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Agronomic Characteristics, Anthocyanin Content, and Antioxidant Activity of Anthocynins Extracted from the Seeds of Black Rice Accessions

Phan Thi Thuy[1], Nguyen Quoc Trung[2] and Do Thi Huong[1]

[1]Faculty of Agronomy, Vietnam National University of Agriculture, Hanoi 131000, Vietnam
[2]Faculty of Biotechnology, Vietnam National University of Agriculture, Hanoi 131000, Vietnam

Abstract

The objectives of this study were to investigate the morphological characteristics, grain yield, and anthocyanin content of 36 black rice accessions that were collected from different locations in Vietnam. The results showed that the black rice accessions varied in growth duration (130 to 150 days), plant height (91.5 to 143.6 cm), morphological characteristics, and yield components. Grain yield of the black rice accessions ranged from 2.8 to 8.7 tons ha^{-1}. The black rice accessions were classified into four groups based on their anthocyanin content: group I > 0.1% anthocyanin, group II 0.05%-0.1%, group III 0.001%-0.05%, and group IV < 0.001%. BR7 had the highest anthocyanin content (0.1438%), followed by BR5 (0.1317%). Anthocyanins with the strongest antioxidant activities were extracted from BR8, BR35, BR6, BR27, BR30, BR32, BR18, BR17, BR19, and BR1 with IC_{50} values less than 2 µg mL^{-1}. Seven promising black rice accessions, namely BR1, BR14, BR17, BR25, BR30, BR34, and BR35, were selected for further research based on their high anthocyanin contents, and good grain yield and yield components.

Keywords

Anthocyanin content, antioxidant activity, black rice accessions, yield

Introduction

Rice (*Oryza sativa* L.) is the most important staple food in the world, feeding more than 50% of the world's population (Huang *et al.*, 2016), and provides up to 76% of the calorific intake of the population in South East Asia (Fitzgerald *et al.*, 2009). The traditional diet in Vietnam as well as in other Asian countries is largely based on polished white rice with a high glycemic index (GI) or glycemic load (GL). The higher dietary GI or GL has been shown

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Correspondence to
Do Thi Huong
dthuong@vnua.edu.vn

to have a significant relationship with diabetes and cardiovascular diseases such as lower levels of HDL-cholesterol, higher triacylglycerol levels, and higher HbA_{1C} levels (Chiu and Taylor, 2011). Nowadays, type 2 diabetes and cardiovascular diseases are major causes of morbidity and mortality around the world, threatening the economies of all nations (Leeder *et al.*, 2004). Therefore, to curb the escalating diabetes epidemic and cardiovascular disease, primary prevention through the promotion of a healthy diet and lifestyle should be a global public policy priority (Frank, 2011).

Black rice is a good source of fibre, minerals, and phytochemicals besides containing basic nutrients. Recently, more and more non-glutinous black rice varieties have been developed and gained increasing popularity as a staple food to replace white rice (Zhang *et al.*, 2010). The color of black rice is caused by anthocyanins which are the group of reddish purple water-soluble flavonoids located in the pericarp, seed coat, and aleurone layer (Kushwaha, 2016). Four different anthocyanins (cyanidin-3-glucoside, peonidin-3-glucoside, cyanidin-3,5-diglucoside, and cyanidin-3-rutinoside) have been identified in black rice (Hou *et al.*, 2013). Anthocyanin is one of the most important antioxidants in preventing cancer and cardiovascular disease (Sancho and Pastore, 2012). Several research articles have shown the positive effects of black rice on human health as an important source of vitamins and minerals (Meng *et al.*, 2005), natural food colorant (Guisti and Wrolstad, 2003; Konczak *et al.*, 2004), anti-inflammatory agent (Min *et al.*, 2010), and health-promoting food ingredient in combating diabetic complications (Yawadio *et al.*, 2007).

As consumers' health awareness increases and their food patterns change, research and the development of special foods such as black rice, colored corn, and soybeans have received much attention (Zhang *et al.*, 2010). However, the phytochemicals in whole grains, including anthocyanins, have not received as much attention as those in fruits and vegetables because many previous studies have underestimated grain phytochemicals (Liu, 2007). Several studies have focused mainly on phytochemical content and antioxidant activity of whole grain white rice or rice bran, and a limited number of studies have reported the antioxidant activity of black rice (Zhang *et al.*, 2010). The concentration of anthocyanins as well as antioxidant activity is different depending on the grain type, variety, and growth conditions (Mpofu *et al.*, 2006; Liu, 2007). However, previous studies did not emphasize different types and varieties of black rice. Therefore, more complete analyses of anthocyanin content and antioxidant activity of diverse varieties of black rice are needed. Knowing the antioxidant activity of different black rice genotypes will give insights to their potential application in promoting health (Zhang *et al.*, 2010). This current study aimed to investigate the anthocyanin content as well as agronomic characteristics and grain yield of several local black rice accessions in Vietnam.

Materials and Methods

Materials

Thirty-six local black rice accessions which were coded from BR1 to BR36 were collected from different locations in Vietnam (Table 1).

Methods

Experimental design

The experiment was conducted in the spring 2017 season in Gia Lam, Hanoi. Rice seedlings were transplanted into the field when they had 2-3 leaves. Hill spacing was 25 cm by 15 cm with 1 seedling per hill. Fertilization (N-P-K) was applied with the ratio 90-90-90 kg ha^{-1}. The chemical fertilizers were urea (46% N), superphosphate (16% P_2O_5), and potassium chloride (60% K_2O).

Measurements and data analysis

The following growth and yield parameters were measured following the methods of IRRI (2013): growth duration, plant height, morphological characteristics, yield, and yield components. To measure the parameters, 5 plants were randomly sampled from each cultivar, excluding plants in the two rows on each side to avoid border effects.

