Black Rice Anthocyanin Content Increases with Increase in Altitude of its Plantation

Abstract
Black rice is the most nutritious heirloom rice landraces. A G60 black rice variety was grown in both terai and hill region of Nepal at 79 meters and 1360 meter altitude from mean sea level in 2015. The important rice qualities such as total anthocyanin content, caryopsis color, Calcium, and iron content were analyzed. It was observed that the anthocyanin content of black rice increased significantly with increase in altitude of its plantation site. The increase in anthocyanin content may be due to favorable environment and environment genotype interaction. This finding also indicates that some other rice quality may also increase with an increase in altitude of its plantation.

Keywords: Black rice; Anthocyanin; Rice quality; Genotype Xenvironment; Quality rice

Introduction
Black rice is an heirloom rice landrace mostly grown in China, Korea, Japan, Thailand, Philippines and India [1]. This rice is highly rated in the market because of its huge nutritional value [2]. The main peculiarity of black rice is its anthocyanin which is both fat and water soluble. Earlier, it was believed that this rice enhance the longevity of life hence this rice is also known as long life rice. General people were forbidden to consume black rice except for emperors and nobles in China, thus this rice is popularly known as forbidden rice. There exists no other rice with a higher nutritional spectrum near black rice. This rice is free of gluten, free of cholesterol, low in sugar, salt, and fat. Black rice is a whole grain, super nutritious type of rice that is high in fiber, anthocyanin, antioxidants, vitamins B and E, iron, thiamine, magnesium, niacin and phosphorous. A huge number of scientific studies show that black rice powder is one of the nature’s most well-balanced superfood and its abilities are truly remarkable. Black rice anthocyanins (BRACs) are extracted from the aleurone layer of black rice which is a major cereal crop existing since ancient times in China and other Eastern Asia countries [3]. American Health Association, the American Cancer Society and the 2005 Dietary Guidelines for Americans recommended an increase in the consumption of black rice to prevent heart disease and certain kinds of cancers [4]. Black rice contains many vitamins and minerals, including iron, vitamin A, and vitamin B, which are beneficial for overall health and the prevention of heart disease [5]. The health benefits of black glutinous rice have recently been reported by several investigators. A recent report showed that anthocyanin supplementation in humans improves LDL and HDL levels [6] and can delay cancer development in rodents models of carcinogenesis [7]. The main purpose of this study was to compare the black rice quality in different altitudes.

Materials and Methods
Black rice G60 variety was grown in both terai and hill region of Nepal maintaining different altitudes at summer season of 2015. First place was selected as Ramban Sarlahi located at 26°52’0N 85°34’0E with an altitude of 79 meters (262 feet) in terai region and the second place was chosen as Khumaltar Lalitpur at an altitude of 1360 meter above mean sea level on 270 40’ N latitude and 850 20’ E longitudes. 25-28 days old seedlings were transplanted in a 5mX2m area with a spacing of 15cmX15cm in normally irrigated condition of July 2015. Fertilizer was recommended @04:03:30 kg/ha and @80:30:30 kg/ha NPK for both terai and hill regions respectively. The basal dose was applied as 50% of total nitrogen content, full dose of phosphorus and potassium and the remaining was applied in split dose as a top dress after 1st wedding and at rice booting stage. Agronomic practices were done as per the recommended practices. Different major traits were measured such as days to maturity, plant height, panicle length and grain yield. Rice quality analysis was done in Food and Technology Division, NARC, Khumaltar, Nepal.

Determination of minerals
The total contents of calcium (Ca) and iron (Fe) were determined in extracts obtained upon mineralization in nitric acid (HNO3 p.a.) with a concentration of 1.40 g•cm−1 in a MARS 5 microwave oven (CEM Corporation, USA), in HP500 Teflon vessels (the parameters of the process, i.e., weight of analytical samples, volume of nitric acid, and temperature of the mineralization process complied with the US-EPA3051 Protocol). Total concentrations of the six analyzed metals were determined by using an inductively coupled plasma (ICP) atomic emission spectrometer (IRISIntrepidXSPI, Thermo, USA). The analysis by use of an ICP atomic emission spectrometer (ICP-AES) was conducted after preparing the standard calibration curves corresponding to each element. Mean and SD were calculated.
Result and Discussion

