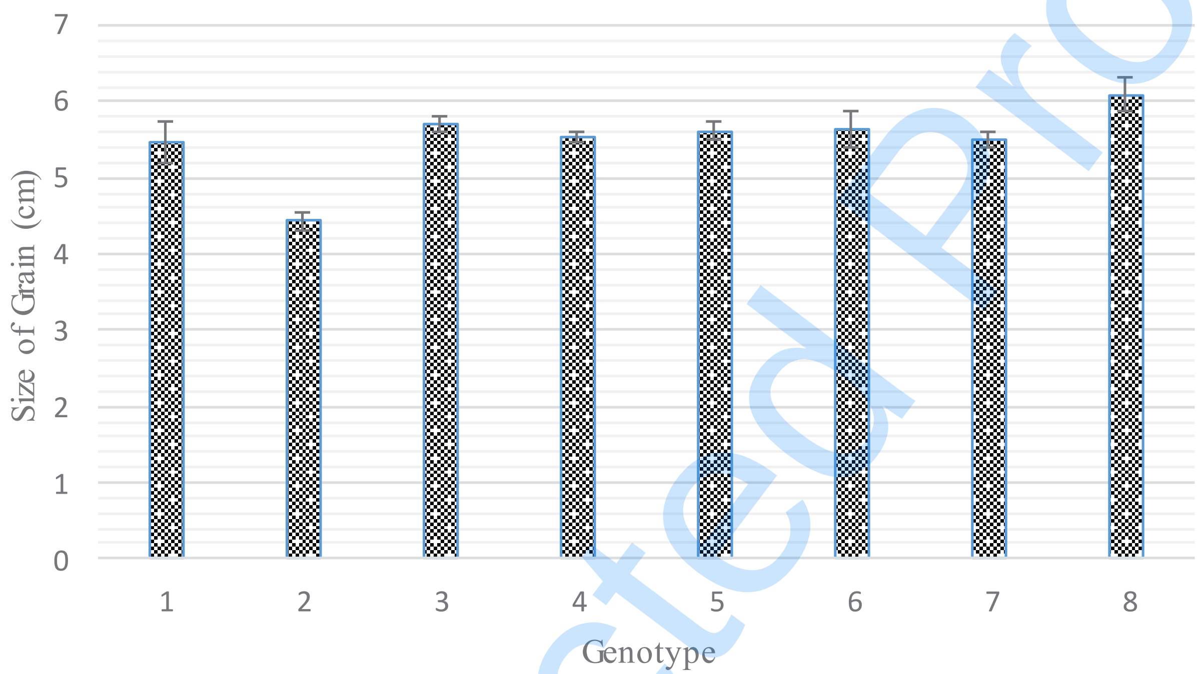

Figure 3. Length Size of F7 Dehulled Rice (Note: No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; while no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134, 487-24-8 lines, respectively)

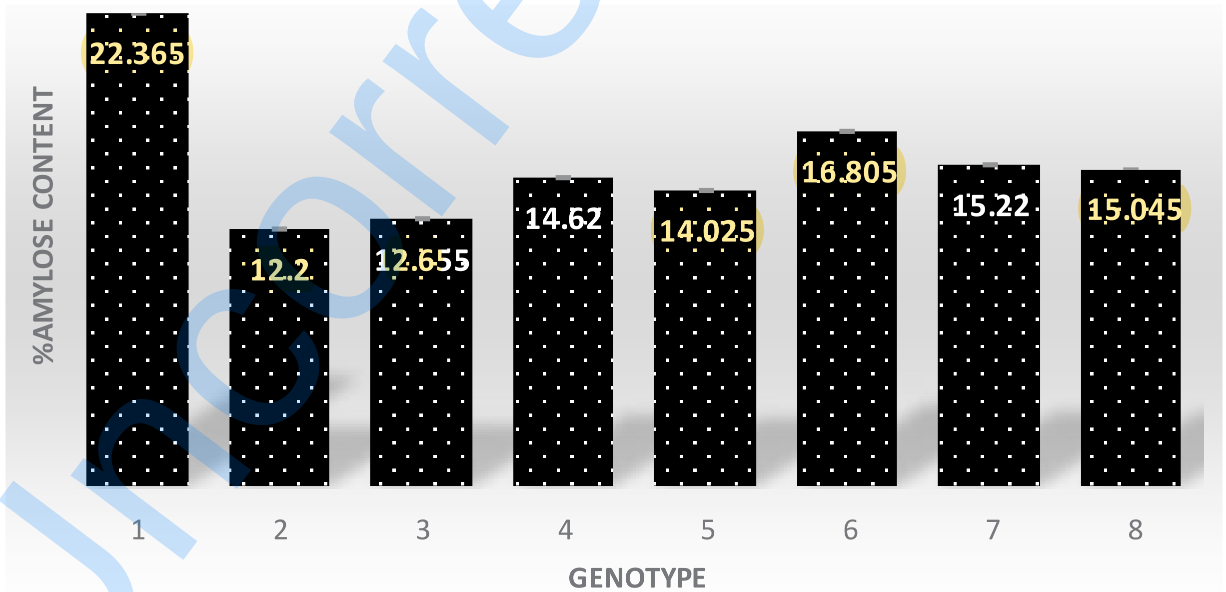

Figure 4. Amylose profile of F7 lines and checked varieties (Note: No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; while no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134, 487-24-8 lines, respectively)

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

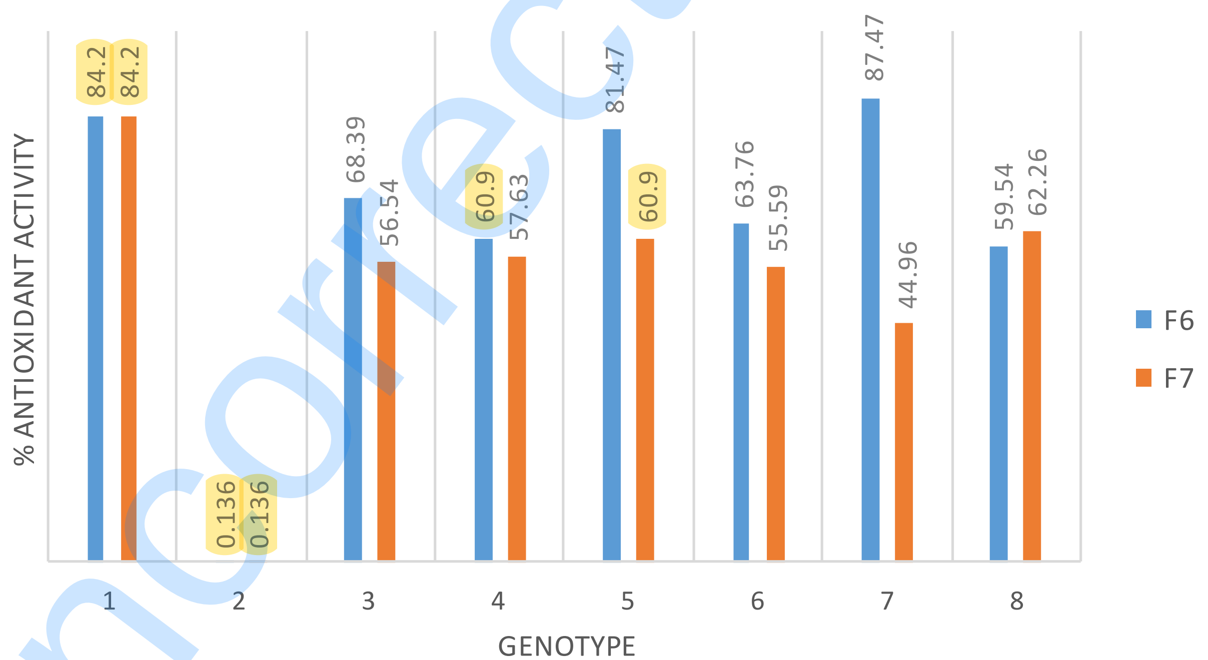

Figure 5. Antioxidant activity of F6 and F7 dehulled rice. (Note: No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134, 487-24-8 lines, respectively)

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 1,000 grains. 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 antioxidants 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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Genetic Parameters, Inter-relationship Among Agronomic Traits and Dehulled Rice Morpho-Biochemical Profile of Promising Black Rice x Mentik Wangi Lines

Eka Oktaviani*, **Suprayogi**

Department of Agrotechnology, Faculty of Agriculture, Universitas Jenderal Soedirman, Banyumas, Indonesia

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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 antioxidants 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

The 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 antioxidants. In that country, the development of black rice to obtain superior traits with low amylose content and high antioxidants has been carried out. The Indonesian Ministry of Agriculture released the low amylose pigmented rice

* Corresponding Author
E-mail Address: oktaviani@unsoed.ac.id

variety (19.6% 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 antioxidants 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 antioxidants can be done by crossing the Black Rice with the Mentik Wangi variety. Rice breeding to obtain lines with high antioxidants and a fluffier texture of rice has reached the 6^{th} line, which was started in 2014.