Total anthocyanin content (TAC) was quantified using the pH differential method reported by Sutharut and Sudarat (2012). TAC was determined from the whole grain rice after dehulling. Samples of 100 μL of grain extract were brought up to 1 mL volume either with 0.025 M potassium chloride buffer (pH = 1.0) or 0.4 M sodium acetate buffer solution (pH = 4.5). The absorbance of each mixture was measured at 510 and 700 nm using a UV-VIS

Table 1. List of the 36 black rice accessions

Code	Type	Source/province collection
BR1	Sticky rice	Cam Thuy - Thanh Hoa
BR2	Sticky rice	Ba Thuoc - Thanh Hoa
BR3	Sticky rice	Sapa - Lao Cai
BR4	Sticky rice	Ngoc Lac - Thanh Hoa
BR5	Sticky rice	Van Chan - Yen Bai
BR6	Sticky rice	Nho Quan - Ninh Binh
BR7	Sticky rice	Bach Thong - Bac Can
BR8	Sticky rice	Ham Yen - Tuyen Quang
BR9	Sticky rice	Na Hang - Tuyen Quang
BR10	Sticky rice	Da Bac - Hoa Binh
BR11	Sticky rice	Ngoc Lac - Thanh Haa
BR12	Ordinary rice	Ba Thuoc - Thanh Hoa
BR13	Sticky rice	Mai Chau - Hoa Binh
BR14	Sticky rice	Plant Resources Center, Vietnam
BR15	Sticky rice	Luc Nam - Bac Giang
BR16	Sticky rice	Nhu Xuan - Thanh Hoa
BR17	Ordinary rice	Field Crops Research Institute (FCRI)
BR18	Sticky rice	FCRI
BR19	Ordinary rice	FCRI
BR20	Sticky rice	FCRI
BR21	Sticky rice	FCRI
BR22	Sticky rice	FCRI
BR23	Sticky rice	FCRI
BR24	Sticky rice	FCRI
BR25	Sticky rice	Vietnam National University of Agriculture
BR26	Sticky rice	Crops Research and Development Institute (CRDI)
BR27	Sticky rice	CRDI
BR28	Sticky rice	CRDI
BR29	Sticky rice	CRDI
BR30	Sticky rice	CRDI
BR31	Sticky rice	CRDI
BR32	Unknown	CRDI
BR33	Unknown	CRDI
BR34	Sticky rice	CRDI
BR35	Unknown	CRDI
BR36	Unknown	CRDI

spectrophotometer and total absorbance was calculated using the following equation:

$$A = ((A_{510\ nm} – A_{700\ nm})_{pH=1.0}) – ((A_{510\ nm} – A_{700\ nm})_{pH=4.5}) \quad (1)$$

where $A_{510\ nm}$ and $A_{700\ nm}$ are the absorbances measured at 510 and 700 nm, respectively. TAC (in mg L^{-1}) was calculated using the following equation:

$$TAC\ (mg\ L^{-1}) = (A \cdot M \cdot DF \cdot 1000).(\varepsilon \cdot 1)^{-1} \quad (2)$$

where A is the absorbance from Eq.1, M is the molecular mass of cyanidin-3-O-glucoside (M = 449.2), DF is the dilution factor (100 µL of sample was diluted to 1 mL, DF = 10), and ε is the molar absorption coefficient of cyanidin-3-O-glucoside (ε = 26 900 L (mol·cm)$^{-1}$). TAC (in %) was calculated using the following equation:

$$TAC\ (\%) = (a.100).(m.(100–w).10^{-2}) \quad (3)$$

where a is TAC in mg L^{-1} from Eq.2, m is the weight of the initial material (g), and w is the moisture content of the initial material (%). Antioxidant capacity was performed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) Free Radical Scavenging Method reported by Yue and Xu (2008). The DPPH reagent (0.025 g) was dissolved in 1000 mL of methanol for preparing the DPPH reagent solution. Two milliliters of the DPPH solution was mixed with 50, 100, and 150 µL of the sample solutions and transferred to a spectrophotometer cuvette. The reactions were carried out at 25°C for 30 min in a dark room and then the absorbance of each reaction mixture was monitored at 515 nm using a UV-visible spectrophotometer. The inhibition percentage of the absorbance of the DPPH solution was calculated using the following equation:

$$Inhibition\ \% = (OD_c – OD_m).(OD_c \times 100)^{-1} \quad (4)$$

where OD_c is the absorbance of DPPH at time zero and OD_m is the absorbance of DPPH after 30 min of incubation for the reaction. The inhibition percentage of the absorbance of DPPH was plotted against each quantity of the extract solution to obtain a regression line. From the regression line, the IC_{50} (inhibitory concentration of 50% of the DPPH radicals) value was derived. The lower the IC_{50}, the higher the antioxidant activity is.

The data were statistically analysed by Microsoft Excel 2013.

Results and Discussion

Growth and morphological characteristics

Growth duration affects the cropping system and is influenced by both genetics and the environment. Early maturing crops evacuate the land early for the next crops and can escape insect pest attacks if handled promptly (Jamal *et al.*, 2009). Nowadays, breeding efforts are underway to develop short lived varieties of rice with high yield potential. In the present study, the growth duration of the local black rice accessions ranged from 130 days to 150 days (Table 2). The low air temperature in the spring season might have caused prolonging of the growth durations of the black rice accessions.

