



Amylose profile and rice grain morphology of selected F6 lines derived from a crossing of Black Rice and Mentik Wangi for the development of waxy pigmented rice

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Abstract

Research on rice plant breeding to get superior black rice varieties with tender rice texture can be carried out by crossing Black Rice and Mentik Wangi variety. The rice lines derived from a crossing of these two varieties have recently reached the F6 line. The texture of rice is distinguished by the amylose content. The lower of the rice amylose, the more tender of the rice texture, and vice versa. This study aimed to find out the profile of amylose content and the grains morphology of the lines that will be developed as waxy pigmented rice. Analysis of amylose content was carried out using iodine-colorimetry methods. The quantification of amylose was measured based on the regression of the standard amylose curve. The results showed that all the F6 lines had the potential to be developed as waxy pigmented rice. Based on the Kruskal Wallis test, there was a variance in the amylose profile average of the eight genotypes. In terms of grains morphology, the line 482-17-7 and 482-17-18 had a combination colour between the two parents. In addition, there was a significant difference in grains size between the genotypes and the grain size of all lines were classified in the medium size. F6 lines resulted from the crossing between Black Rice and Mentik Wangi are recommended to be used as superior varieties of waxy pigmented rice.

INTRODUCTION

Rice, with natural color pigments, has been known to contain various types of flavonoid compounds, tannins, phenols, sterols, oryzanols, and essential oils (Limtrakul et al., 2019). Pratiwi and Purwestri (2017) have shown that pigmented rice, especially black rice, has antioxidant activity, antihyperlipidemia, antihyperglycemia, and cancer prevention. Goufo and Trindade (2014) reported that antioxidant compounds played an important role in determining the color differences in grains of four types of pigmented rice (brown, black, white, and red). The antihyperlipidemic and antihyperglycemic activities in black rice have been known to be related to the regulation of lipogenic

enzyme activity in the liver (Um et al., 2013). Meanwhile, the cancer prevention activities of pigmented rice have been reported due to the content of the main compounds in the form of flavonoids and phenols (Pratiwi and Purwestri, 2017). In fact, Pratiwi et al. (2013) have further reported that this compound content could inhibit the invasion of cancer into other tissues.

In line with the increasing prevalence rate of Diabetes Mellitus, especially type 2 (DM2) and cancer in Indonesia, the need for pigmented rice as a functional food is becoming increasingly important. Based on the data released by Ministry of Health Data and Information (2013), it was reported that the prevalence rate of DM2 increased from 5.7 %

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(2007) to 6.9 % (2013). In addition, from the same study, the number of diabetic patients in Indonesia reached 10 million, and Indonesia was the country with the seventh highest diabetic prevalence rate in the world. Furthermore, the number of diabetic patients would rise to 642 million by 2040 in the world (World Health Organization, 2016). Meanwhile, the prevalence rate of cancer in Indonesia is no less. In 2013, the prevalence of cancer patients in the population of all ages was 1.4 ‰, with Central Java Province being the second highest (2.1 ‰) after the Special Region of Yogyakarta (DIY) (4.1 ‰). In 2012, various types of cancer became the cause of death for approximately 8.2 million people (Ministry of Health Republic of Indonesia, 2015).

As a country with abundant biodiversity, Indonesia is also home for pigmented rice. Pratiwi and Purwestri (2017) have documented 24 varieties of black rice from Java, Nusa Tenggara, Sulawesi and Kalimantan. Meanwhile, Dwiatmini and Afza (2018) have used 27 types of red rice originating from various regions in Indonesia such as Kalimantan, Java, Sumatra, Riau, and East Nusa Tenggara to conduct research on anthocyanin content in rice accessions. Afza (2016) reported that there were 54 accessions of brown rice preserved at the Center for Research and Development of Agricultural Biotechnology and Genetic Resources, Bogor.

However, the texture of rice cooked from black rice has known to be dry, hard and not tender at all, so that they are less preferred (Mardiah et al., 2016). Meanwhile, the Mentik Wangi variety has been reported to have soft and sticky texture, as well as fragrant aroma (Yunus et al., 2018). The development of superior black rice with a stickier texture could be done by crossing Black Rice with the Mentik Wangi variety.

According to Aliawati (2003), the quality of rice grain is determined based on the profile of amylose and amylopectin. Amylose profile has been used to predict tenderness in cooked rice grains and to select genotype of interest in rice breeding program (Avaro et al., 2011). 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).

Researches to develop black rice with superior traits had been carried out by researchers from various countries. Zhang et al. (2018) 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 intra-specific 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. In addition, 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. Until now, there has been a Jeliteng variety, which is the first superior black rice variety released by Indonesian Agency for Agricultural Research and Development in 2019, derived from a crossing between Ketan Hitam and Pandan Wangi Cianjur. This variety has a soft and sticky rice texture, with an amylose content of 19.6 %. Research on the development of soft, tender, and waxy black rice from crossing Black Rice and Mentik Wangi is expected to enrich the resource of novel superior black rice germplasm in Indonesia.