This study aimed 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 6^{th} lines and checked varieties were cultivated until harvest to obtain the 7^{th} lines. Dehulled rice of the 7^{th} line was analyzed for size, cumulative color, amylose content, and antioxidant activity. Meanwhile, the dehulled rice of the 6th line was also subjected to the same analysis.

2.1. Field Trial of F6 Lines

Field trial of the 6^{th} 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) blocks and 5 (five) replications in each block, so the total of genotypes was 120 polybags. The growth media contained ultisols soil, roasted rice husks, and manure (4:1:1, respectively). The growth media was fertilized 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 F7 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 7th line 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 6th and 7th 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 6th lines, dehulled rice length of the 7th lines, and amylose content of the 7th 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 7th and the 6th 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 different effect in each line. 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

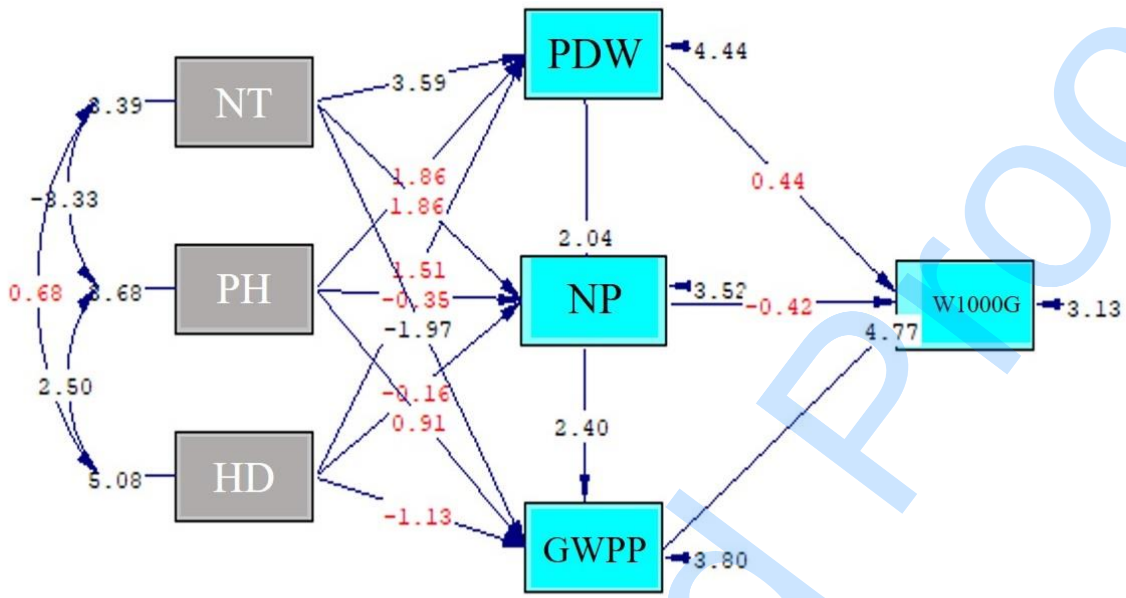

Figure 1. Path diagram of inter-relationship traits (p-value = 0.19579). (Notes: NT: number of tillers, PH: plant height, HD: heading date, PDW: plant dry weight, NP: number of panicles, GWPP: grain weight per panicle, W1000G: weight of 1,000 grain)

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)

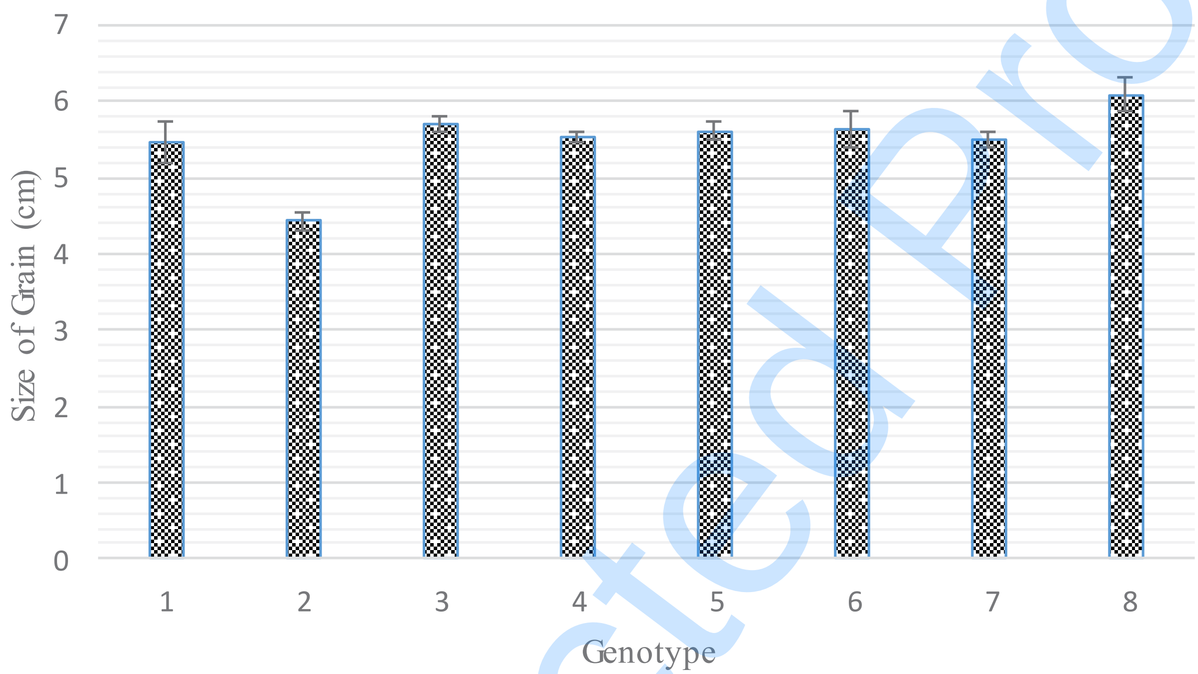

Figure 3. Length Size of F7 Dehulled Rice (Note: No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; while no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134, 487-24-8 lines, respectively)

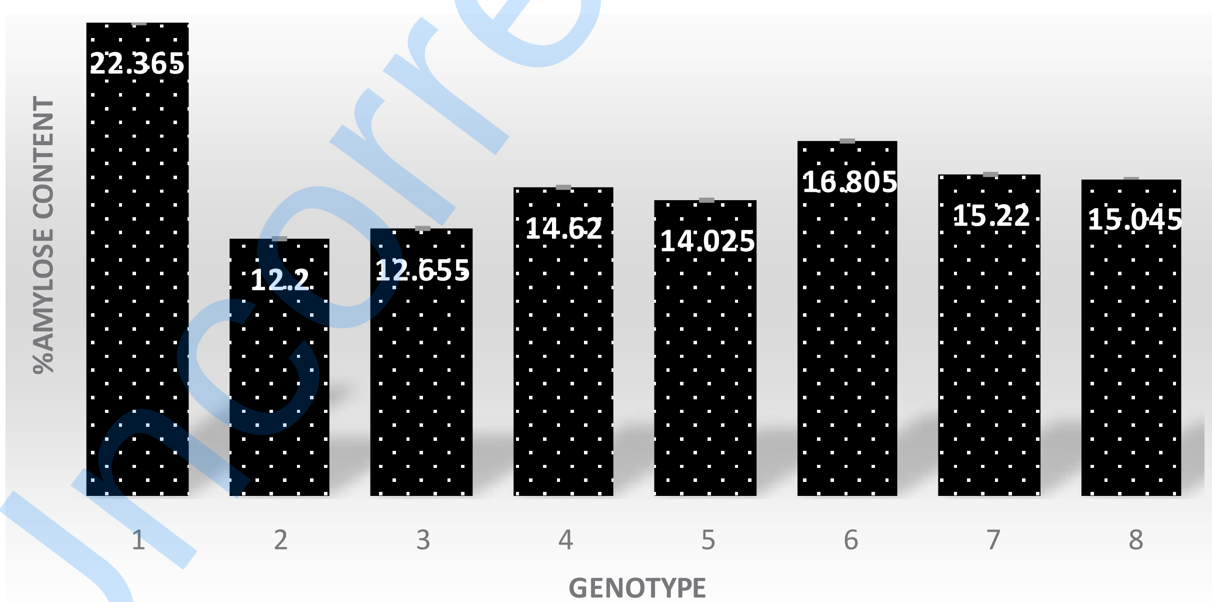

Figure 4. Amylose profile of F7 lines and checked varieties (Note: No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; while no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134, 487-24-8 lines, respectively)

