Anthocyanin Content in Some Black Rice Cultivars

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Abstract. Rice is one source of staple foods that have high health benefits, especially black rice, because of the presence of anthocyanin, carbohydrates, fats, proteins, fibre, and minerals. At present, people are starting to pay attention to nutritious foods with the contents and benefits that support health for their diet pattern. This greatly provides a great opportunity for the development of black rice as one of the cultivars which has high anthocyanin content. Anthocyanin is one of the phenolic compounds that enter flavonoid compounds and function as an antioxidant that has a role for the plant itself and for humans as black rice consumers. Anthocyanin in black rice is the black pigment found in pericaps and tegmen (skin layers) of rice. Some are also found in all layers of rice. This research aimed to determine the anthocyanin content in some black rice cultivars. The analysis used to determine the anthocyanin content is the pH difference method. The results of the experiment showed that from 10 cultivars tested, the highest anthocyanin content was found in Toraja black rice cultivars of 21.12 mb/g, followed by Banjarnegara of 19.3 mg/g and melik black rice of 19.06 mg/g. The results of this research are essential for a breeder for the initial selection of the development of superior black rice varieties to choose the rice plant parent with high anthocyanin content.

1. Introduction

One of the staple foods of Asian people is rice. The colour and shape of rice is so various, both in plants and in rice. The type and colour of rice are white, and some have red and black pigments. Each type of rice has health benefits, which is caused by the presence of micronutrients, fibres and proteins. Black rice has a high anthocyanin content when compared to white and red rice so that it becomes a chemo-preventive agent and has antioxidant and anti-inflammatory effects [1]. The black colour of black rice is found not only in pericap but also in the grain, stem and leaves. In some types of black rice, it is only found in aleurone or the outer layer of the rice and the grinding process can affect the peeling of the aleurone layer [2].

Along with public awareness of health, the food patterns also become one thing to consider; for example: the selection of black rice as a substitute for white rice because of its high anthocyanin content. The research results of Ref.[3] show that anthocyanin has been proven can reduce the risk of stroke, heart disease and cancer. According to the research results of [4], anthocyanins found in aleurone were cyanidin 3-O-glucodiglucoside and peonidin 3-O-glukoside [5-7], reported that triglyceride levels, total cholesterol in the blood of mice that were tested, could be lowered by administering black rice aleurone layer extract. The availability of anthocyanin in addition to some types of rice is also present in several...
varieties of vegetables and fruits such as corns, carrots, red spinach, roseapples and other agricultural products [8]. However, the development of black rice cultivation is still relatively small and the number of superior varieties of black rice is still not as much as that of white and red rice. Therefore, black rice is very likely to continue to be developed with several properties of superior nutritional content that really needs a lot of genetic diversities to assemble these superior varieties. This becomes a challenge and opportunity for plant breeders.

This research aimed to determine the anthocyanin content of some black rice cultivars and obtain the best cultivars from black rice which have high anthocyanin content useful for breeders in selecting plant varieties as parents in the assembly of new varieties.

2. Materials and Method
This research is divided into four stages, namely: collecting several black rice cultivars, cultivating black rice, observing the color of cultivated black rice, and analysing the anthocyanin content found in black rice. The cultivars used in this research were 10 cultivars obtained mostly from several regions in Indonesia consisting of Banjarnegara black rice, Purbalingga black rice, Pari Ireng, Purwokerto black rice, Hare Lahok black rice, Bantul black rice, Lumajang black rice, Toraja black rice, Melik black rice, and Blitar black rice. The design used in this research was a randomized block design for the treatment of 10 black rice cultivars with three-time repetitions. The colour of rice observed is in brown rice. Rice colour codes: 1 = White, 2 = Brown, 3 = Red Brown, 4 = Blackish Purple.

The method used to test the anthocyanin content was the pH difference method and the results of subsequent observations were analysed with F Test statistics. If the F value in the treatment is greater than the F table value of 5 percent, then DNMRT is used at the 5 percent level. The use of the pH difference method for anthocyanin analysis was carried out with, first, grinding the dried black rice seed sample as much as 0.1 g. Furthermore, the sample was extracted using 40 ml of ethanol: 0.1 M HCl (85: 5%, v:v). The total anthocyanin content in the black rice seed extract was determined using the pH difference method [9]. It needs 2 buffer solutions. The first solution is a solution for pH 1.0 using the KCl buffer and the second one for pH 4.5 using Sodium Acetate Monohydrate. 3 ml of black rice seed extract was dissolved with 5 ml pH 1.0 KCl buffer and the Sodium Acetate Monohydrate pH 4.5 was then incubated for ±30 minutes at the room temperature. Then, the solution of different pH conditions was measured by using a UV-Vis spectrophotometer at a wavelength of 520 nm and 700 nm. The total anthocyanin content is calculated using the following formula:

\[
\text{Total anthocyanin (mg/L)} : \frac{A \times \text{MW} \times \text{DF} \times 10^3}{E \times 1}
\]

Remark:
A : \((A_{520 \text{ nm}} - A_{700 \text{ nm}}) \) pH 1 – \((A_{520 \text{ nm}} - A_{700 \text{ nm}}) \) pH 4.5
E : Molar extinction coefficient
MW : Molecular weight
DF : Dilution factor.

