



## Assessment of nutritive and antioxidant properties of some indigenous pigmented hill rice (*Oryza sativa* L.) cultivars of Assam

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### ABSTRACT

Fourteen pigmented hill rice cultivars along with a non-pigmented one were studied for ascertaining the extent of their nutritional and genetic diversity. Moisture contents of the fourteen pigmented hill rice cultivars ranged from 7.49 to 10.10 % along with the contents (% dry weight) of nutrients viz., crude fat (4.37 – 5.27) and crude protein (9.27 - 11.42). The corresponding range of values for the contents (mg/100g dry weight) of microminerals Zn and Fe were from 3.42 to 4.28 and from 3.21 to 4.18 respectively. The pigmented germplasm had anthocyanin content (mg cyanidine 3-O glucoside /100g) ranging from 3.58 to 7.86, total phenolic content (mg GAE/100 g) from 67.89 to 89.43, flavonoid content (mg QE/100g) from 57.75 to 78.74 and antioxidant activity from 19.56 to 29.29 %. Analysis of variance indicated that the varietal effect on each of the parameters studied was highly significant. There was significant correlation amongst the different parameters of the rice genotypes analysed. Results of the biochemical screening of these cultivars may lead to selection of promising candidates that can be used for developing improved lines in future breeding programmes.

**Key words:** Antioxidant, Assam, Hill, Pigmented, Rice.

### INTRODUCTION

Pigmented rice (*Oryza sativa* L.) has been consumed since time immemorial, in most of the countries of Southeast Asia (Tananuwong and Tewaruth 2010). Nowadays, whole grain pigmented rice has been categorized as one of the potent functional foods since it contains high amounts of phenolic compounds (Yawadio *et al.*, 2007). The phenolic compounds have been found as a major active component for antioxidation. Anthocyanins have been recognized as health-promoting functional food ingredients due to their antioxidant activity (Philpott *et al.*, 2006), anticancer (Zhao *et al.*, 2004), hypoglycemic (Tsuda *et al.*, 2003), and anti-inflammatory effects (Tsuda *et al.*, 2002). Rice is also a good source of various essential minerals and vitamins. Microminerals viz., zinc and iron present in rice play important roles in body regulatory functions. Identification of rice cultivars with a high anthocyanin level, good agronomic traits and nutritional quality may facilitate rice breeding. Nutritional quality of rice has received more attention in the developing countries, where monotonous consumption of rice may lead to deficiencies of essential minerals, vitamins, phytochemicals and other nutritional compositions.

Rice can be grown practically any where, even on a steep hill or mountain. In Assam, the Hill zone constitutes 20% of the geographical area of the state with 1.24 lakh ha area and 1.74 lakh tonnes production for rice (Baishya *et al.*,

2010). The Hill zone constitutes only 5% of the total rice area in the state. A traditional method of cultivation practiced by hill community of Northeast India is called “*jhum*” cultivation. For this type of cultivation, the forest land is usually cleared in December to January by slashing shrubs and cutting trees. The slashed vegetation is then allowed to dry for two months before burning the tract of land in March. In addition to clearing the land, the burning of the leftover vegetation enriches the soil with mineral nutrients present in the ash and destroying pests leading to enhanced yield of the succeeding crop i.e., rice, the seeds of which are then sown. The practice is usually driven by groups of villages. A large number of traditional cultivars of rice with either red or black coloured outer layer of the grains are grown in hill region of Assam. However, there is lack of systematic investigations aiming at ascertaining the biochemical composition of those cultivars. This led the authors to undertake the present project involving nutritive and phytochemical evaluation of a few selected hill rice genotypes with good agronomic traits for providing improved materials to be used in future rice breeding programmes.

### MATERIALS AND METHODS

For the present study, a total of 15 numbers of hill rice cultivars were collected including 14 pigmented viz., Bairing Gurmu, Joradhan, Miren, Maibee, Maichukik, Dimrou, IRAT-141, Bairing, Sakbothung, Buarcha, Sakcharap, Vijoy Bijor and Pakai and one non-pigmented

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hill rice cultivar 'Vandana' from Regional Agricultural Research Station (RARS), Diphu, Assam, during 2014 (Table 1). The rice grains were stored at 4°C. Around 200g of rice sample of each cultivars were collected, dehusked and then ground using a mechanical grinder to make it fine powder dried in an oven at 100°C for few hours.