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

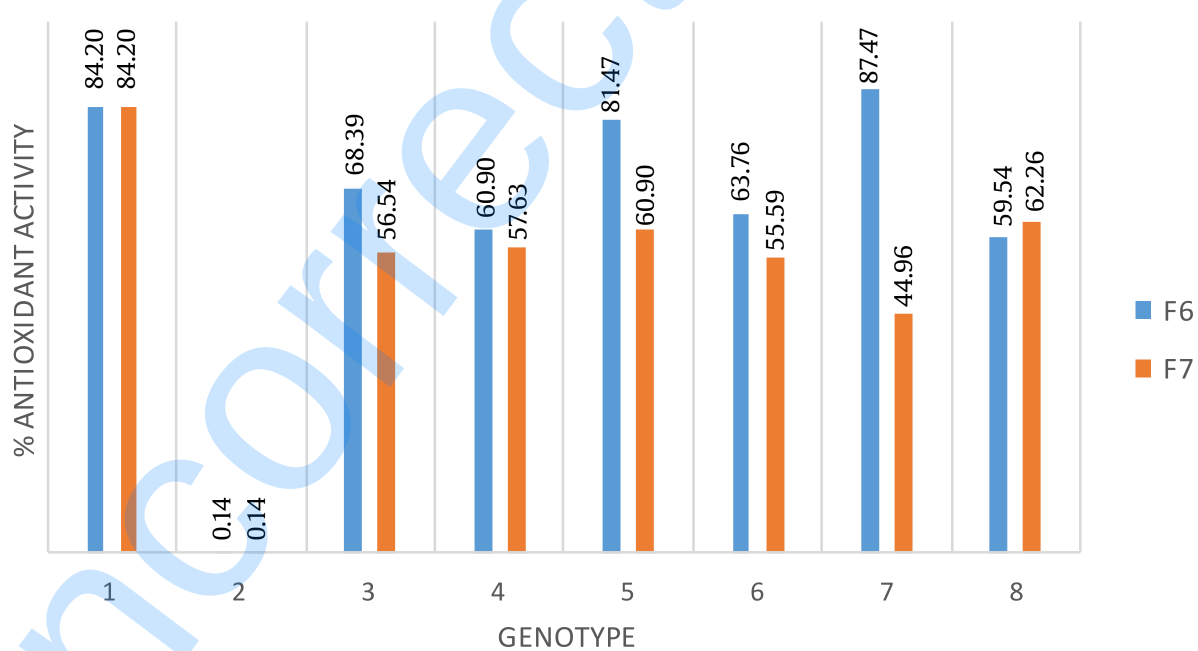

Figure 5. Antioxidant activity of F6 and F7 dehulled rice. (Note: No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134, 487-24-8 lines, respectively)

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

Eka Oktaviani*, Suprayogi

Department of Agrotechnology, Faculty of Agriculture, Universitas Jenderal Soedirman, Banyumas, Indonesia

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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 antioxidants 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

The 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 antioxidants. In that country, the development of black rice to obtain superior traits with low amylose content and high antioxidants has been carried out. The Indonesian Ministry of Agriculture released the low amylose pigmented rice

* Corresponding Author
E-mail Address: oktaviani@unsoed.ac.id

variety (19.6% 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 antioxidants 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 antioxidants can be done by crossing the Black Rice with the Mentik Wangi variety. Rice breeding to obtain lines with high antioxidants and a fluffier texture of rice has reached the 6th line, which was started in 2014.

This study aimed 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 6th lines and checked varieties were cultivated until harvest to obtain the 7th lines. Dehulled rice of the 7th line was analyzed for size, cumulative color, amylose content, and antioxidant activity. Meanwhile, the dehulled rice of the 6th line was also subjected to the same analysis.

2.1. Field Trial of F6 Lines

Field trial of the 6th 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) blocks and 5 (five) replications in each block, so the total of genotypes was 120 polybags. The growth media contained ultisols soil, roasted rice husks, and manure (4:1:1, respectively). The growth media was fertilized 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 F7 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 7th line 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 6th and 7th 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 6th lines, dehulled rice length of the 7th lines, and amylose content of the 7th 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 7th and the 6th 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 different effect in each line. 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

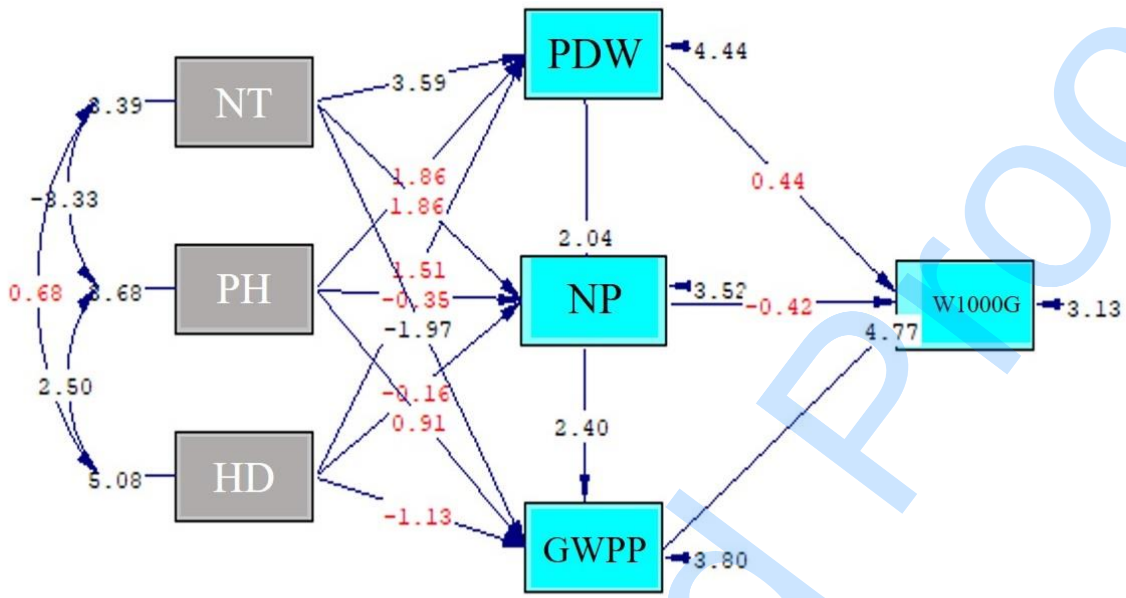

Figure 1. Path diagram of inter-relationship traits (p-value = 0.19579). (Notes: NT: number of tillers, PH: plant height, HD: heading date, PDW: plant dry weight, NP: number of panicles, GWPP: grain weight per panicle, W1000G: weight of 1,000 grain)

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)