Plant height is one of the most important bio-agronomic traits and is closely related to photosynthetic capacity, lodging resistance, and the fertilization response ability of a plant. When the plant height is too short, the plant produces less dry matter; whereas, if it is too high, it is vulnerable to lodging and less responsive to N fertilization (Yoshida, 1981). The increase of plant height is usually due to the elongation of stem internodes. Lines having longer internodes produce taller plants (Ashrafuzzaman *et al.*, 2009). Plant height is governed by the genetic makeup of the cultivar as well as environmental factors (Hussain *et al.*, 2014). Plant height of the local black rice accessions in our research varied from 91.5 cm to 143.6 cm (Table 2). Of the 36 black rice accessions we studied, 13.9% belonged to the semi-dwarf group with plant heights less than 100 cm, 44.4% belonged to the intermediate group with plant heights from 100 to 120 cm, and 41.7% belonged to the tall group with plant heights more than 120 cm.

The basic characteristics of rice plants such as flag leaf length and width, panicle length, and leaf angle, etc. affect the growth, development, photosynthetic rate, and final productivity of the plant and are often used to select the best phenotypic variety which increases the yield and plant resistance. The results of the morphological trait measurements of the local black rice accessions are presented in Table 2. The flag leaf plays a crucial role in grain yield, spikelet fertility, panicle size, and grain size and weight. Rice varieties with larger flag leaf areas can perform more photosynthetic activities (Ashrafuzzaman *et al.*, 2009). The flag leaf dimension varies depending on genotype, air temperature, photoperiod, and other traits such as plant height and plant population density (Jamal *et al.*, 2009). In our study, flag leaf length of the 36 black rice accessions fluctuated between 23.1 cm and 57.6 cm. BR5 and BR27 had the shortest flag leaves (23.1 and 24.8 cm, respectively), and were classified into the short flag leaf group. Twenty black rice accessions belonged to the intermediate flag leaf group (flag leaf lengths from 25.0 cm to 35.0 cm), accounting for 55.6% of the accessions. The remaining accessions (38.8%) belonged to the long flag leaf group with flag leaf lengths of more than 35.0 cm. The breadth of the flag leaves ranged from 1.45 cm to 1.93 cm and no accession belonged to the narrow leaves group.

Panicle length has indirectly contributed to increments in rice grain yield by increasing the number of panicles per unit area and the number of spikelets per panicle. According to Fageria *et al.* (2010), panicle length has a significant quadratic relationship with grain yield. Panicle lengths of the black rice accessions in the present study varied from 21.8 cm to 37.6 cm, respectively. Eight accessions were having a long panicle length (more than 30.0 cm), accounting for 22.2%; 18 accessions had an intermediate panicle length (26.0-30.0 cm), accounting for 50.0%; and the remaining (27.8%) had short panicles lengths (20.0-25.0 cm). No accession was assigned to the very short panicle group (panicle lengths less than 20 cm) (Table 2).

Panicle base length is an important trait for improving panicle architecture and grain yield in rice. When the length of the panicle base is positive, the panicle rises completely out of the flag leaf; in contrast, when panicle base length is negative, the panicle is clenched in the flag leaf, leading to an increase in the unfilled grain percentage. Panicle bases that are too long increase the chance that the panicle will be easily broken during ripening. The results in Table 2 show that the panicles of all the black rice accessions were completely out of their flag leaves with the panicle base lengths ranging from 1.44 cm to 13.68 cm.

The panicle base diameters of the black rice accessions varied from 0.18 cm (BR24) to 0.32 cm (BR8). A larger panicle base diameter corresponds to a higher vascular number in the panicle base which leads to more primary and secondary panicle branches. This is a good premise to achieve a high number of grains per panicle and increase grain yield (Lee *et al.*, 1992).

Grain yield and yield components

One of the main objectives of any breeding program is to produce high yielding and good quality lines for release as cultivars to farmers. Of the top four yield components, panicle number per unit area is considered as one of the most important factors in increasing rice yield (Fageria *et al.*, 2010). In conformity with the results of that study, Gebrekidan and Seyoum (2006) demonstrated that panicle number was associated positively (r = 0.61) with grain yield. The number of panicles per hill of the local black rice accessions had huge fluctuations from 2.5 to 13.4 panicles. Classification results showed that 11 black rice accessions (accounting for 30.6%) belonged to the little panicle group (number of panicles per hill was less than 5); 12 accessions (accounting for 33.3%) belonged to the intermediate group (number of panicles per hill was between 5 and 8); and 13 accessions (36.1%) had many panicles (number of panicles per hill was more than 8) (Table 3).

The number of spikelets has been shown to be associated positively and highly significantly with grain yield and panicle length (Gebrekidan and Seyoum, 2006). Spikelet number per unit area determines the sink size of the rice because the variability of a single grain weight is small within a genotype. Spikelet number is the product of the panicle number per unit area and spikelet number per panicle. A negative correlation is generally observed between these two numbers. Thus, recent efforts for breeding high-yield rice genotypes have been directed to those types having a larger number of spikelets per panicle (Kobayasi *et al.*, 2001). The majority

Table 2. The growth and morphological characteristics of the 36 black rice accessions in spring season 2017