#### Nutritive quality

Protein content was estimated by method of Scales and Harrison (1920). Crude fat was estimated from oven dried sample using a Soxhlet apparatus (AOAC, 1970). The mineral solution was prepared according to the method as described by AOAC (1970). This solution was used for the estimation of zinc (Zn) and iron (Fe) using a double beam atomic absorption spectrophotometer (Chemito, model AA203D).

#### Phytochemicals and antioxidant activities

**Sample extraction :** Finely powdered dehusked grain samples of hill rice cultivars were extracted following the method of Atala *et al.* (2009) for subsequent analysis of phytochemicals and antioxidant activity. Briefly, 10 g of rice powder sample was extracted with 100 ml of extraction solvent (75:25 v/v, acetone: water). Extracts were shaken in a water bath at 25°C for 2.5 hours and then centrifuged at 10,000 rpm for 10 min (Sigma laboratory centrifuge, 3K30). The supernatant was then stored at 20°C until further analysis of total phenolics, flavonoids, anthocyanins and antioxidant activity.

**Determination of total anthocyanin content:** The sample extracts were used to determine total anthocyanin content. It was determined by the pH differential method (Giusti and Wrolstad, 2005). To measure the absorbance at pH 1.0 and 4.5, the crude extract was diluted 20 times with pH 1.0 potassium chloride buffer and pH 4.5 sodium acetate buffers, respectively. The total anthocyanin content of crude extract was calculated in terms of cyanidin-3-O-glucoside (CG). The concentration of monomeric anthocyanin pigment was calculated by the following equation.

**Monomeric anthocyanin pigment (mg/L) =**

$$[A \text{ diff} \times MW \times DF \times 1000] / \epsilon$$

where MW represents molecular weight of cyanidin-3-glucoside (449.2), DF is the dilution factor  $\epsilon$  is molar absorptivity of cyanidin-3-glucoside (26,900 l/mol cm) and A diff was calculated from the following equation:

$$A \text{ diff} = (A_{513} - A_{700})_{\text{pH}1.0} - (A_{513} - A_{700})_{\text{pH}4.5}$$

**Determination of DPPH activity :** The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the extracts was measured according to the method of Brand-Williams *et al.*, (1995). Precisely, 100  $\mu$ L of extracts were added to 1.4 mL DPPH radical methanolic solution (10<sup>-4</sup> M). The absorbance at 517 nm was measured at 30 min against blank (100  $\mu$ L methanol in 1.4 mL of DPPH radical solution). The results

were expressed in terms of radical scavenging activity using the following equation:

$$\text{Radical scavenging activity (\%)} = \frac{(A_0 - A_s)}{A_0} \times 100$$

Where, A<sub>0</sub> is the absorbance of control blank, and A<sub>s</sub> is the absorbance of sample extract.

**Determination of total phenolic content :** A modified version of the Folin-Ciocalteu assay (Slinkard & Singleton, 1977) was used to determine the total phenolic content in the extracts from the rice samples. Gallic acid standard curve was made with appropriate concentrations of aqueous gallic acid solution. For the analysis, 20  $\mu$ L each of extract, gallic acid standard or blank were taken in separate test tubes and to each 1.58 mL of distilled water was added, followed by 100  $\mu$ L of Folin-Ciocalteu reagent, mixed well and within 8 min, 300  $\mu$ L of sodium carbonate was added. The samples were vortexed immediately and allowed to incubate in dark for 30 min at 40°C. The absorbance was measured at 765 nm. The phenolic content was expressed in mg GAE/100 g.