MATERIALS AND METHODS

Preparations of grains for analysis

The six lines were cultivated in Wanarejan Utara, Taman District, Pemalang Regency, Central Java Province. Then, the grain preparations for analysis were carried out from May to November 2020. The grain samples were mixed from some panicles of some rice plants, including the six lines and two checked varieties (Black Rice cv Cilacap and Mentik Wangi). A total of 200 mg of grains were taken for amylose content analysis, and a total of three grains from each line and checked varieties were used for grains size determination.

Determination of amylose content and size of grains

Iodine-colorimetric method was used to quantify the amylose content in rice grains (Juliano, 1971). Analytical repetition was carried out two times. The grains were measured by calipers. Repetition was carried out three times.

Quantitative analysis of amylose was measured by making a standard amylose curve first. Pure amylose (40 mg) was put into the test tube. Then, 95 % ethanol (1 mL) and 1N NaOH (9 mL) were mixed to the tube. The mixture was heated in boiling

water for about 10 minutes until all the chemical formed a gel. After that, it was cooled. The whole mixture was put to a 100 mL measuring flask, until it was right on the calibration mark. Each mixture was taken as much as 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL, and 1 ml, then each was added with 2 mL of Iodine solution. Each mixture was put in a measuring flask, reaching the calibration mark, and left for 20 minutes. Spectrophotometer (λ 625 nm) was used to measure the intensity of blue color. A standard curve was then created, with an x vs y axis (amylose concentration vs absorbance).

The rice grain (100 mg) sample in the form of flour (the sample consists mostly of starch, if it contains many other components, first extract the starch, then just analyze the amylose) was put into a test tube. Then, 95 % ethanol (1 mL) and 1 N NaOH (9 mL) were mixed to the sample. The whole gel was displaced to a 100 mL measuring flask, shaken out, then adjusted to the calibration mark. After that, as much as 5 mL of the solution was taken and put into a 100 mL measuring flask, added with 1 N acetic acid (1 mL) and iod solution (2 mL), then adjusted to the calibration mark, shaken, and let stand for 20 minutes. Spectrophotometer (λ 625 nm) was used to measure the intensity of sample color. The amylose quantification in the sample was then measured based on the linear regression equation in standard curve.

Data analysis and methods of selection

Normality and homogeneity tests were carried out on amylose content and size of grains data. The normality test aimed to determine whether the data were normally distributed, while the homogeneity

test aimed to determine whether the data variations from the population have a uniform variance (homogeneous or not). If these two conditions were met, then the analysis was proceeded to the parametric One Way Analysis of Variance (ANOVA) statistical test at $\alpha = 5\%$, to distinguish if there was a difference in the average of the data consisting of more than two groups. Meanwhile, if the two requirements above were not met, then the Kruskal Wallis non-parametric statistical test was performed to determine whether the average data from more than two groups tested had a difference.

The selection of lines was carried out based on the amylose content data, in accordance with the purpose of plant breeding, namely to obtain lines of black rice with soft and sticky rice texture, which could be indicated by lower amylose content in grains.

RESULTS AND DISCUSSION

Amylose content in selected lines and checked varieties

The test for normality and homogeneity of the amylose content data showed that the data were not normally distributed and were not homogeneous. Then, based on the Kruskal Wallis test, there was a variance in the amylose content average of the 8 genotypes (the Asymp. Sig value of 0.038 <0.05). Figure 1 shows the amylose content average of each genotype. One of the determinants of rice grains quality is the amylose content (Traore, 2005). Biosynthesis amylose in endosperm is catalyzed by the granule bound starch synthase (GBSS) enzyme (waxy protein), encoded by waxy (Wx) gene (Wang

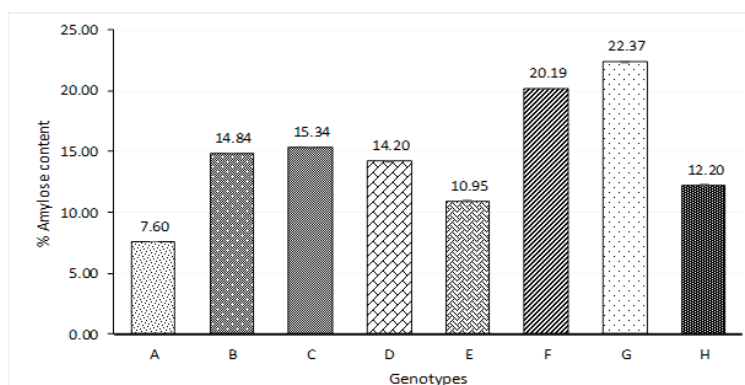


Figure 1. Amylose content in the 8 (eight) genotypes (A: line 482-1-14; B: line 487-24-8; C: line 482-9-134; D: line 482-1-4; E: line 482-17-7; F: line 482-17-18; G: Black Rice cv Cilacap and H: Mentik Wangi)

et al., 2014; Traore, 2005; Wang et al., 1995; Rohde et al., 1988). Generation of F1 with a high amylose content corresponds to an increase in the dose of genes involved in amylose biosynthesis in parents with a higher amylose content, although this doesn't always have a linear graphic (Khumar & Khush, 1986). Variation in the amylose content of the lines indicates a complex pattern of inheritance from parents to generations. He et al. (1999) concluded that grain quality traits were inherited in a more complex pattern than agronomic traits in cereals due to the effect of extranuclear inheritance, the triploid nature of the endosperm, and epistasis. In addition, rice grain quality is regulated by nuclear genes of endosperms, cytoplasmic genes, and nuclear genes of maternal plants (Chen and Zhu, 1999). Various studies also reported that high level of amylose was managed either by complete or partial dominance. Amylose content was regulated by a single locus in Shanyou 63 hybrid rice when the trait of high amylose content

was dominant (Tan et al, 1999). Meanwhile, Heda and Reddy (1986) reported that it was controlled by two pairs of gene when the trait of high amylose was partially dominant over the low amylose.