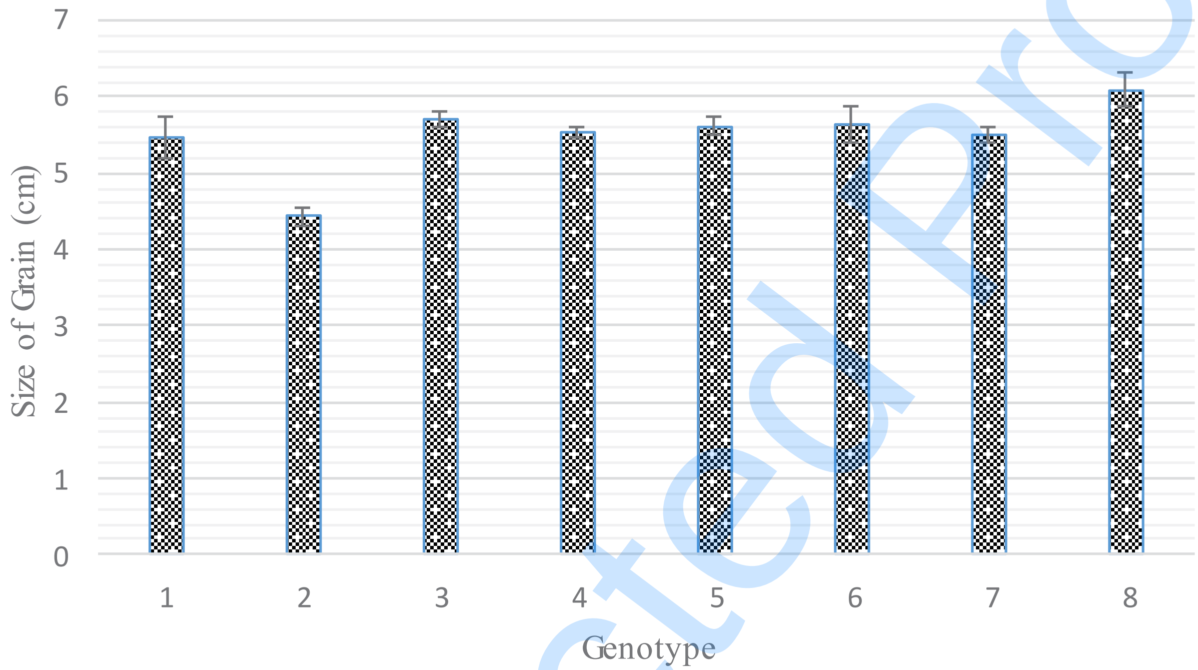

Figure 3. Length Size of F7 Dehulled Rice (Note: No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; while no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134, 487-24-8 lines, respectively)

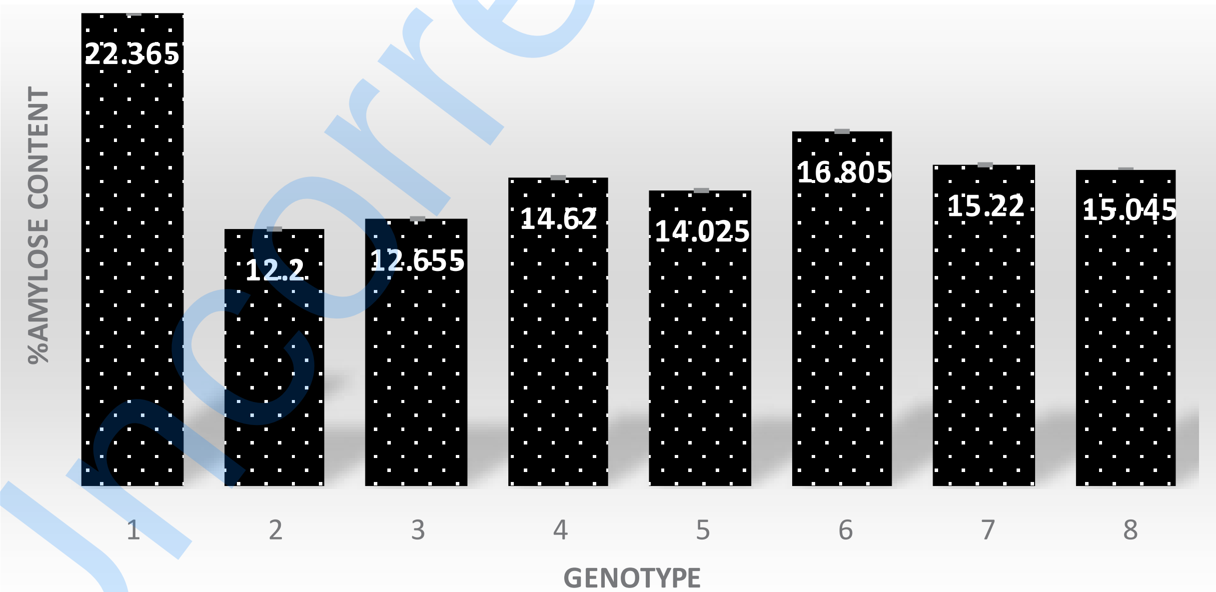

Figure 4. Amylose profile of F7 lines and checked varieties (Note: No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; while no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134, 487-24-8 lines, respectively)

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

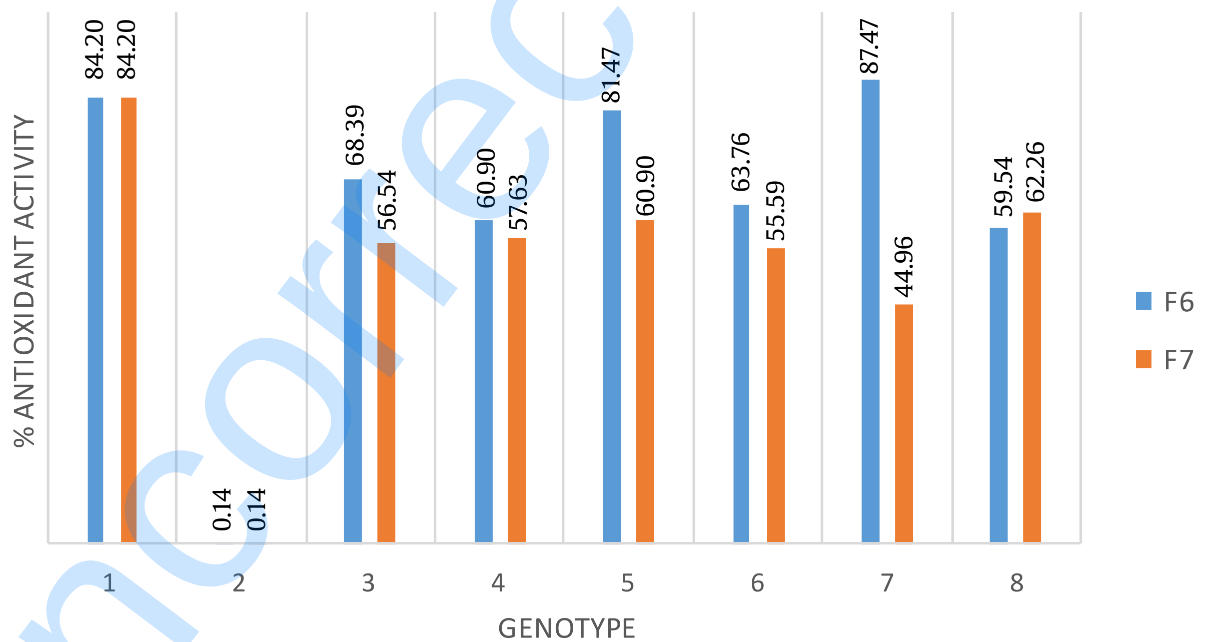

Figure 5. Antioxidant activity of F6 and F7 dehulled rice. (Note: No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134, 487-24-8 lines, respectively)

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

Eka Oktaviani*, **Suprayogi**

Department of Agrotechnology, Faculty of Agriculture, Universitas Jenderal Soedirman, Banyumas, Indonesia

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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%

* Corresponding Author
E-mail Address: oktaviani@unsoed.ac.id

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 6th 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 ($\alpha = 0.05$) 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 different. 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

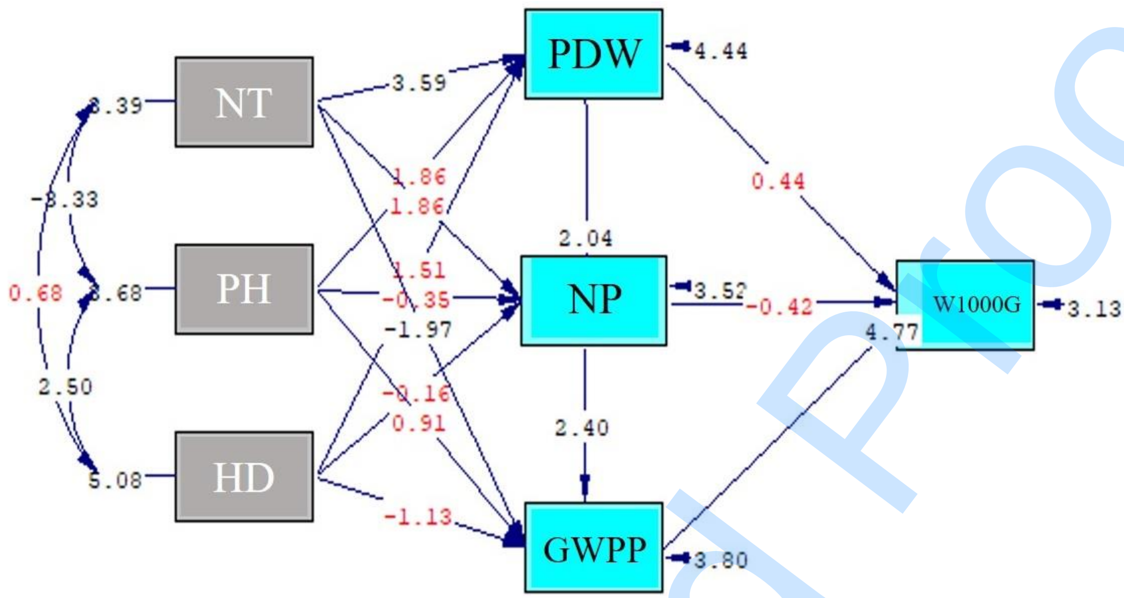

Figure 1. Path diagram of inter-relationship traits (p-value = 0.19579). (Notes: NT: number of tillers, PH: plant height, HD: heading date, PDW: plant dry weight, NP: number of panicles, GWPP: grain weight per panicle, W1000G: weight of 1,000 grain)