**Determination of total flavonoid content:** The method of Dewanto *et al.* (2002) with slight modification was followed for estimation of the flavonoid content. Briefly, to 0.5 mL of the extract in a test tube, 2.25 mL of distilled water and 0.15 mL of 5% sodium nitrite solution were added and the contents were mixed well and kept for 6 min. To this, 0.3 mL of a 10% aluminium trichloride hexahydrate solution was added and contents were mixed well and allowed to stand for 5 min followed by addition of 1 mL of 1 M sodium hydroxide. The mixture was vortexed and the absorbance was measured immediately at 510 nm. Results were expressed as quercetin equivalent (mg QE/100 g) of sample.

#### Physical properties

**Colour value of rice grain:** The colour of different rice grain sample was measured using Colour Measurement Spectrophotometer (Hunter Color Lab Ultrascan Vis). Color measurements were expressed as tristimulus parameters, L\*, a\*, and b\*. where 'L\*' indicates degree of lightness or darkness (L\*=0 indicates perfect black and L\*=100 indicates most perfect white); 'a\*' indicates degree of redness (+) and greenness (-); whereas 'b\*' indicates degree of yellowness (+) and blueness (-). Hue angle and Chroma were determined using respective formula as shown below.

$$\text{Chroma} = \sqrt{a^2 + b^2}$$

$$\text{Hue angle}(\theta) = \tan^{-1} b/a$$

#### RESULTS AND DISCUSSION

**Nutritive quality :** The nutritive quality parameters were shown in the Table 1. The fat content values ranged from 4.37% ('Buaracha') to 5.27% ('Vijoy') with an average 3.91% as compared to 'Vandana' (3.50%). A slightly higher fat

**Table-1:** Contents of different nutritional and phytochemical principles, antioxidant activity and colour properties of hill rice cultivars

Rice Cultivar	Moisture (%)	Crude protein (%)	Crude fat (%)	Zn (mg/100 g)	Fe (mg/100 g)	Anthocyanin mg/100g C3G	Antioxidant activity % DPPH	Total Phenol mg GAE/100 g	Flavonoid mg QE/100g	L*	a*	b*	Hue	Chroma
Bairing Gurmu	10.1	9.88	4.99	3.73	3.49	4.7	24.34	78.56	67.12	48.69	7.47	8.14	56.13	11.04
Joradhan	7.49	10.67	5.15	3.94	3.76	6.96	29.29	89.43	71.24	46.26	6.86	7.76	57.83	10.35
Miren	8.3	11.06	5.06	4.23	3.21	6.18	22.11	81.11	69.21	46.36	6.19	7.87	61.44	10.01
Maibee	8.87	11.15	5.04	3.91	3.29	3.58	20.68	76.56	59.22	50.27	9.09	9.87	54.46	13.41
Maichukik	9.45	10.06	4.97	3.97	3.98	4.5	19.56	67.89	57.75	49.58	9.2	9.57	53.47	13.28
Dimrou	8.96	10.75	5.18	4.23	3.72	7.23	26.47	78.76	70.47	47.47	7.98	8.02	53.67	11.3
IRAT-141	9.29	9.8	5.19	3.94	4.18	4.27	21.43	71.34	58.34	49.78	8.84	9.76	55.22	13.22
Bairing	8.96	11.02	5.1	3.42	3.88	6.35	23.69	79.46	69.61	50.93	10.11	10.24	52	14.36
Sakbothung	8.64	11.42	4.82	3.86	3.48	7.86	25.64	85.23	78.74	46.11	6.83	7.85	58.15	10.52
Buarcha	8.65	10.49	4.37	3.53	3.46	6.23	26.73	80.16	70.76	49.25	8.17	8.77	55.03	12.02
Sakcharap	8.29	11.19	4.76	3.97	3.59	5.45	20.43	77.94	68.23	49.02	7.87	8.59	55.77	11.7
Vijoy	9.05	10.75	5.27	3.46	3.81	6.44	26.61	86.48	69.89	48.58	8.16	8.06	53.06	11.44
Bijor	8.5	9.27	4.75	4.14	3.94	4.23	22.43	78.9	63.87	49.23	9.13	9.06	52.21	12.82
Pakai	7.7	11.06	4.84	4.28	3.56	6.56	23.79	86.45	70.48	49.47	8.54	9.79	56.3	13.07
Vandana	9.47	10.84	3.5	2.58	2.67	0.98	ND	33.23	24.58	84.78	3.67	6.89	61.74	8.1
MEAN	8.78	10.63	4.87	3.81	3.60	5.43	23.80	76.77	64.63	51.05	7.87	8.68	55.77	11.76
	<b>(8.73)†</b>	<b>(10.61)</b>	<b>(4.96)</b>	<b>(3.90)</b>	<b>(3.67)</b>	<b>(5.75)</b>	<b>(23.80)</b>	<b>(79.88)</b>	<b>(67.50)</b>	<b>(48.64)</b>	<b>(8.17)</b>	<b>(8.81)</b>	<b>(55.34)</b>	<b>(12.04)</b>
C.V.	1.893	0.605	1.185	2.536	1.192	3.218	0.123	0.069	0.096	0.117	0.770	0.486	0.123	0.406
S.E.M.	0.096	0.037	0.033	0.056	0.025	0.101	0.016	0.030	0.036	0.034	0.035	0.024	0.040	0.028
C.D. 5%	0.279	0.107	0.096	0.161	0.072	0.293	0.046	0.088	0.104	0.100	0.101	0.071	0.115	0.080