Code	Growth uration (day)	Plant height (cm)	Flag leaf length (cm)	Flag leaf breadth (cm)	Panicle length (cm)	Panicle base diameter (cm)	Panicle base length (cm)
BR1	143	102.2 ± 3.9*	37.5 ± 4.3*	1.59 ± 0.09*	27.7 ± 3.0*	0.26	11.84
BR2	148	101.7 ± 3.1	31.8 ± 2.5	1.66 ± 0.11	27.5 ± 2.0	0.25	3.48
BR3	141	100.5 ± 3.7	32.7 ± 2.3	1.57 ± 0.09	27.5 ± 1.5	0.22	4.76
BR4	150	94.2 ± 3.3	30.1 ± 6.2	1.55 ± 0.13	25.8 ± 2.8	0.23	5.62
BR5	145	103.0 ± 2.8	23.1 ± 3.3	1.61 ± 0.09	24.2 ± 2.0	0.21	1.44
BR6	144	118.4 ± 3.7	30.1 ± 1.7	1.59 ± 0.08	26.1 ± 1.2	0.23	7.04
BR7	143	130.2 ± 4.0	28.6 ± 3.8	1.71 ± 0.15	28.4 ± 2.5	0.24	5.43
BR8	147	128.3 ± 6.3	36.5 ± 2.3	1.72 ± 0.07	37.6 ± 4.8	0.32	5.52
BR9	143	138.2 ± 3.2	34.5 ± 3.0	1.47 ± 0.05	27.5 ± 1.8	0.22	9.56
BR10	145	124.4 ± 3.1	36.5 ± 2.7	1.79 ± 0.13	28.2 ± 1.7	0.21	13.68
BR11	135	121.0 ± 3.1	30.2 ± 5.8	1.55 ± 0.11	29.0 ± 1.4	0.24	8.25
BR12	141	91.5 ± 2.3	33.1 ± 2.4	1.53 ± 0.11	26.3 ± 1.2	0.19	2.26
BR13	143	143.6 ± 2.3	41.3 ± 3.4	1.82 ± 0.09	37.4 ± 3.8	0.29	13.46
BR14	143	103.7 ± 2.5	33.8 ± 3.3	1.93 ± 0.04	29.5 ± 1.4	0.22	6.44
BR15	142	97.7 ± 2.5	35.7 ± 3.1	1.61 ± 0.11	28.9 ± 1.7	0.30	4.54
BR16	145	91.6 ± 2.3	33.7 ± 6.7	1.57 ± 0.08	26.1 ± 1.8	0.25	7.39
BR17	145	110.5 ± 2.4	38.0 ± 5.6	1.52 ± 0.12	28.9 ± 2.4	0.25	3.82
BR18	140	103.4 ± 2.5	27.8 ± 3.5	1.76 ± 0.07	24.3 ± 1.4	0.27	7.32
BR19	130	94.0 ± 2.6	27.0 ± 3.0	1.45 ± 0.11	24.5 ± 2.2	0.21	3.58
BR20	138	123.4 ± 2.4	30.7 ± 3.4	1.79 ± 0.11	28.3 ± 3.5	0.26	8.16
BR21	133	141.3 ± 2.5	32.0 ± 3.8	1.64 ± 0.11	32.6 ± 1.5	0.25	8.88
BR22	136	101.5 ± 1.7	29.1 ± 4.9	1.52 ± 0.04	21.8 ± 1.9	0.19	5.22
BR23	131	126.4 ± 2.8	57.6 ± 8.9	1.53 ± 0.11	34.6 ± 2.3	0.22	5.20
BR24	131	125.5 ± 2.6	41.0 ± 7.3	1.71 ± 0.16	32.9 ± 3.8	0.18	4.40
BR25	146	104.7 ± 3.1	27.4 ± 4.9	1.68 ± 0.13	25.0 ± 2.6	0.22	4.37
BR26	143	104.4 ± 3.1	30.2 ± 3.8	1.55 ± 0.09	24.3 ± 1.8	0.22	6.37
BR27	131	102.4 ± 2.6	24.8 ± 5.3	1.58 ± 0.12	27.2 ± 2.0	0.21	5.51
BR28	134	115.5 ± 2.4	36.6 ± 6.6	1.73 ± 0.09	29.4 ± 3.0	0.21	5.51
BR29	130	109.5 ± 2.9	32.8 ± 2.4	1.54 ± 0.07	23.0 ± 1.7	0.21	5.63
BR30	132	125.6 ± 2.7	37.5 ± 7.2	1.66 ± 0.22	27.3 ± 2.6	0.25	6.32
BR31	130	131.9 ± 3.4	32.3 ± 8.0	1.49 ± 0.07	24.3 ± 1.8	0.19	1.48
BR32	149	123.5 ± 3.5	42.0 ± 6.6	1.79 ± 0.07	29.1 ± 2.6	0.21	10.88
BR33	138	125.7 ± 2.6	45.5 ± 4.6	1.63 ± 0.18	32.3 ± 1.9	0.27	6.16
BR34	132	117.2 ± 4.0	28.6 ± 4.8	1.57 ± 0.08	30.1 ± 1.8	0.21	5.81
BR35	135	105.3 ± 2.4	35.2 ± 4.7	1.61 ± 0.07	22.0 ± 1.9	0.22	6.36
BR36	145	135.2 ± 1.8	47.2 ± 5.9	1.73 ± 0.05	30.1 ± 2.3	0.27	5.58

*Note: * The data are presented as mean ± standard deviation (SD).*

of the local black rice accessions in our study had a small number of grains per panicle, while only 11 accessions (accounting for 30.6%) had high data for this trait (more than 200 grains per panicle) (Table 3).

A high number of filled grains increases the panicle weight, leading to enhanced grain yield. This parameter depends on many factors such as fertilization, light intensity, air temperature, and other climate conditions, especially during the panicle initiation period. In our study, the filled grain percentage values of all the black rice accessions were very high (more than 90%). The warmer daily temperatures which occurred during the flowering stage of the rice crop between April and May might have contributed