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)

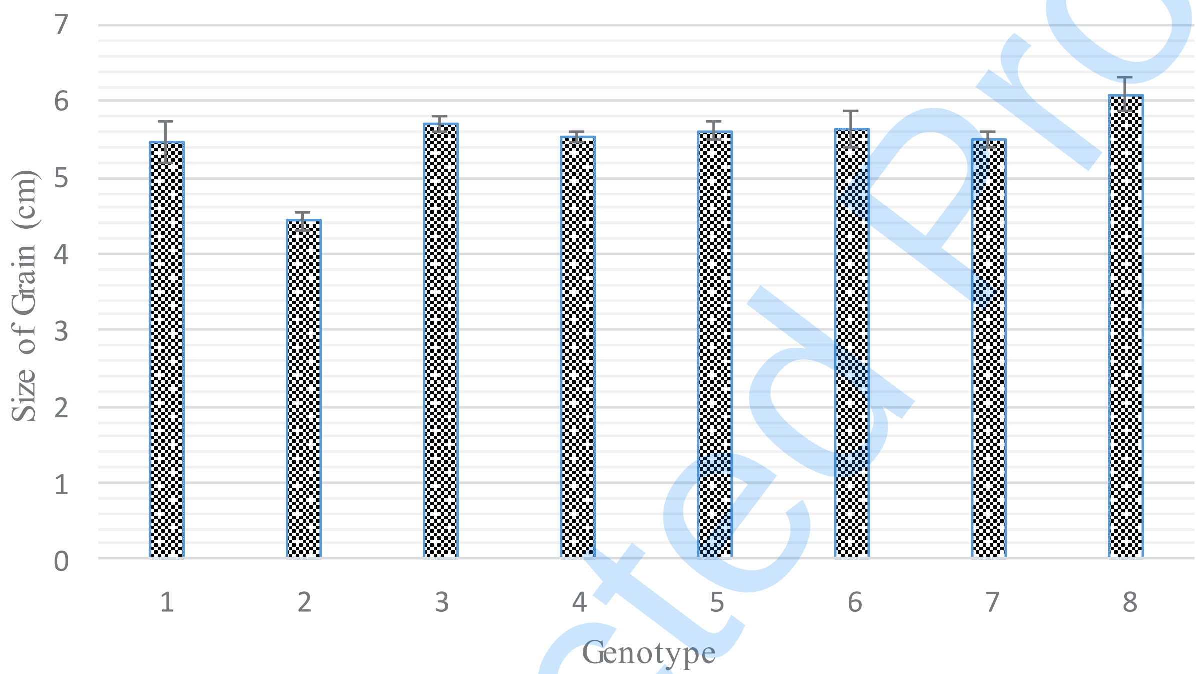

Figure 3. Length Size of F7 Dehulled Rice (Note: No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; while no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134, 487-24-8 lines, respectively)

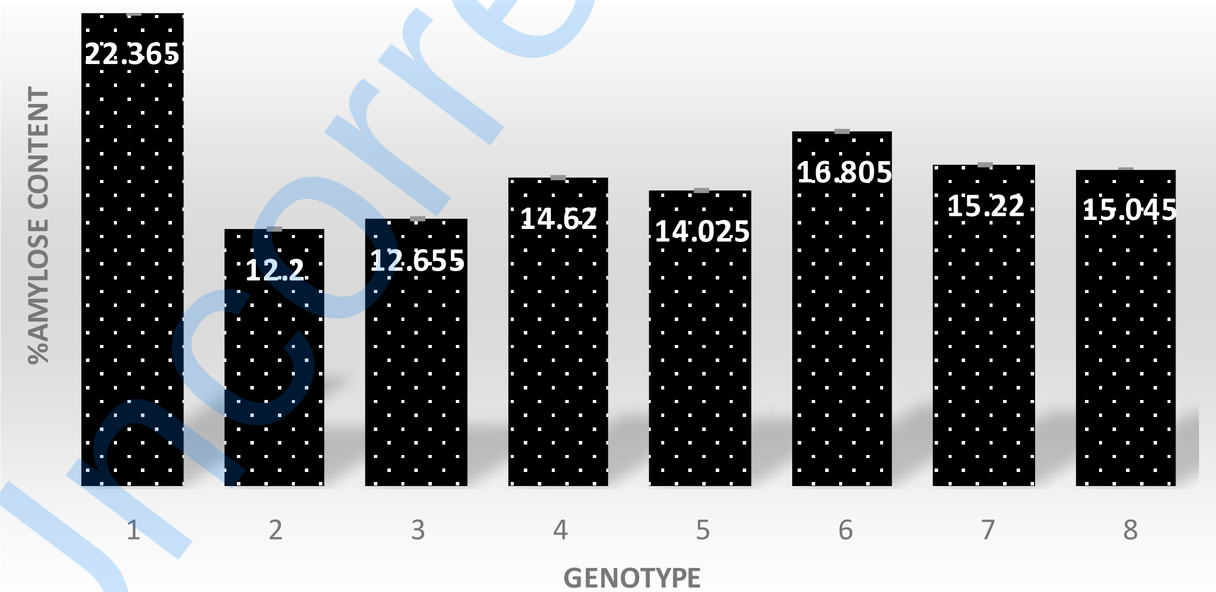

Figure 4. Amylose profile of F7 lines and checked varieties (Note: No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; while no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134, 487-24-8 lines, respectively)

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

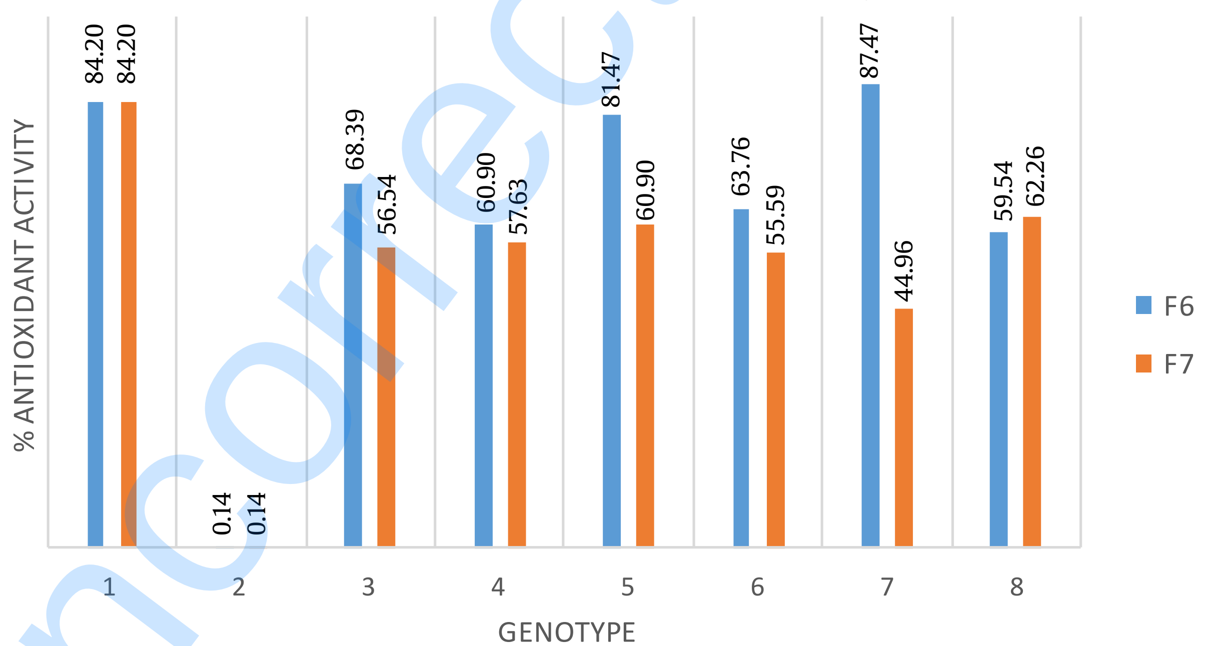

Figure 5. Antioxidant activity of F6 and F7 dehulled rice. (Note: No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134, 487-24-8 lines, respectively)