†Values in the parentheses represent the mean value of 14 pigmented hill rice cultivars excepting non-pigmented 'Vandana'.

content was reported by Bhagabati (2000), who found the range of crude fat content varying from 3.34% to 3.51% in glutinous rice genotypes of Assam. Saikia *et al.* (2012) also found higher amounts of fat in pigmented rice as compared to the non pigmented rice varieties, which is in good agreement with the results of the present investigation. This might be due to the concentration of phenols, polyphenols, anthocyanins and other fatty substances (Pantindol *et al.*, 2006). The highest protein content was recorded in 'Sakbothang' (11.42%) and lowest in 'Bijor' (9.27%) with a mean 10.61% against 'Vandana' (10.84%). The similar findings were also reported by Bhagabati (2000). The variation in the protein content of rice grains may primarily be ascribed to cultivar differences, genetic makeup of the cultivars, climatic condition, and nutrient status of the soil as well as the analytical methods used (Baishya *et al.*, 2010). Effects of such factors on protein content of rice was observed by Juliano *et al.* (1964). The highest (4.28 mg/100g) Zn content was found in 'Pakai' and lowest (3.42 mg/100g) in 'Bairing' with an average 3.90 mg/100 g whereas 'Vandana' contained 2.58 mg/100g. The highest Fe content was recorded in 'IRAT-141' (4.18 mg/100g) and the lowest in 'Miren' (3.21 mg/100g) with an average of 3.67 mg/100 g as against 'Vandana' (2.67 mg/100g). The Fe content of the cultivars as recorded in the present study was found to be similar with the findings of Borua *et al.* (2004) but higher than the findings of Yodmanee *et al.* (2011). The pigmented cultivars in the present study were having higher Zn and Fe contents than the non-pigmented hill rice cultivar 'Vandana' and similar results were also reported by several researchers (Pathak, 2015). The red rice has approximately eight times the amount of zinc contained in the polished rice, as reported by Juliano (1985). Milling of rice generally decreases the mineral content in rice. The complete milling and polishing that converts brown rice into white rice destroy 60% of the iron content (Oko *et al.*, 2012).

#### Phytochemicals and antioxidant activity

**Total phenolic content :** The range of total phenolic content of 14 pigmented hill rice cultivars was 67.89–89.43 mg GAE/100 g with a mean value of 79.88 mg GAE/100 g against 'Vandana' (33.23 mg GAE/100 g) scoring much less than 50% (Table 1). Yodmanee *et al.* (2011) observed the polyphenol content of eight pigmented rice varieties of Thailand ranging from 58–329 mg GAE/100 g sample. Lum *et al.* (2012) and Saikia *et al.* (2012) reported higher amount of total phenolic acids in some pigmented rice. Tananuwong and Tewaruth (2010) found 188 mg GAE/100g total phenolic content in the crude extract of one black glutinous rice 'Kam Doi Saked' of Thailand. The total phenolic content of present investigation was found to be higher than that of white rice as reported by Saikia *et al.* (2012). Bordiga *et al.* (2014) reported the phenolic content of six pigmented rice varieties ranging from 140.4 to 1187.2 mg GAE/100 g of rice.