Table 3. Grain yield and yield components of the 36 black rice accessions

Code	Number of panicles per hill	Number of grains per panicle	Percentage of filled grains (%)	P1000◇ (g)	Yield (tons ha^{-1})
BR1	5.4	148.5 ± 1.4*	95.8 ± 0.8*	28.5	4.3
BR2	7.4	218.9 ± 2.2	95.2 ± 0.6	22.1	4.9
BR3	8.6	204.7 ± 1.6	95.9 ± 0.5	22.3	4.7
BR4	10.8	212.8 ± 2.5	94.2 ± 2.2	21.9	4.7
BR5	9.2	171.1 ± 2.7	94.5 ± 0.7	19.9	3.4
BR6	10.8	206.2 ± 2.2	94.9 ± 0.3	28.9	6.1
BR7	4.4	121.4 ± 3.0	95.0 ± 0.5	31.2	3.9
BR8	5.4	285.8 ± 3.4	96.8 ± 0.6	29.3	8.7
BR9	8.8	179.9 ± 2.0	95.3 ± 0.6	29.2	5.4
BR10	5.0	151.8 ± 1.9	93.1 ± 0.8	25.2	3.8
BR11	4.6	171.8 ± 2.0	93.9 ± 0.2	24.3	4.2
BR12	11.4	146.7 ± 1.7	96.4 ± 0.5	27.1	4.1
BR13	3.8	147.1 ± 2.0	95.4 ± 0.6	35.3	5.3
BR14	4.8	113.7 ± 1.8	92.7 ± 1.0	31.9	3.6
BR15	13.4	267.8 ± 2.4	95.4 ± 0.4	24.3	6.6
BR16	11.6	167.9 ± 2.5	95.6 ± 0.6	24.9	4.3
BR17	9.2	133.1 ± 2.8	92.0 ± 0.7	34.4	4.5
BR18	9.4	215.7 ± 4.6	96.5 ± 1.1	22.5	5.1
BR19	8.8	141.8 ± 3.8	94.3 ± 0.8	27.2	3.9
BR20	7.2	136.5 ± 2.6	95.6 ± 0.5	25.9	3.6
BR21	4.2	209.2 ± 1.9	95.8 ± 0.4	29.1	6.2
BR22	8.0	109.7 ± 2.5	93.9 ± 0.7	33.9	3.7
BR23	3.8	170.9 ± 1.1	94.6 ± 0.3	30.1	5.2
BR24	3.6	132.5 ± 1.6	92.1 ± 0.9	33.9	4.4
BR25	7.8	152.1 ± 1.0	95.1 ± 0.4	26.5	4.1
BR26	7.2	235.2 ± 1.5	95.5 ± 0.3	23.1	5.6
BR27	9.0	201.2 ± 1.0	95.3 ± 0.2	16.8	3.5
BR28	3.6	133.0 ± 0.6	93.6 ± 0.8	29.2	3.9
BR29	6.4	130.6 ± 1.9	95.4 ± 0.6	35.1	4.7
BR30	3.2	129.2 ± 3.2	94.2 ± 0.3	30.2	3.9
BR31	8.0	215.0 ± 0.5	93.3 ± 0.6	22.1	4.7
BR32	2.5	104.8 ± 0.8	94.0 ± 0.6	26.2	2.8
BR33	4.2	135.9 ± 1.1	93.1 ± 0.2	29.3	4.0
BR34	6.4	139.5 ± 1.5	93.9 ± 0.1	27.1	3.8
BR35	5.4	119.1 ± 1.6	94.7 ± 0.4	23.2	4.0
BR36	9.6	198.3 ± 1.2	95.2 ± 0.3	34.3	6.9

*Note: *The data are presented as mean ± standard deviation (SD); ◇P1000: Thousand-grain weight.*

to the increase in the fertility of the spikelets. Nishiyama (1995) also reported that the prevalence of cool air temperatures during the flowering stage increases sterility in rice crops by affecting pollination and fertilization.

The thousand-grain weight (P1000) is the final important yield-forming attribute of grain yield. Bharali and Chandra (1994) reported the correlation and influence of the thousand-grain weight with the flag leaf area. Other factors like adaptability, temperature, soil fertility, transplantation season, and time might also be responsible for the thousand-grain weight (Jamal *et al.*, 2009). However, the thousand-grain weight is the most stable factor under strict hereditary control. Therefore, this important characteristic is often used to classify a variety into a group and select better phenotypic varieties. In the 36 black rice accessions, only BR13 and BR29 had very high thousand-grain weights (35.3 g and 35.1 g, respectively). Meanwhile, BR5 and BR27 had very low thousand-grain weights (less than 20.0 g). The BR5 and BR27 accessions also had short flag leaf lengths among the 36 surveyed accessions.

Grain yield of the local black rice accessions in the present study varied from 2.8 to 8.7 tons ha^{-1}. The BR8 accession had the highest grain yield because it had the highest values in panicle length, number of grains per panicle, and percentage of filled grains. In contrast, BR32 showed the lowest grain yield with the smallest number of panicles per hill and number of grains per panicle.

Total anthocyanin content and antioxidant activity of black rice accessions

Total anthocyanin content

The results of the anthocyanin content in the 36 black rice accessions are presented in Table 4.

Among the 36 black rice accessions, BR1, BR5, BR7, BR14, BR17, BR25, BR27, BR30, BR34, and BR35 had high anthocyanin contents (more than 0.1%); and of these, BR7 had the highest anthocyanin content (0.1438%), followed by BR5 (0.1317%). BR4 and BR24 had the lowest

Table 4. Total anthocyanin content of the 36 black rice accessions

Code	Anthocyanin content (%)	Code	Anthocyanin content (%)
BR1	0.1152	BR19	0.0053
BR2	0.0364	BR20	0.0592
BR3	0.0016	BR21	0.0043
BR4	0.0015	BR22	0.0766
BR5	0.1317	BR23	0.0644
BR6	0.0949	BR24	0.0015
BR7	0.1438	BR25	0.1166
BR8	0.0137	BR26	0.0047
BR9	0.0000	BR27	0.1130
BR10	0.0062	BR28	0.0256
BR11	0.0000	BR29	0.0000
BR12	0.0574	BR30	0.1012
BR13	0.0000	BR31	0.0119
BR14	0.1013	BR32	0.0672
BR15	0.0022	BR33	0.0064
BR16	0.0557	BR34	0.1015
BR17	0.1032	BR35	0.1064
BR18	0.0563	BR36	0.0039