The average days to rice heading and maturity of black rice were 83 days and 129 days at 1360 meter height whereas days to heading and maturity at 79 m were 78 days and 116 days. The longer days to maturity in hill region may be due to low solar radiation and low growing degree days compared to terai region. Some other rice parameters like plant height and tiller number did not differ significantly from both sites. Similarly, the grain yield of different rice varieties also varies with different locations. Grain yield was found higher in high altitude compared to low altitude. The increase in grain yield in high altitude is due to longer duration of photosynthesis uptake by plants compared to terai region. Similar results were also found by Kushwaha et al. [8] and Khakwani et al. [9] who suggested that highest paddy yields are obtained in early transplanting. The reason could be that this might be due genotype genetic superiority, an appropriate temperature for growth and development, nutrients absorption, the proper root system of the genotype and proper time of transplanting which leads to providing optimum duration for seed filling. The mineral content (Ca and Fe) of black rice from different two locations were analyzed and no significant difference was found from both altitude of plantations. But black rice possessed more Ca and Fe than other rice varieties. Kushwaha [8] also reported that black rice contains a huge amount of minerals than other rice varieties. Chen et al. [10] reported that red and black rice both contained higher total phenols content than white rice. Therefore, it is reasonable to presume that the higher amounts of minerals in red and black rice derive from a phenolic compound that promotes accumulation of divalent and trivalent minerals, such as Cu, Fe, Mg, and Zn. The color of black glutinous rice is caused by anthocyanins which are a group of reddish purple water-soluble flavonoids [11] located on pericarp, seed coat, and aleurone layer [12]. Major rice grain quality like total anthocyanin content and caryopsis color were also analyzed. It was found that Khumaltar (1360m) location (3.39) had significantly higher anthocyanin content than Ramban-Sarlahi (69m) location (2.03) (Table 1-4). The grain color was also visually observed and caryopsis color of high altitude rice was found darker than low altitude rice (light black) (Figure 1). Similar results were also reported by Kushwaha [8] and Finocchiaro et al. [13] who reported that on average, the pigmented rice had a TAC four times higher than the white ones.

![Figure 1: Black rice caryopsis color (1-light dark and 2-heavy dark) from 79 m and 1360 m altitude from mean sea level respectively.](image)

| S.No | Genotypes           | Days to Heading | Days to Maturity | Plant Height | Tiller Number | Grain Yield |
|------|---------------------|-----------------|------------------|--------------|---------------|-------------|
| 1    | Karo                | 99              | 140              | 118.8        | 11.2          | 6.07        |
| 2    | Darmali             | 99              | 141              | 114.8        | 13            | 6.33        |
| 3    | Salidhan            | 94              | 131              | 124          | 11.8          | 5.77        |
| 4    | chandannath-3       | 89              | 131              | 125          | 13.6          | 4.56        |
| 5    | Lekali-3            | 90              | 133              | 122.2        | 0             | 6.11        |
| 6    | Black rice (G60)    | 83              | 129              | 57.6         | 0             | 3.81        |
| 7    | 98046-TR196-2-1-1   | 88              | 133              | 86.2         | 19            | 6.8         |

Table 1: Rice different parameters are taken during rice growing season of 2015 at 1360 meter under high hill observation nursery trial at Agriculture Botany Division, Khumaltar, Nepal.
Table 2: Rice different parameters are taken during rice growing season of 2015 at 79 meter level at Ramban Sarlahi Nepal.

| S.No | Genotypes       | Days to Heading | Days to Maturity | Plant Height | Tiller Number | Grain Yield |
|------|-----------------|-----------------|------------------|--------------|---------------|-------------|
| 1    | Karo            | 80              | 125              | 115.5        | 10            | 4.05        |
| 2    | Darmali         | 82              | 122              | 110.8        | 11            | 4.33        |
| 3    | Salidhan        | 85              | 118              | 112          | 10            | 5           |
| 4    | chandannath-3   | 84              | 120              | 115          | 12            | 4.5         |
| 5    | Lekali-3        | 83              | 116              | 121          | 14            | 6           |
| 6    | Black rice (G60)| 78              | 116              | 67           | 11            | 3           |
| 7    | 98046-TR196-2-1-1| 82              | 119              | 89           | 13            | 4.8         |

Table 3: Content of two mineral elements in different locations with different varieties.

| Cultivars       | Khumaltar (1360 meter) | Ramban (79 meter) |
|-----------------|------------------------|-------------------|
|                 | Ca (mg /100 g)         | Fe (mg /100 g)    | Ca (mg /100 g) | Fe (mg /100 g) |
| Blackrice G60   | 53.01±1.82h            | 5.90±1.43d        | 52.60±0.19a    | 5.02±4.41e     |
| Karo            | 20.29±1.49c            | 10.35±2.15f       | 19±1.24b       | 8.35±4.03f     |
| Darmali         | 53.27±54.74g           | 3.67±0.95c        | 51.80±0.21b    | 3.00±7.08g     |
| Salidhan        | 20.34±1.33b            | 4.24±1.40b        | 18.02±0.38d    | 4.45±2.32b     |
| Chandannath-3   | 20.56±3.16a            | 8.56±2.61e        | 19.21±3.37d    | 7.30±2.41h     |
| Lekali Dhan-3   | 30.47±2.41f            | 4.24±0.97a        | 30.37±0.82bc   | 4.54±4.56c     |
| 98046-TR196-2-1-1| 26.69±3.02e            | 1.44±0.76a        | 25.24±0.79b    | 1.04±1.30a     |

