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Original Research Article

Changes of Anthocyanidin, Growth Characteristics and Brown Rice Yield of Red Colored Rice at Two Region of Eastern Coast in Gyeongsangbuk-do Province

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Abstract - The study was carried out to determine the changes of growth characteristics, anthocyanidin, and brown rice yield grown at coast and inland areas apart from the east coastline of Gyeongsangbuk-do province. Number of spikelets per panicle was much more increased in inland area. Regional difference in number of spikelets per panicle was observed in Jeongjinju cultivar. Among these red rice cultivars, the highest brown rice yield was Jeongjinju rice cultivar having 702 ㎏ in inland area and 692 ㎏ in coast area, respectively. Anthocyanidin content ranged 524 to 610 ㎍/g dry weight basis. Cyanidin content was 11.4 to 14.0 times higher than that of delphinidin under coast and inland area. Anthocyanidin content was higher in rice cultivar grown at coast area. Highest head brown rice rate was only observed in Geonganghongmi to 95.2 at coast area and 95.4 inland area. In considering brown rice yield and pigment content, Hongjinju rice cultivar was recommended in optimal pigment rice cultivar in eastern coast of Gyeongsangbuk-do Province.

Key words − Amylose, Anthocyanidin, Head rice, Protein, Red rice

Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world, especially in Asian countries. Pigmented rice such as black and red rice consumptions are rapidly growing presently due to their healthy functional food ingredients in Korea. Pigmented rice or colored rice is distinguished by the rice grain having red brown or dark purple color in its covering layers. Pigments, which are located in the aleurone layer of rice grain, have been reported as a mixture of anthocyanin compounds, which belong to the family of flavonoids (Yawadio *et al*., 2007). For pigmented rice, the main substance of phenolic compounds has been reported as anthocyanins (Iqbal *et al*., 2005; Zhang *et al*., 2006; Yawadio *et al*., 2007). Anthocyanins in pigmented rice have been identified. They are cyanidin-3-glucoside and peonidin-3-glucoside (Hu *et al*., 2003); malvidin, pelargonidin-3, 5-diglucoside, cyanidin-3-glucoside and cyanidin-3, 5-diglucoside (Zhang *et al*., 2006); cyanidin-3-glucoside, pelargonidin-3-glucoside (Yawadio *et al*., 2007). Anthocyanins, a group of reddish or purple flavonoids, are reported to be the primary pigments in these rice varieties (Abdel-Aal and Hucl, 1999; Mazza and Gao, 2005; Moreno *et al*., 2005). Anthocyanins have been recognized as health-promoting functional food ingredients due to their antioxidant activity (Nam *et al*., 2006; Philpott *et al*., 2006), anticancer (Hyun and Chung, 2004; Zhao *et al*., 2004), and anti-inflammatory effects (Tsuda *et al*., 2002), and these functions provide synergic effects with various nutrients *in vivo*. At present, red rice is commonly used as a food colorant in bread, ice cream, and liquor (Yoshinaga, 1986). Anthocyanins are located in certain layers of the rice grain, which could be separated into anthocyanin-rich fractions for use as functional colorants or functional food ingredients. Although an extensive scientific literature (Francis, 2000; Mazza and Gao, 2005) on the composition of anthocyanins in fruits and vegetables exists, little is known about anthocyanin composition in pigmented rice. Early studies have shown cyanidin-3-glucoside (C3G) as a major anthocyanin in black rice, but the minor ones were reported either malvidin-3-glucoside (Yoon *et al*., 1995) or peonidin-3-glucoside (Pt3G)

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(Choi *et al.*, 1994).