Table 5. Classification of black rice accessions by anthocyanin content

Group	Anthocyanin content (%)	Number of accessions	Codes
I	> 0.1	10	BR1, BR5, BR7, BR14, BR17, BR25, BR27, BR30, BR34, BR35
II	0.05-0.1	8	BR6, BR12, BR16, BR18, BR20, BR22, BR23, BR32
III	0.001-0.05	14	BR2, BR3, BR4, BR8, BR10, BR15, BR19, BR21, BR24, BR26, BR28, BR31, BR33, BR36
IV	< 0.001	4	BR9, BR11, BR13, BR29

anthocyanin contents with 0.0015%. No anthocyanin was measured in BR9, BR11, BR13, and BR29. In the research of Shao *et al.* (2018), anthocyanins were detected in black rice with contents ranging from 15.57 to 1417.12 mg kg^{-1} depending on the genotype. Based on the different anthocyanin contents, we divided the black rice accessions into four groups as shown in Table 5.

Combining anthocyanin content with the observations of grain color, we found that in general, the samples with a darker grain color had a higher anthocyanin content such as BR1, BR5, BR17, and BR30. According to Pereiracaro *et al.* (2013), coloration of rice is derived from the accumulation of anthocyanins. Many studies have reported that colored rice varieties are rich of anthocyanins and other polyphenolic compounds much more abundantly than non-colored rice varieties (Moko *et al.*, 2014). Trung *et al.* (2016) showed that a rice sample with a black shell (N20) and a sample with the red-brown shell (N25) contained the highest and the lowest anthocyanin contents (more than 0.2% and less than 0.01%, respectively). Nevertheless, we observed several special cases such as BR7, which had a brown color but belonged to the highest anthocyanin content group (group I), and BR8, which was completely black but the anthocyanin content was lower than that of BR27. It is possible that due to the nature and chemical compositions of the anthocyanins, changes in pH, for example, led to variations in the grain color instead of black or dark brown as usual. However, further research is required to confirm the exact causes of these specific cases. It has not been concluded clearly whether ordinary rice or sticky rice has a higher anthocyanin content.

Antioxidant activity

The antioxidant activity of anthocyanins in the black rice accessions was determined using the DPPH assay and expressed in IC_{50} values, which are the inhibitory concentrations of 50% of the DPPH radicals (Figure 1). Lower IC_{50} values show higher antioxidant activity. In our study, anthocyanins with the strongest antioxidant activity were extracted from ten black rice accessions, namely BR8, BR35, BR6, BR27, BR30, BR32, BR18, BR17, BR19, and BR1. Most of these accessions also had a high anthocyanin content and black or dark brown color. Our results were consistent with those reported by Zhang *et al.* (2010). According to their study, total antioxidant activity in black rice was significantly correlated to the content of total phenolics, total flavonoids, and anthocyanins. The antioxidant properties of colored rice and non-colored rice were determined by Hu *et al.* (2003) and Chakuton *et al.* (2012), who showed a significant positive correlation between pigmented varieties and their antioxidant activity. The antioxidant properties of colored rice bran were higher than those of non-colored rice bran because of the presence of anthocyanins, which are potent reducing agents and possess strong radical scavenging activities (cited by Moko *et al.*, 2014).

Jiao *et al.* (2012) researched the antioxidant capacity of an anthocyanin extract from purple sweet potato (*Ipomoea batatas* L.) which resulted in an IC_{50} value of 6.94 µg mL^{-1}. The red rice variety investigated by Moko *et al.* (2014) had the highest DPPH scavenging radical activity (88.29 ± 5.62%), with the lowest IC_{50} value (26.26 ± 0.95 µg mL^{-1}) and the highest total anthocyanin content (68.61 ± 1.98 mg g^{-1}). These data showed that the anthocyanin extracts from the black rice lines in our study had higher antioxidant activities than the purple sweet potato and several other rice varieties. In black rice, cyanidin-3-glucoside (Cy-3-Glc) has been reported to be one of the major antioxidant compounds (Ryu *et al.*, 1998), and the anthocyanin cyanidin-3-glucoside has been shown to have strong superoxide radical scavenging activities (Ichikawa *et al.*, 2001). Based on the anthocyanin content as well as the growth characteristics and grain yield, we selected seven promising black rice accessions for further research, namely BR1, BR14, BR17, BR25, BR30, BR34, and BR35, as shown in Table 6.

All the selected black rice accessions had high anthocyanin contents (more than 0.1%), and relatively high productivity (from 3.6 to 4.5 tons ha^{-1}). The most promising accessions belong to the semi-dwarf group or intermediate group to establish lodging resistance and fertilization responsive ability of the plants.

Conclusions

The black rice accessions varied in growth duration, plant height, morphological characteristics, and yield components. Grain yield of the black rice accessions varied from 2.8 to 8.7 tons ha^{-1}. Using the pH differential method to measure anthocyanin content, we