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

Eka Oktaviani*, **Suprayogi**

Department of Agrotechnology, Faculty of Agriculture, Universitas Jenderal Soedirman, Banyumas, Indonesia

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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%

* Corresponding Author
E-mail Address: oktaviani@unsoed.ac.id

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 different. 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

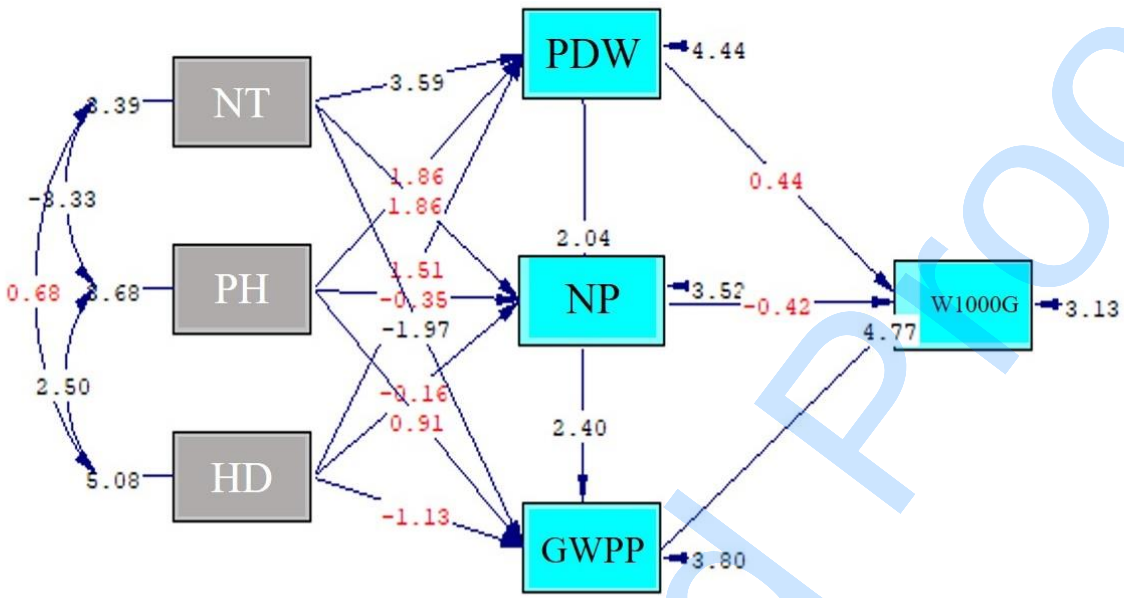

Figure 1. Path diagram of inter-relationship traits (p-value = 0.19579). (Notes: NT: number of tillers, PH: plant height, HD: heading date, PDW: plant dry weight, NP: number of panicles, GWPP: grain weight per panicle, W1000G: weight of 1,000 grain)

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)

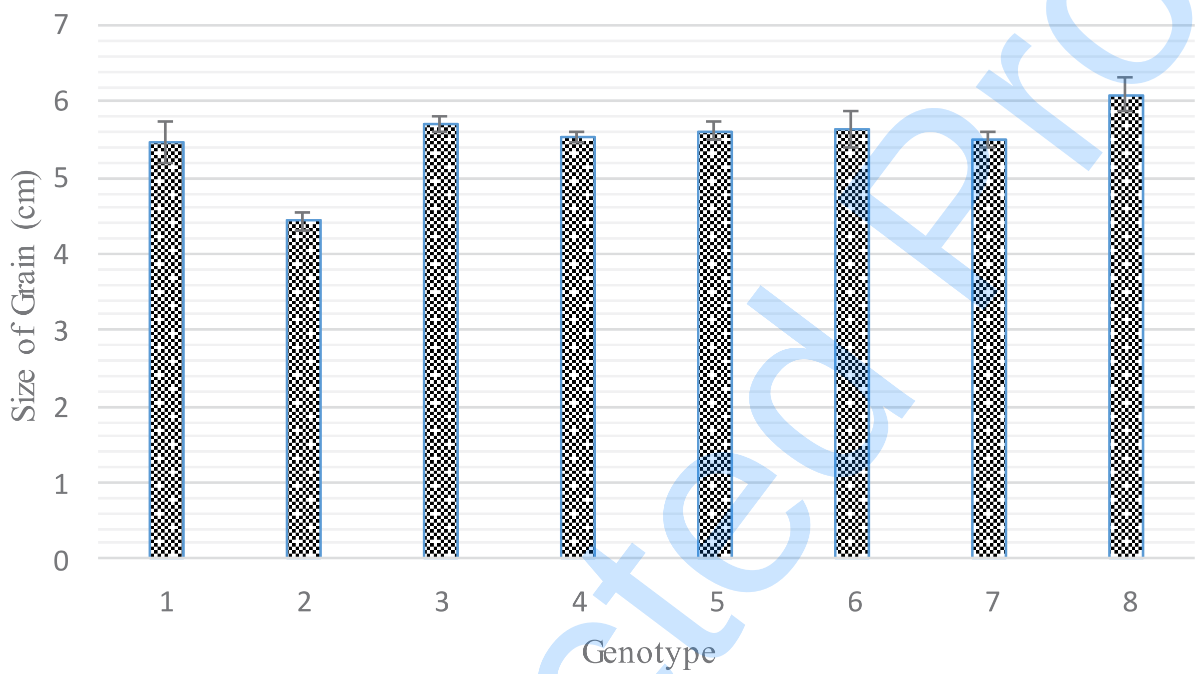

Figure 3. Length Size of F7 Dehulled Rice (Note: No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; while no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134, 487-24-8 lines, respectively)

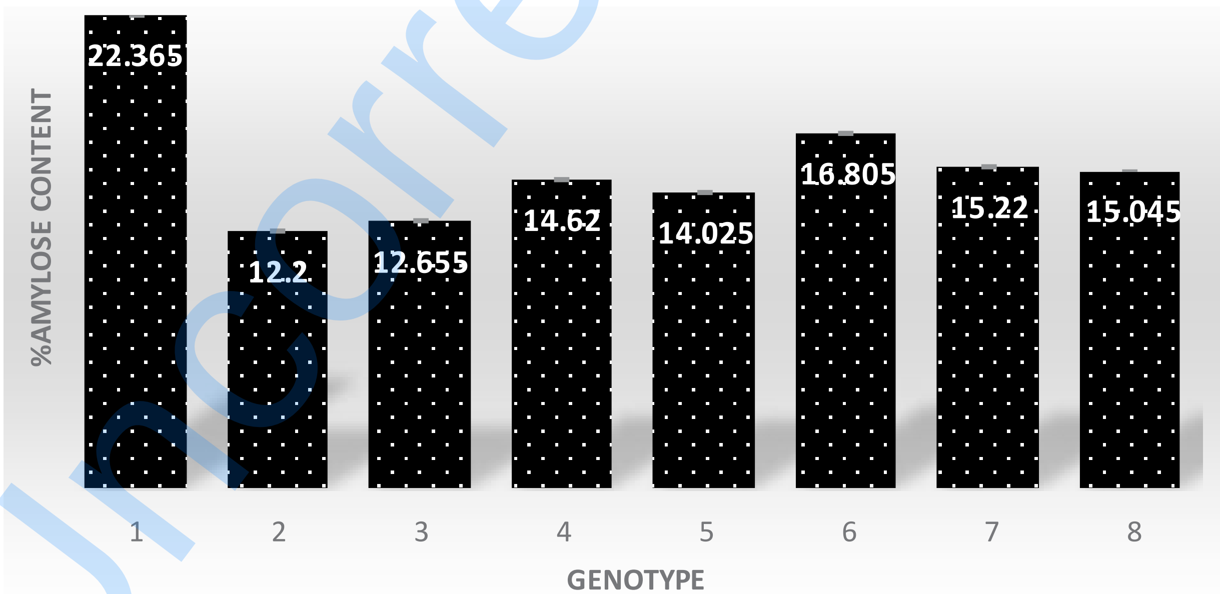

Figure 4. Amylose profile of F7 lines and checked varieties (Note: No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; while no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134, 487-24-8 lines, respectively)