Rice could be categorized into 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 %) (Khush and Cruz, 2000). Based on these parameters, the lines observed in this study can be classified into very low amylose (line 482-1-14), low amylose (lines 487-24-8, 482-9-134, 482-1-4, and 482-17-7) and medium amylose (line 482-17-18). The amylose content was one of the parameters used to predict the quality of processed rice (Juliano et al., 1965; Bhattacharaya and Juliano, 1985) and to select the genotype of interest in rice breeding program (Avaro et al., 2011). The lower the amylose content, the more tender the texture of the rice, and vice versa (Panesar and Kaur, 2016). Li et al. (2016a) reported that

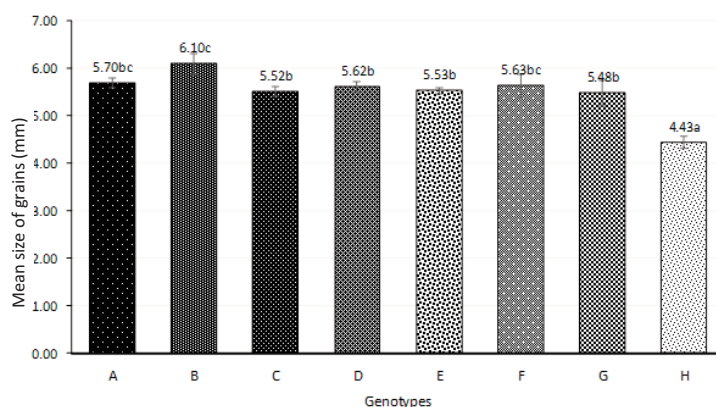


Figure 2. The average grain size of each line compared to the checked varieties (A: line 482-1-14; B: line 487-24-8; C: line 482-9-134; D: line 482-1-4; E: line 482-17-7; F: line 482-17-18; G: Black Rice cv Cilacap and H: Mentik Wangi)



Figure 3. Grain morphology of the selected lines and two checked varieties, (A)= line 482-1-14; (B)= line 487-24-8; (C)= line 482-9-134; (D)= line 482-1-4; (E)= line 482-17-7; (F)= line 482-17-18; (G)= Black Rice cv Cilacap and (H)=: Mentik Wangi)

the molecular size and chain-length distribution of amylose were significant determinants of the hardness of cooked rice. Li et al. (2016b) also reported other factors affecting the texture of rice were post-harvest processing and cooking methods.

Size of grains in selected lines and checked varieties

The grain morphology of the lines studied can be seen in Figure 2. The data on grain size average are homogeneous (Sig.> 0.05) and normally distributed (Sig.> 0.05). Based on the One Way of ANOVA (Analysis of Variance), there was a significant difference in grain size average between the eight genotypes (Sig <0.05). Mentik Wangi variety has grain size average that is significantly different from the other genotypes. The average size of black rice grain does not differ significantly from the grain size average of the line 482-1-14, 482-9-134, 482-1-4, 482-17-7, and 482-17-18. The line 487-24-8 is significantly different from the Mentik Wangi variety, black rice variety, line 482-9-134, 482-1-4, and 482-17-7, however, it is not significantly different from the line 482-1-14 and 482-17-18.

In addition, based on International Rice Research Institute (2013), the standard of grain size of the lines studied are in the medium category, while that of those checked varieties are in the short category. A bar chart showing the size comparison of each line with checked varieties can be seen in Figure 2 (in mm). Kush and Cruz (2000) stated that grain size was the initial selection criteria in plant breeding program for commercial rice.

Based on the data of grain morphology, line 482-17-7 and line 482-17-18 have the mixed color of the grains from combination of two parents (Figure 3). Kim et al. (2007) reported that two main anthocyanin pigments stored in the seed pericarps of black rice were cyanidin-3-O-glucoside and peonidin-3-O-glucoside. Rahman et al. (2013) detected that the level of cyanidin-3-glucoside was defined by the copy number of the Pp allele. However, the number of genes involved in the appearance of this trait, the segregation pattern and molecular mechanism of this inheritance were not further analyzed.

CONCLUSIONS

The F6 lines from the crossing of Black Rice and Mentik Wangi have the prospect of being used as superior varieties of waxy pigmented rice. However, further research is needed regarding the organoleptic

status and traits stability across environments and seasons (year) to evaluate the performance of rice lines, as well as the genetic mechanism of specific traits expression.

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