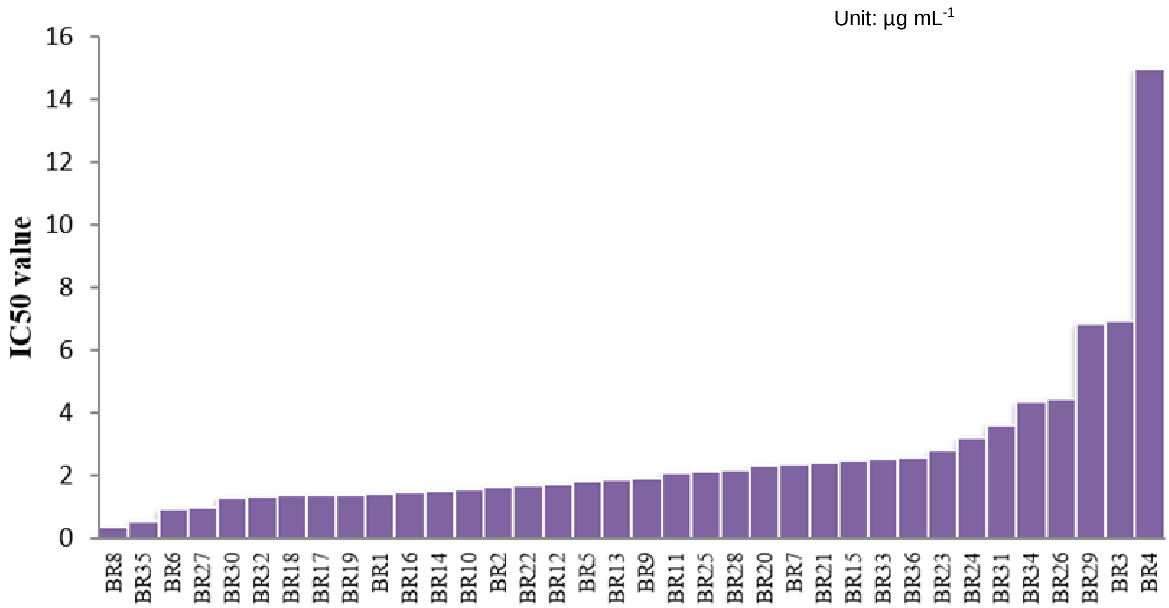

Figure 1. IC_{50} values of the 36 black rice accessions

Table 6. Characteristics of the promising black rice accessions selected in the spring of 2017

Code	Anthocyanin content (%)	Yield (tons ha^{-1})	Number of panicles per hill	Number of grains per panicle	Percentage of filled grains (%)	P1000 (g)	Plant height (cm)
BR1	0.1152	4.3	5.4	148.5	95.8	28.5	102.2
BR14	0.1013	3.6	4.8	113.7	92.7	31.9	103.7
BR17	0.1032	4.5	9.2	133.1	92.0	34.4	110.5
BR25	0.1166	4.1	7.8	152.1	95.1	26.5	104.7
BR30	0.1012	3.9	3.2	129.2	94.2	30.2	125.6
BR34	0.1015	3.8	6.4	139.5	93.9	27.1	117.2
BR35	0.1064	4.0	5.4	119.1	94.7	23.2	105.3

divided the black rice accessions into four groups: group I > 0.1% anthocyanin, group II 0.05%-0.1%, group III 0.001%-0.05%, and group IV < 0.001%. BR7 had the highest anthocyanin content (0.1438%), followed by BR5 (0.1317%). Anthocyanins with the strongest antioxidant activity were extracted from ten black rice accessions, namely BR8, BR35, BR6, BR27, BR30, BR32, BR18, BR17, BR19, and BR1, with IC_{50} values less than 2 µg mL^{-1}. As far as antioxidants and their activity are concerned, it was found that in general, dark-coloured rice contained more anthocyanin and antioxidant activity than light-colored rice. The seven black rice accessions selected for further study, BR1, BR14, BR17, BR25, BR30, BR34, and BR35, are promising sources of antioxidative phytochemicals and have the potential to be released as cultivars to farmers. The differences in anthocyanin content and antioxidant activity among the diverse black rice genotypes opened a door for scientists to breed and choose varieties with higher anthocyanin contents and grain yields, as well as for the food industry to develop new products to compete in today's functional food markets.

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References

Ashrafuzzaman M., Islam M. R., Ismail M. R., Shahidullah S. M. and Hanafi H. M. (2009). Evaluation of six aromatic rice varieties for yield and yield contributing characters. International Journal of Agriculture and Biology. Vol 11 (5). pp. 616-620.

Bharali B. and Chandra K. (1994). Effect of low light on dry matter production, harvest index and grain yield of rice (*Oryza sativa* L.) in wet season. Neo Botanica. Vol 2 (1). pp. 11-14.

Chiu C. J. and Taylor A. (2011). Dietary hyperglycemia, glycemic index and metabolic retinal diseases. Prog Retin Eye Research. Vol 30 (1). pp. 18-53.

Fageria N. K., de Morais O. P. and dos Santos A. B. (2010). Nitrogen use efficiency in upland rice genotypes. Journal of Plant Nutrition. Vol 33. pp. 1696-1711.

Fitzgerald M. A., McCouch S. R. and Hall R. D. (2009). Not just a grain of rice: the quest for quality. Trends in Plant Science. Vol 14 (3). pp. 133-139.

Frank B. H. (2011). Globalization of diabetes: The role of diet, lifestyle, and genes. Journal of Diabetes Care. Vol 34. pp. 1249-1257.

Gebrekidan H. and Seyoum M. (2006). Effects of mineral N and P fertilizer on yield and yield components of flooded lowland rice on vertisols of fogera plain, Ethiopia. Journal of Agriculture and Rural Development in the Tropics and Subtropics. Vol 107 (2). pp. 161-176.

Guisti M. M. and Wrolstad R. E. (2003). Acylated anthocyanins from edible sources and their application in food systems. Biochemical Engineering Journal. Vol 14. pp. 217-225.

Hou Z., Qin P., Zhang Y., Cui S. and Ren G. (2013). Identification of anthocyanins isolated from black rice (*Oryza sativa* L.) and their degradation kinetics. Food Research International. Vol 50 (2). pp. 691-697.

Huang L., Yu J., Yang J., Zhang R., Bai Y., Sun C. and Zhuang H. (2016). Relationships between yield, quality and nitrogen uptake and utilization of organically grown rice varieties. Pedosphere. Vol 26 (1). pp. 85-97.

Hussain S., Fujii T., McGoey S., Yamada M., Ramzan M. and Akmal M. (2014). Evaluation of different rice varieties for growth and yield characteristics. The Journal of Animal and Plant Sciences. Vol 24 (5). pp. 1504-1510.