Recently, black rice was analyzed to contain a wide range of total anthocyanin content, with C3G being the most common anthocyanin (0~470 ㎎/100 g) in most of the 10 varieties studied, whereas Pt3G (0~40 ㎎/100 g) was the second dominant anthocyanin (Ryu *et al.*, 1998). Researchers have reported that the contents of the functional components of pigmented rice were significantly increased according to the level of N-fertilizer, and normal planting conditions showed significantly higher effects than early or late plantings and the contents were different according to the planting region (Jung *et al.*, 2003; Oh *et al*., 2017). The present study was aimed to investigate whether different regions apart from eastern coast of Gyeongsangbuk-do province affect the different contents of pigment (delphinidin, cyanidin) components and brown rice yield in three red colored rice cultivars.

Materials and Methods

Plant material and culture

The study was conducted to select the red colored rice cultivar highly adaptable for two regions which are located 0.7 and 2 ㎞ apart from an eastern coast in Gyeongsangbuk-do Province. The three red colored rice cultivars, Jeogjinju, Hongjinju and Geonganghongmi were transplanted on 25th May at two different regions. Planting distance was 30×14 ㎝ and fertilizer amount was $N\text{-}P_2O_5\text{-}K_2O$ = 9-4.5-5.7 ㎏/10a and fertilizer split application was basal-tillering stage-panicle initiation = 50-25-25 ratio. In order to analyze rice quality and pigment content from each cultivar, rice grains were harvested 30 days after heading. The experiment was laid out following a randomized complete block design with three replications.

Analysis of anthocyanidin

For quantification and identification of anthocyanidin pigments, anthcyanidins were extracted from the samples by weighing 5.0 g of pigmented rice grains. The flours of pigmented rice grains were transferred into 50 ㎖ graduated cylinder and 5 ㎖ of extraction solution (80/20 acetonitrile/0.3% phosphoric acid in distilled water) was added. The samples were centrifuged for 10 min and added 200 ㎕ of concentrated HCl. The aglycons of anthocyanins were prepared by acid hydrolysis of anthocyanins isolated from pigmented rice flours. The anthocyanin mixtures (200 ㎕) in 100 ㎕ of 2 N HCl were hydrolyzed under an atmosphere of nitrogen for 3 h at 98℃. The analysis of anthocyanins in pigmented rice seeds was carried out using a HPLC system (Waters 2487, USA) equipped with a C_{18} reverse phase column (4.6 ㎜ × 250 ㎜). The extract (200 ㎕) was mixed with 100 ㎕ of 2 N HCl in 40% methanol solution, and then incubated at 100℃. Samples were taken at various time periods prior to injection. HPLC was run by isocratic elution mode using 18% solvent B (0.4% TFA in acetonitrile) in solvent A (0.4% TFA in distilled water) at a flow rate of 1.0 ml/min. The elution profile was monitored by UV-detection at 530 ㎚. UV-Vis spectra of anthocyanin extract from pigmented rice seeds were obtained in buffer solutions with different pH values (Choung *et al*., 2001). Small aliquots of the extract were diluted in either 200 mM phosphate : acetonitrile, 2:1 volume ratio, pH 1.5 or 30 mM borate, 100 mM SDS, pH 9.0. The spectrum was recorded in the range of 300 ㎚ to 700 ㎚ using a UV-Vis spectrophotometer (Shimadzu, Japan).

Determination of color value

Color of hulled grain was determined by Hunter-Lab (Ultrascan XE, Hunter-Lab, USA). Prior to color measurements, the instrument was calibrated with light tap and white calibration tile. The colorimeter was set to an illuminant condition D65 and a 10° standard observer. Each sample was put in a cuvette and replaced in to the specular port site, the color parameters (a, b and L) were then read (Lamberts *et al*., 2007). Three replicates for each sample were determined.