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

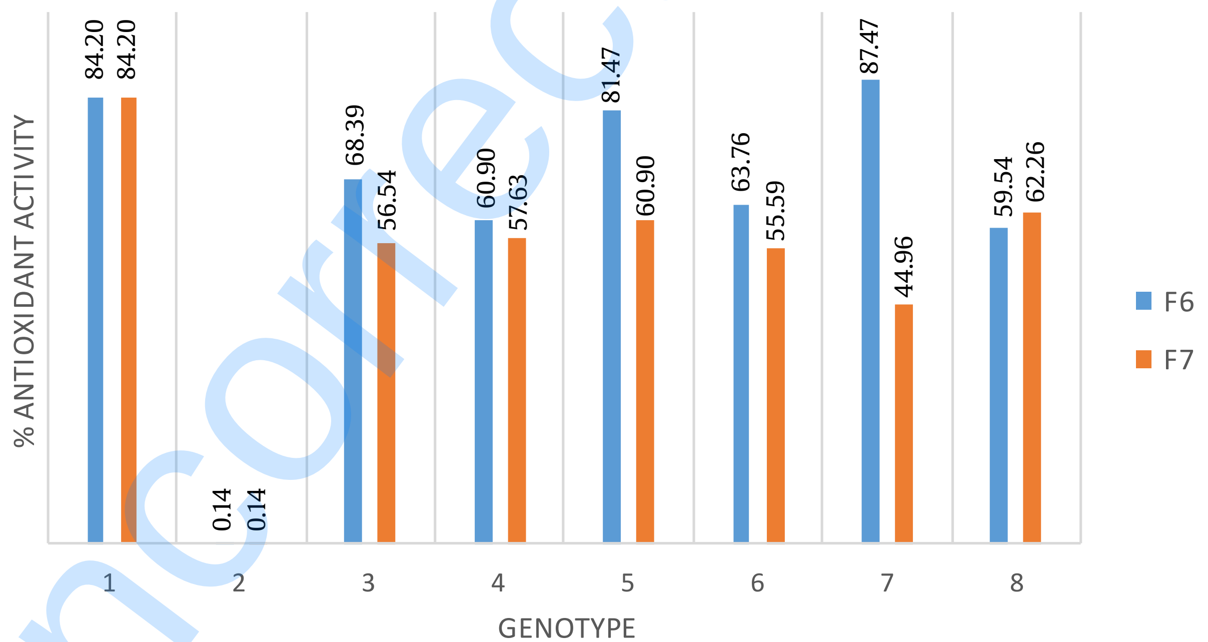

Figure 5. Antioxidant activity of F6 and F7 dehulled rice. (Note: No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134, 487-24-8 lines, respectively)

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

Eka Oktaviani*, **Suprayogi**

Department of Agrotechnology, Faculty of Agriculture, Universitas Jenderal Soedirman, Banyumas, Indonesia

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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%

* Corresponding Author
E-mail Address: oktaviani@unsoed.ac.id

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 6th 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 ($\alpha = 0.05$) 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

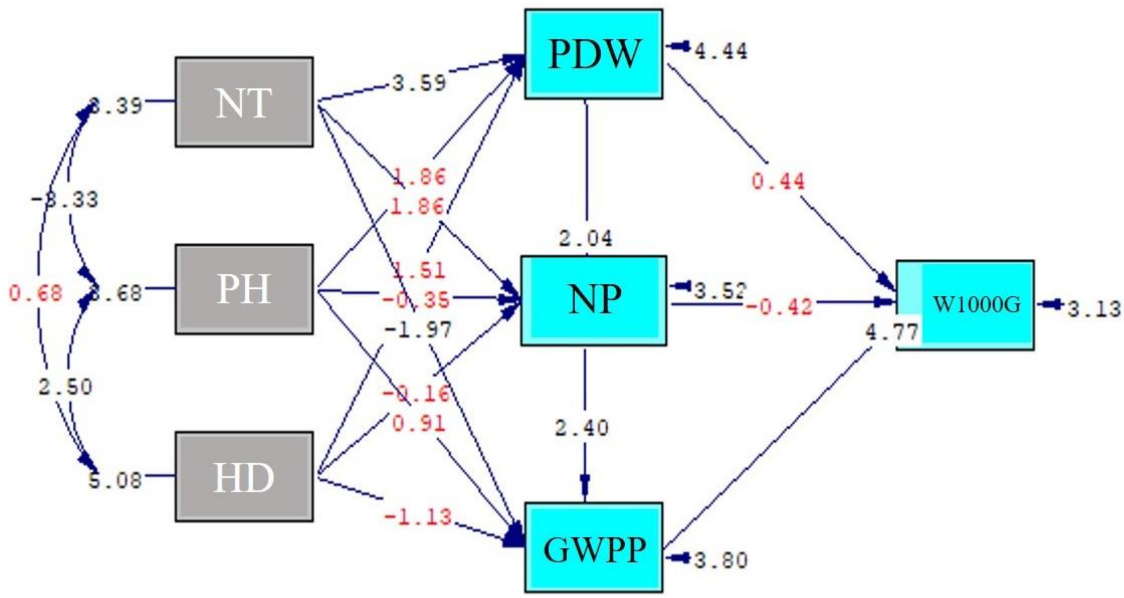

Figure 1. Path diagram of inter-relationship traits (p-value = 0.19579). (Notes: NT: number of tillers, PH: plant height, HD: heading date, PDW: plant dry weight, NP: number of panicles, GWPP: grain weight per panicle, W1000G: weight of 1,000 grain)

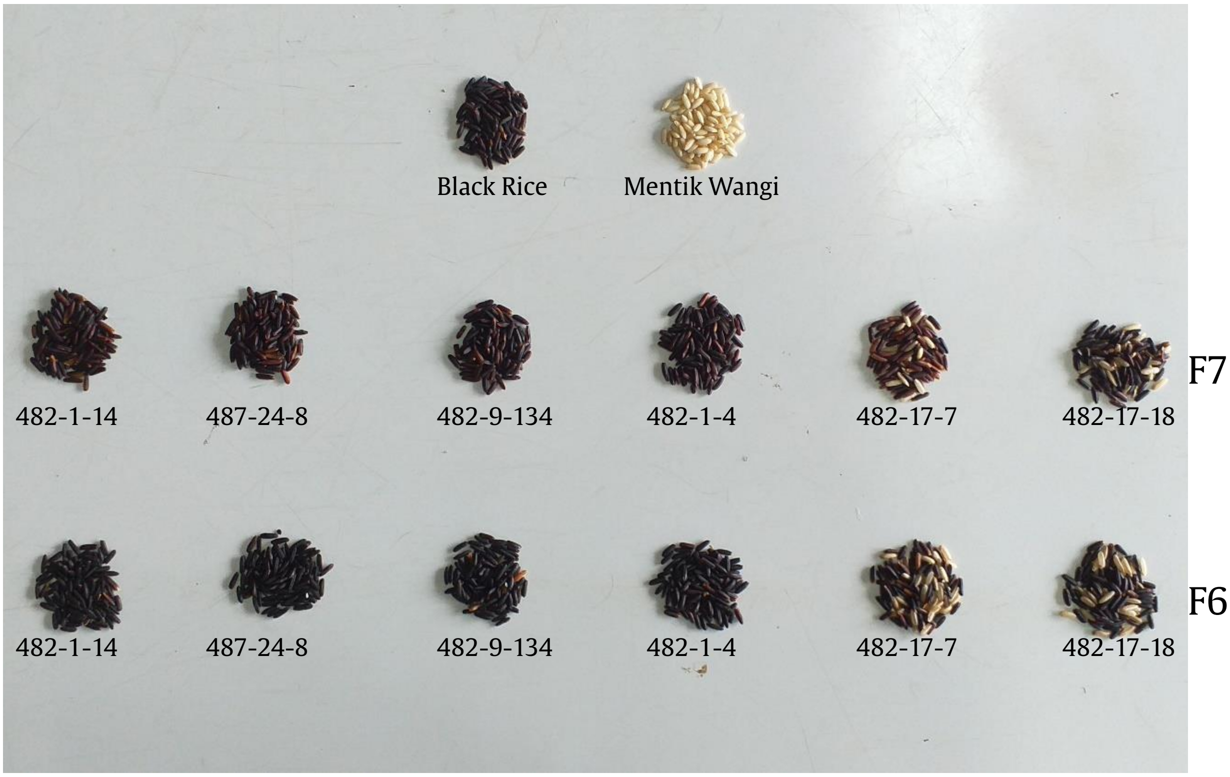

Figure 2. Color of the dehulled rice of lines and checked varieties

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)