**Flavonoid content :** The range of flavonoid content (Table 1) of the 14 pigmented hill rice cultivars under the present study was 57.75–78.74 mg QE/100g with an average 67.50 mg QE/100g, whereas 'Vandana' contained 24.58 mg QE/100 g. The range of flavonoid content is lower than the findings of the Saikia *et al.* (2012). They reported that the flavonoid content of two pigmented rice varieties were 220.50 mg QE/100 g ('Chak-hao-amubi') and 123.75 mg QE/100 g ('Poreiton chakhao'). This might be because of the differences in the rice genotypes used for the studies (Pathak 2015).

**Anthocyanin content :** The range of anthocyanin content of 14 pigmented hill rice cultivars was 3.58 ('Maibee') - 7.86 mg Cyanidine 3-*O* glucoside/100 g ('Sakbothang') with a mean value of 5.75 mg Cyanidine 3-*O* glucoside/100 g as compared to much lower value in 'Vandana' (0.98 mg Cyanidine 3-*O* glucoside/100 g) (Table 1). Saikia *et al.* (2012) reported the anthocyanin content of 'Poreiton chakhao' to be 35.87 mg cyanidin-3-glucoside equivalent 100/ g whereas the variety 'Chak-hao-amubi' contained 1.81 mg (cyanidin-3-glucoside equivalent 100/ g). The anthocyanin content in brown rice having lower in non pigmented rice than the pigmented rice, which is also a good agreement with the outcome of present investigation. The red pigmented rice is having mean content of 5.75 mg Cyanidine 3-*O* glucoside/100 g. Similar results were also reported earlier by few workers [Shen *et al.*, 2009; Yodmanee *et al.*, 2011 and Pathak, 2015]

#### Antioxidant activity (Radical DPPH scavenging activity) :

The range of radical DPPH scavenging activity of 14 pigmented hill rice cultivars was 19.56 ('Maichukik')–29.29 ('Joradhan') % with an average value of 23.80 % whereas no DPPH activity has been detected in non-pigmented rice cultivar 'Vandana' (Table 1). The range of DPPH activity was lower than the findings of Saikia *et al.* (2012), Lum *et al.* (2012) which might be because of differences in the genetic makeup of the materials and extraction procedure used for the study. Bordiga *et al.* (2014) reported that pigmented rice showed higher contents of phenolics, flavonoids, and anthocyanins showing higher antioxidant activity as compared to white rice.

#### Physical properties

**Colour value of rice grain :** The value of the colour parameters were shown in the Table 1. The L\* values, which expresses the brightness of 14 pigmented hill rice cultivar was in the range of 46.11 ('Sakbothang') - 50.93 ('Bairing') with a mean value 48.64 against 'Vandana' (84.78). It indicated that the 'Sakbothang' is darker in nature among the 14 pigmented hill rice genotypes. Saikia *et al.* (2012) reported that L\* value of pigmented rice ranged from 48.58 to 62.12 which is in a good agreement with the results of the present investigation. The a\* value of 14 pigmented hill rice