3. Result and Discussion

3.1. Colour of Black Rice
The observation results of the rice colour of black rice from 10 different cultivars are as follows: Rice colour grouping has been regulated in the System Evaluation Standard for IRRI Rice [10] including white, light brown, small-spotted brown, brown, red, variate purple, and purple rice.
Fig 1. Morphology of Rice Colour. 1: Black Banjarnegara, 2: Black Purbalingga, 3: Pari Ireng, 4: Black Purwokerto, 5: Hare Lahok, 6: Black Bantul, 7: Black Lumajang, 8: Black Toraja, 9: Black Melik, 10: Black Blitar

Table 1. Colour 10 Cultivar of Black Rice

| Black Rice Cultivar      | Colour of Rice     |
|--------------------------|--------------------|
| Toraja                   | Blackish Purple    |
| Banjarnegara             | Blackish Purple    |
| Melik                    | Blackish Purple    |
| Lumajang                 | Blackish Purple    |
| Purwokerto               | Blackish Purple    |
| Purbalingga              | Blackish Purple    |
| Pari Ireng               | Blackish Purple    |
| Blitar                   | Blackish Purple    |
| Bantul                   | Blackish Purple    |
| Hare Lahok               | Brown              |

Table 2. The value of anthocyanin analysis in 10 black rice cultivars

| Black Rice Cultivar      | Anthocyanin Content mg/g |
|--------------------------|--------------------------|
| Toraja                   | 21.12 a                  |
| Banjarnegara             | 19.31 ab                 |
| Melik                    | 19.06 b                  |
| Lumajang                 | 11.54 c                  |
| Purwokerto               | 11.17 c                  |
| Purbalingga              | 10.81 cd                 |
| Pari Ireng               | 9.71 d                   |
| Blitar                   | 8.74 d                   |
| Bantul                   | 8.62 d                   |
| Hare Lahok               | 5.95                     |

3.2. The results of the anthocyanin analysis in black rice.
The results of the anthocyanin analysis were then tested based on the value of variance and anthocyanin content in 10 cultivars showing different results.
The anthocyanin levels in black rice using the pH difference method were then measured with a spectrophotometer. Based on the previous research, there are many anthocyanin levels in black rice compared to those in white rice and brown rice [4], [11-12]. The colour difference of in the rice will affect the levels or values of anthocyanins contained in it stated that [13], the darker and more concentrated the colour of black rice is, the higher the anthocyanin level will be, as shown in Table 1 (rice colour of black rice) and Table 2 (the result of anthocyanin analysis). The Toraja black rice cultivar showed the highest value of 21.12 mg/g compared to other cultivars, but it was not significantly different from the black rice of Banjaregara cultivar which had a value of 19.31mg/g. This was in accordance with the results of the observation of the rice colour which shows blackish dark purple as the result of high anthocyanin content. The lowest value of anthocyanin content was found in Here Lahok cultivar of 5.95 mg/g.

In Figure 2, the bar chart shows the average value of anthocyanin content which seems very clear from the highest value in Toraja black rice and the lowest in Here Lahok black rice. Anthocyanins in black rice were found in aleuron and endosperm layers which can produce anthocyanins in relatively large quantities so that the morphology of the rice that appears is blackish purple. The anthocyanin pigments in black rice have inhibitor compounds that can inhibit antioxidants (as antioxidants) so that human health has enormous benefits which are useful for preventing the pulverization of arteries, because black rice pigments contain much more active flavonoids compared with white rice [14]. Thus, the colour difference of the rice can be used as a reference to predict the anthocyanin content in rice [6], [15]. Anthocyanin pigments in black rice from the research results. It is known that the pigments can reduce the risk of oxidative damage from low density lipoprotein in humans. Besides, the pigment in black rice can reduce the formation of nitric oxide synthase in macrophage cells which serves to prevent DNA damage. With the high anthocyanin content in black rice, it can be made as one of the superior properties of one type of cultivars that needs to be maintained and developed again into a superior new variety.

4. Conclusion
From the results of the analysis and discussion, it can be concluded that the highest anthocyanin value of the 10 cultivars tested was in the Toraja black cultivar and the lowest in the Here Lahok black rice. The high anthocyanin content in black rice cultivars can be used as a reference for the selection of parents for the assembly of new varieties that have superior properties for the development of new black rice varieties.

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Acknowledgement
This research is fully supported by using the scholarship funds of the Government of the Republic of Indonesia through the Indonesian Institute of Education Scholarship Fund Management Institution (LPDP BUDI-DN) Indonesia. Number: PRJ-4951/LPDP.3/2016.