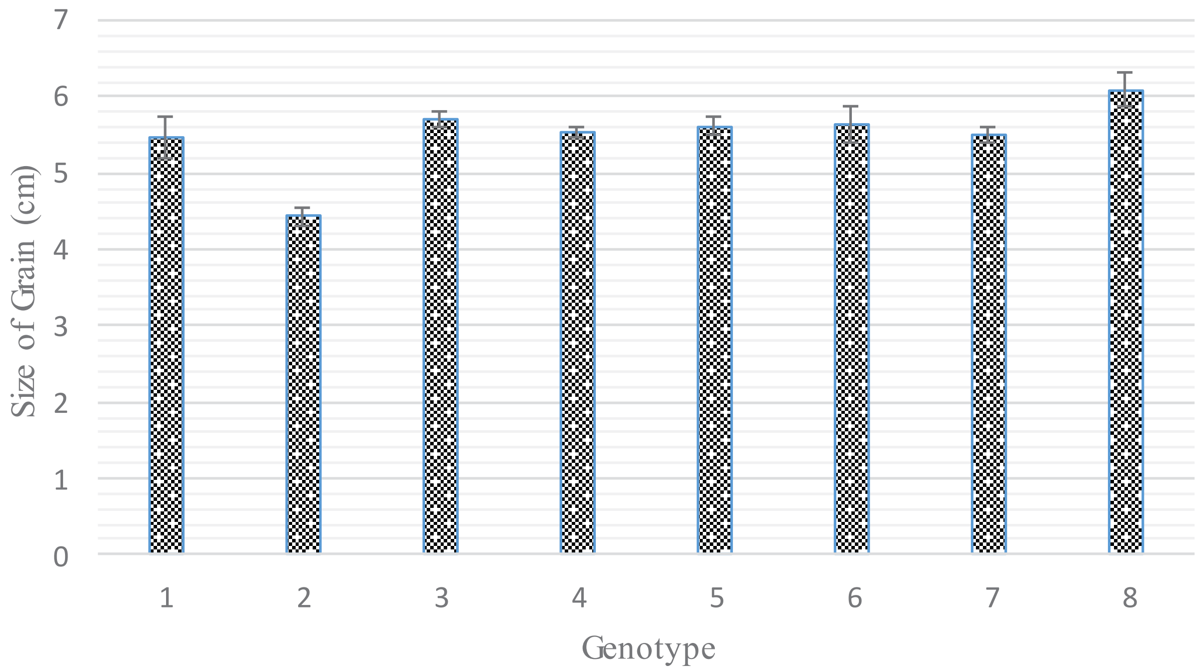

Figure 3. Length Size of F7 Dehulled Rice (Note: No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; while no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134, 487-24-8 lines, respectively)

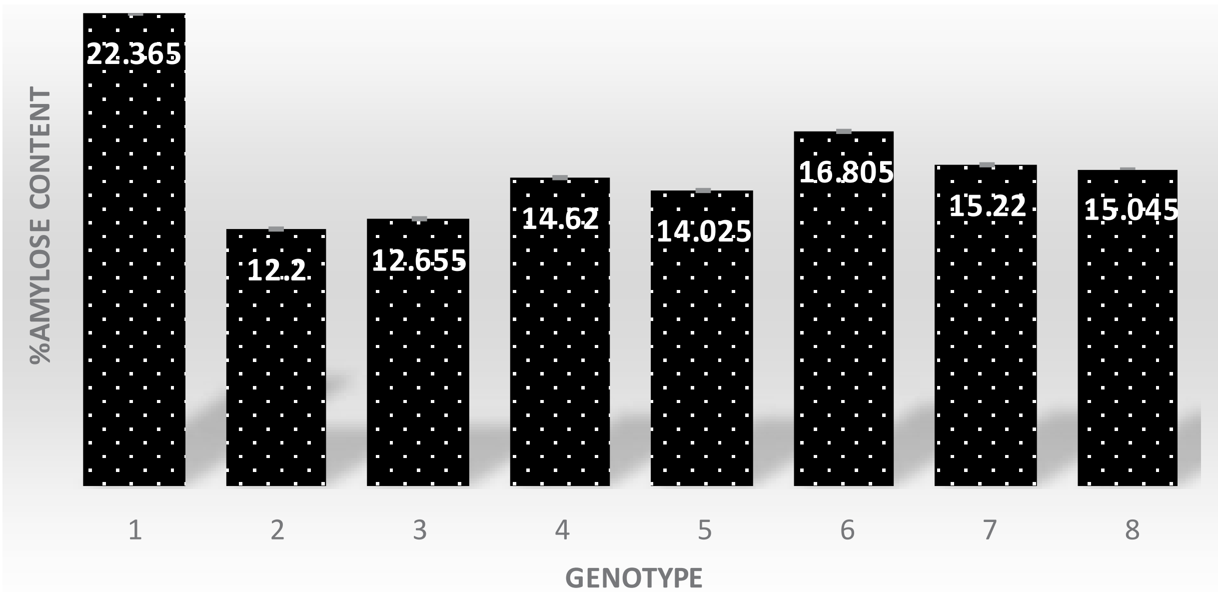

Figure 4. Amylose profile of F7 lines and checked varieties (Note: No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; while no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134, 487-24-8 lines, respectively)

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

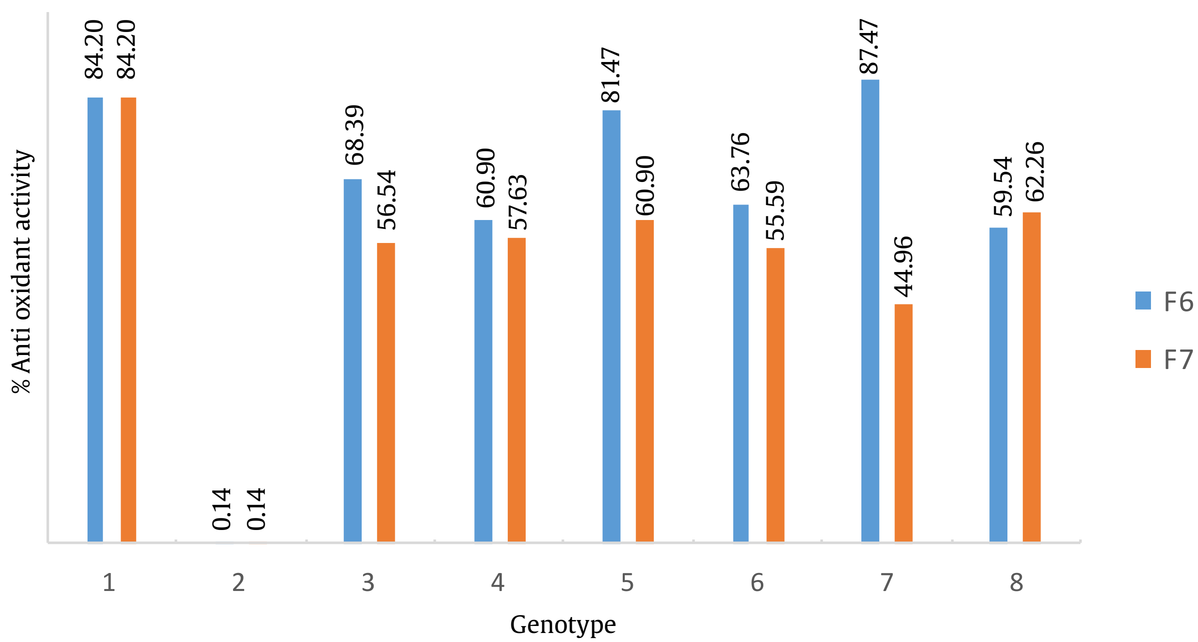

Figure 5. Antioxidant activity of F6 and F7 dehulled rice. (Note: No. 1 and 2 are Black Rice and Mentik Wangi varieties, respectively; no. 3, 4, 5, 6, 7, and 8 are PHMW 482-1-14, 482-17-7, 482-1-4, 482-17-18, 482-9-134, 487-24-8 lines, respectively)

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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