Table 4: Total anthocyanin content and caryopsis color of different rice varieties at different altitudes.

| Cultivars       | Khumaltar (1360 meter) | Ramban (79 meter) |
|-----------------|------------------------|-------------------|
|                 | Total Anthocyanin Content | Caryopsis Colour   | Total Anthocyanin Content | Caryopsis Colour   |
| Blackrice756    | 3.93±0.12a              | Dark black        | 2.03±0.12a              | Light Black       |
| Karo            | 0.81±0.76d              | White             | 0.81±0.76d              | White             |
| Darmali         | 0.76±0.69a              | White             | 0.76±0.69a              | White             |
| Salidhan        | 0.69±0.71bc             | White             | 0.69±0.71bc             | White             |
| Chandannath-3   | 0.02±0.34b              | White             | 0.02±0.34b              | White             |
| Lekali Dhan-3   | 0.87±1.06c              | White             | 0.87±1.06c              | White             |
| 98046-TR196-2-1-1| 0.38±0.03bc             | White             | 0.38±0.03bc             | White             |

Conclusion

Black rice is packed with high nutrition. Among several rice quality, anthocyanin is the one which causes black rice color black. The results reveal that black rice anthocyanin content increase with an increase in altitude of its plantation with a favourable environment.

References

1. Kong L, Wang Y, Cao Y (2008) Determination of Myo-inositol and D-chiro-inositol in black rice bran by capillary electrophoresis with electrochemical detection. J Food Composition and Analysis 21(6): 501-504.
2. Kushwaha UKS, Khatiwada SP and Upreti HK, (2016) Delayed transplanting of aged rice seedlings cause the yield reduction in farmer’s field. Genomics and Applied Biology 7(1): 1-9.
3. Ling WH, Wang LL, Ma J (2002) Supplementation of the black rice outer layer fraction to rabbits decrease atherosclerotic plaque formation and increases antioxidant status. J Nutr 132(1): 20-26.
4. USA Rice Federation.
5. Chen CC, Hsu JD, Wang SF, Chiang HC, Yang MY, et al. (2003) Hibiscus sabdariffa extract inhibits the development of atherosclerosis in cholesterol-fed rabbits. J Agric Food Chem 51(18): 5472-5477.
6. Qin Y, Xia M, Ma J, Hao Y, Liu J, et al (2009) Anthocyanin supplementation improves serum LDL and HDL-cholesterol concentrations associated with the inhibition of cholesteryl ester transfer protein in dyslipidemic subjects. Am J Clin Nutr 90(3): 485-492.

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7. Thomasset S, Teller N, Cai H, et al. (2009) Do anthocyanins and anthocyanidins, cancer chemopreventive pigments in the diet, merit development as potential drugs? Cancer Chemother Pharmacol 64(1): 201-211.

8. Kushwaha, UKS (2016) Black Rice-Research, History, and Development, Springer International Publishing, Switzerland, p. 21-47.

9. Khakwani AA, Zubair M, Mansoor M, Naveed K, Shah IH, et al. (2006) Agronomic and morphological parameters of rice crop as affected by date of transplanting. J Agron 5(2): 248-250.

10. Chen XQ, Nagao N, Itani T, Irifune K (2012) Anti-oxidative analysis, and identification and quantification of anthocyanin pigments in different colored rice. Food Chemistry 135(12): 2783-2788.

11. Shen Y, Jin L, Xiao P, Yan L, Jinsong B (2009) Total phenolics, flavonoids, antioxidant capacity in rice grain and their relation to grain color size and weight. Journal of Cereal Science 49(1): 106-111.

12. Sompong R, Siebenhandl ES, Linsberger MG, Berghofer E (2011) Physicochemical and antioxidative properties of red and black rice varieties from Thailand, China, and Sri Lanka. Food Chemistry 124(1): 132-140.

13. Finocchiaro F, Ferrari B, Gianinetti A (2010) A study of biodiversity of flavonoid content in the rice caryopsis evidencing simultaneous accumulation of anthocyanins and proanthocyanidins in a black-grained genotype. J Cereal Sci 51(1): 28-34.