Results and Discussion

There was no any remarkable difference in heading date of each red rice cultivar grown in coast and inland area apart from the eastern coast of Gyeongsangbuk-do province (Table 1). Mean temperatures from heading to harvesting periods were lower in rice plants grown at coast region than in rice plants grown at inland region. Among these rice plants, two red rice cultivars, Jeogjinju and Hongjinju, were higher than that of Geonganghongmi resulting from late heading time. Like a tendency of mean temperature, accumulated temperature

Table 1. Heading date, mean and accumulative temperature in three red rice cultivars grown at two different regions of eastern coast in Gyeongsangbuk-do province

Cultivars	Area[z]	Heading date	Mean temp. (℃)	Accumulated temp. (℃)
Jeogjinju	Coast	8.11	22.1	663
	Inland	8.12	21.4	642
Hongjinju	Coast	8.11	21.8	654
	Inland	8.11	20.5	615
Geonganghongmi	Coast	8.18	19.5	585
	Inland	8.18	18.9	567

[z]Coast and inland area is located at 0.7 and 2.0 ㎞ apart from the coastline, respectively.

Table 2. Changes of growth characteristics and yield in three red rice cultivars grown at two different regions of eastern coast in Gyeongsangbuk-do province

Cultivars	Area[z]	Culm length (㎝)	No. of tiller per plant	No. of spikelets per panicle	1000 grain weight (g)	Ripened grains (%)	Brown rice yield (㎏/10a)
Jeogjinju	Coast	78	19.5	88	21.5	84.6	692a
	Inland	77	19.1	98	22.7	85.1	702a
Hongjinju	Coast	79	18.7	85	21.3	87.0	642b
	Inland	76	19.0	93	22.4	86.7	676ab
Geonganghongmi	Coast	93	21.8	73	21.7	79.7	586c
	Inland	92	21.6	82	22.8	77.6	579c

[z]Coast and inland area is located at 0.7 and 2.0 ㎞ apart from the coastline, respectively.
In each column, same letters do not differ significantly at P < 0.05 as per DMRT.

ranged from 567 to 654℃. It was always lower in inland regions than in coast region caused by valley wind. When temperature drops from 24 to 21, there was a sharp increase in days to heading (Yoshida *et al*., 1981).

The tallest culm length was observed in Geonganghongmi cultivar grown in coast area, and culm length was always taller in coast area than that of inland area among these three red rice cultivars. However, number of tiller per rice plant was not affected by different cultural area. Number of spikelets per panicle was much more increased in inland area. Regional difference in number of spikelets per panicle was only observed in Jeongjinju cultivar. The number of tillers per square meter during the early growth period was generally larger under high temperature and the maximum tillering stage was earlier than under normal temperature conditions. At maturity, the number of tillers was found to be lower in high-temperature conditions than in ambient conditions inside a temperature gradient chamber (Oh *et al*., 2007). Among these red rice cultivars, the highest brown rice yield was Jeongjinju rice cultivar having 702 ㎏/10a in inland area and 692 ㎏/10a in coast area, respectively (Table 2). Temperature influences rice yield by directly affecting the physiological processes involved in grain production. During the reproductive stage, the spikelet number per plant increases as the temperature drops. In general, the optimal temperature shifts from high to low as growth advances from the vegetative to the reproductive stages. It was reported that the mean optimum temperature for ripening of japonica rice ranged to about 20~22℃. Although temperature during ripening affects the weight per grain, the 1000-grain weight of a particular cultivar is considered to be almost constant under different environments and cultural practices (Matsui *et al*., 2005).

Table 3 shows the color parameters (L^*, a^*, and b^*) of three pigmented brown rice and grain color degree as affected by different cultural areas. The L^* values, which expresses the brightness, were in the range of 36.3~36.7, 36.7~37.0, and 38.7~39.6 in Jeogjinju, Hongjinju, Geonganghongmi rice cultivars, respectively. The a^* values, which expresses the redness positively and greenness negatively were in the range of 12.3~12.8, 12.2~12.3 and 12.1~11.9 in Jeogjinju, Hongjinju,

Table 3. Color value in three red rice cultivars grown at two different regions of eastern coast in Gyeongsangbuk-do province

Cultivars	Area^z	Brown rice color		
		L^*	a^*	b^*
Jeogjinju	Coast	36.3	12.3	13.8
	Inland	36.7	12.8	14.0
Hongjinju	Coast	36.7	12.2	13.6
	Inland	37.0	12.3	13.5
Geonganghongmi	Coast	38.7	12.1	14.9
	Inland	39.6	11.9	14.7

^z Coast and inland area is located at 0.7 and 2.0 ㎞ apart from the coastline, respectively.