Table-2: Correlation among different parameters studied

	Moisture	Crude protein	fat	Zn	Fe	Anthocyanin	Antioxidant activity	Total Flavonoid Phenol	L*	a*	b*	Hue	Chroma	
Moisture	1	-0.65966 *	-0.79814 *	-0.51094	0.0661	-0.72762 *	-0.54588 *	-0.71754 *	0.68746 *	0.4846	0.39485	-0.53609 *	0.44379	
Crude protein		1	0.54395 *	0.58495 *	-0.60668 *	0.14692	-0.03692	0.36463	-0.22589	-0.19601	0.05236	0.4284	-0.07916	
Crude fat			1	0.28714	-0.07397	0.65548 *	0.64935 *	0.76704 *	-0.59328 *	-0.46008	-0.41039	0.46715	-0.45088	
Zn				1	-0.23005	0.41479	-0.20496	0.082	-0.47955	-0.37264	0.15485	0.62008 *	-0.28069	
Fe					1	0.08712	0.13303	-0.23432	0.113	0.3654	0.16443	-0.50758	0.26954	
Anthocyanin						1	0.75798 *	0.87483 *	-0.97113 *	-0.84670 *	-0.86032 *	0.76559 *	-0.86493 *	
Antioxidant activity							1	0.89577 *	-0.67371 *	-0.61401 *	-0.74127 *	0.39042	-0.68910 *	
Total Phenol								1	-0.77109 *	-0.75965 *	-0.76072 *	0.65140 *	-0.77558 *	
Flavonoid									1	-0.90898 *	-0.96926 *	0.82147 *	-0.97390 *	
L*										1	0.90815 *	-0.88678 *	0.93106 *	
a*											1	0.95484 *	-0.94443 *	
b*												1	-0.81173 *	
Hue													1	
Chroma														1

\* represent the significant value of correlation

cultivar ranged from 6.19 ('Miren') to 10.11 ('Bairing') with a mean value of 8.17 as compared to 'Vandana' (3.67) and was similar with the findings of Mir *et al.* (2013) who found the range of a\* from 4.23 to 7.73 in brown rice. The b\* value of 14 pigmented hill rice cultivar ranged from 7.76 ('Joradhan') to 10.24 ('Bairing') with an average of 8.81 against 'Vandana' (6.89). A slightly wider range of b\* values 3.30-15.71 was reported for pigmented rice by Yodmanee *et al.* (2011). The Hue angle of 14 pigmented hill rice cultivar ranged from 52 to 61.44 as compared to non-pigmented 'Vandana' (61.74). The chroma of 14 pigmented rice ranged from 10.01 to 14.36 as compared to non-pigmented 'Vandana' (8.1). Saikia *et al.* (2012) reported slightly lower but with wider range of Hue values (35.62 – 63.35) and a lower value of Chroma (3.04-9.83) for some pigmented rice varieties.

**CORRELATION**

The correlation values of different parameters were shown in the Table 2. Moisture was found to be negatively correlated with Protein, fat, anthocyanin content, antioxidant activity, total phenol, flavonoid and hue and positively correlated with only L\* value. A positive correlation was observed in between protein and fat content. The crude fat content was found to be positively correlated with anthocyanin, antioxidant activity, total phenol and flavonoid content. There was a negative correlation between fat and L\* value. Zn was found to be positively correlated with hue. The anthocyanin content was positively correlated with antioxidant activity, total phenol and flavonoid content. The anthocyanin content was negatively correlated with all the colour value like L\*, a\*, b\* and chroma. Yodmanee *et al.* (2011) also reported that rice grain color parameters (L\*, a\* and b\*) had negative correlations ( $p < 0.01$ ) with polyphenol ( $r = -0.893, -0.851$  and  $-0.928$ ) and antioxidant capacity ( $r = -0.794, -0.629$  and  $-0.770$ ). Similarly, phenol was found to be positively correlated with flavonoid content and negatively correlated with all the colour values like L\*, a\*, b\* and chroma except Hue. Jin *et al.* (2009) reported that the phenolic content was positively correlated with the flavonoid content. A significant correlation among the different colour parameters was also observed. Jin *et al.* (2009) also reported that these color parameters were interrelated. The correlation value among different parameters will be useful for breeder to go for simultaneous improvement of pigmented hill rice cultivars.

The pigmented rice cultivars analysed in the present study appear to be a good source of phytochemicals like anthocyanin, phenol, flavonoid and found to have antioxidant activity. These genotypes are also rich in important minerals like Fe and Zn as compared to the other common rice

cultivars grown in the plane region of Assam. No single genotype was found to be superior over the other in terms of all the parameters studied.

The genetic diversity found in the cultivars under the present investigation may be considered as a need to

select the best performers amongst themselves with respect to the contents of nutritive and antioxidant principles as well as the antioxidant activity. Promising candidates thus selected, may be utilized for developing improved lines in future breeding programmes.

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