Ichikawa H., Ichiyanagi T., Xu B., Yoshii Y., Nakajima M. and Konishi T. (2001). Antioxidant activity of anthocyanin extract from purple black rice. Journal of Medicinal Food. Vol 4 (4). pp. 211-218.

IRRI (2013). Standard evaluation system for rice (SES). 5^{th} Edition. International Rice Research Institute, Los Banos, Phillippines.

Jamal, Khalil I. H., Bari A., Khan S. and Zada I. (2009). Genetic variation for yield and yield components in rice. Journal of Agricultural and Biological Science. Vol 4 (6). pp. 60-64.

Jiao Y., Jiang Y., Zhai W. and Yang Z. (2012). Studies on antioxidant capacity of anthocyanin extract from purple sweet potato (*Ipomoea batatas* L.). African Journal of Biotechnology. Vol 11 (27). pp. 7046-7054.

Kobayasi K., Imaki T. and Horie T. (2001). Relationship between the size of the apical dome at the panicle initiation and the panicle components in rice. Plant Production Science. Vol 4 (2). pp. 81-87.

Konczak I. and Zhang W. (2004). Anthocyanins-more than nature's colours. Journal of Biomedicine and Biotechnology. Vol 5. pp. 239-240.

Kushwaha U. K. S. (2016). Black rice anthocyanin content increases with increase in altitude of its plantation. Advances in Plants and Agriculture Research. Vol 5 (1). pp. 1-4.

Lee D. J., Benito S. V., Oscar B. Z., Kim B. K. and Chae J. C. (1992). Development of vascular bundles in the peduncle of different tillers and its relationship to panicle characteristics in rice. Korean Journal of Crop Science. Vol 37. pp. 155-165.

Leeder S., Raymond S., Greenberg H., Liu H. and Esson K. (2004). A race against time: The challenge of cardiovascular disease in developing economies. 2nd edition, Centre for Chronic Disease Control, New Delhi, India, pp. 9-10.

Liu R. H. (2007). Whole grain phytochemicals and health. Journal of Cereal Science. Vol 46 (3). pp. 207-219.

Meng F., Wei Y. and Yang X. (2005). Iron content and bioavailability in rice. Journal of Trace Elements in Medicine and Biology. Vol 18 (4). pp. 333-338.

Min S. W., Ryu S. N. and Kim D. H. (2010). Anti-inflammatory effects of black rice, cyanidin-3-O-β-D-glycoside, and its metabolites, cyanidin and protocatechuic acid. International Immunopharmacology. Vol 10 (8). pp. 959-966.

Moko E. M., Purnomo H., Kusnadi J. and Ijong F. G. (2014). Phytochemical content and antioxidant properties of colored and non-colored varieties of rice bran from Minahasa, North Sulawesi, Indonesia. International Food Research Journal. Vol 21 (3). pp. 1053-1059.

Mpofu A., Sapristein H. D. and Beta T. (2006). Genotype and spring wheat. Journal of Agricultural and Food Chemistry. Vol 54. pp. 1265-1270.

Nishiyama I. (1995). Physiological basis of the damage caused by unfavorable climatic conditions, disease and insect pests. I. Damage due to extreme temperatures. In: Matsuo T., Kumazawa K., Ishii R., Ishihara K. and Hirata H. (Ed.). Science of the Rice Plant. Food and Agriculture Policy Research Center, Tokyo, 2. pp.769-810.

Pereiracaro G., Cros G., Yokota T. and Crozier A. (2013). Phytochemical profiles of black, red, brown, and white rice from the Camargue region of France. Journal of Agricultural and Food Chemistry. Vol 61 (33). pp. 7976-7986.

Ryu S. N., Park S. Z. and Ho C. T. (1998). High performance liquid chromatographic determination of anthocyanin pigments in some varieties of black rice. Journal of Food and Drug Analysis. Vol 6 (4). pp. 729-736.

Sancho R. A. S. and Pastore G. M. (2012). Evaluation of the effects of anthocyanins in type 2 diabetes. Food Research International. Vol 46. pp. 378-386.

Shao Y., Hu Z., Yu Y., Mou R., Zhu Z. and Beta T. (2018). Phenolic acids, anthocyanins, proanthocyanidins, antioxidant activity, minerals and their correlations in non-pigmented, red, and black rice. Food Chemistry. Vol 239. pp. 733-741.

Sutharut J. and Sudarat J. (2012). Total anthocyanin content and antioxidant activity of germinated colored rice. International Food Research Journal. Vol 19 (1). pp. 215-221.

Trung N. Q., Hue N. T., Dung P. T., Thuy T. T. T., Thao T. T., Anh P. H. and Nam N. H. (2016). Identification of DNA marker for *Rc* gene conferring anthocyanin production in brown rice. Summary report of research project at university level, Vietnam National University of Agriculture, Hanoi (in Vietnamese).

Yawadio R., Tanimori S. and Morita N. (2007). Identification of phenolic compounds isolated from pigmented rices and their aldose reductase inhibitory activities. Food Chemistry. Vol 101 (4). pp. 1616-1625.

Yoshida S. (1981). Fundamentals of Rice Crop Science. Los Banos, Philippines: International Rice Research Institute.

Yue X. and Xu Z. (2008). Changes of anthocyanins, anthocyanidins, and antioxidant activity in bilberry extract during dry heating. Journal of Food Science. Vol 73 (6). pp. 494-499.

Zhang M. W., Zhang R. F., Zhang F. X. and Liu R. H. (2010). Phenolic profiles and antioxidant activity of black rice bran of different commercially available varieties. Journal of Agricultural and Food Chemistry. Vol 58. pp. 7580-7587.