Table 4. Changes of anthocyanidin content in three red rice cultivars grown at two different regions of eastern coast in Gyeongsangbuk-do province

Cultivars	Area^z	Anthocyanidin content (㎍/g DW)		
		Delphinidin	Cyanidin	Sum
Jeogjinju	Coast	44	566	610b
	Inland	38	532	570c
Hongjinju	Coast	47	600	647a
	Inland	44	555	599b
Geonganghongmi	Coast	48	546	594b
	Inland	36	488	524d

^z Coast and inland area is located at 0.7 and 2.0 ㎞ apart from the coastline, respectively.
In each column, same letters do not differ significantly at $P < 0.05$ as per DMRT.

Geonganghongmi rice cultivars, respectively. In addition, the b* values, which expresses the yellowness positively and blueness negatively were in the range of 13.8~14.0, 13.6~13.5 and 14.9~14.7 in Jeogjinju, Hongjinju, Geonganghongmi rice cultivars, respectively. Consequently, it showed more red-brown colors. It was reported that the differences in the grain color of these pigmented rices could depend on the form of anthocyanins and rice genotypes (Yawaido *et al*., 2007; Escribano-Bailon *et al*., 2004).

Three pigmented rice cultivars, Jeogjinju, Hongjinju, and Geonganghongmi as affected by different cultural areas were sampled at 30 days after heading to determine total anthocyanin contents. Anthocyanidin content ranged 524 to 610 ㎍/g dry weight basis (Table 4). Cyanidin content was 11.4 to 14.0 times higher than that of delphinidin under coast and inland area. Among these rice cultivars, anthocyanidin content was always higher in rice cultivar grown at coast area. It is known that dihydroflavonol 4-reductase (DFR) is a key enzyme in pelargonidin biosynthesis and color expression of husk, leaf, and flower (Nakamura *et al*., 2010). Expression of genes for DFR, which catalyzes dihydrokaempferol to leucopelargonidin in petunia mutants that are deficient in F3'H and F3'5'H, results in pelargonidin production (Tanaka *et al*., 1995).

Table 5. Changes of rice qualities in three red rice cultivars grown at two different regions of eastern coast in Gyeongsangbuk-do province

Cultivars	Area^z	Head brown rice (%)	Protein (%)	Amylose (%)
Jeogjinju	Coast	92.9b	6.6b	16.4c
	Inland	92.4b	6.3b	17.0b
Hongjinju	Coast	92.4b	7.0a	16.2c
	Inland	92.5b	6.6b	17.4b
Geonganghongmi	Coast	95.2a	7.4a	17.2b
	Inland	95.4a	7.2a	19.0a

^z Coast and inland area is located at 0.7 and 2.0 ㎞ apart from the coastline, respectively.
In a column, same letters do not differ significantly at $P < 0.05$ as per DMRT.

The rice quality such as head brown rice, protein and amylose content was determined in three red rice cultivars grown in coast and inland area (Table 5). Highest head brown rice rate was only observed in Geonganghongmi to 95.2 at coast area and 95.4 inland area, respectively. Protein content was always higher in coast area than that of inland area. The lowest protein content was measured in Jeogjinju and amylose content was relatively increased in inland area compared to that of coast area. Consequently, in considered with brown rice yield and pigment content, Hongjinju rice cultivar was recommended in optimal pigment rice cultivar in eastern coast of Gyeongsangbuk-do Province.

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