

Genetic Analysis of Agronomic Traits and Grain Anthocyanin and Micronutrient (Zinc and Iron) Content in Rice (*Oryza sativa* L.)

Mohammad Zahir Ullah, Prince Biswas and Mohammad Shariful Islam

Bangladesh Institute of Research and Training on Applied Nutrition, Head Office, Araihasar, Narayanganj, Bangladesh

ABSTRACT

Background and Objective: Consumption of coloured rice has been associated with decreased risk of many diseases and may exert positive effects on human health in the presence of anthocyanin and micronutrients. This experiment was carried out for genetic analysis of agronomic traits, anthocyanin and micronutrients (Zn, Fe) content of rice. **Materials and Methods:** Five coloured pericarp rice genotypes were included in a field experiment for eleven agronomic traits and lab tests for three nutrients measurement. The anthocyanin content of rice grain was determined by the HPLC method and micronutrient (Zn, Fe) content was determined by Atomic Absorption Spectrophotometer (AAS) method. **Results:** The results revealed that the highest grains per panicle (302) and grain yield per plant (60 g) were observed in the genotype RS 31. Genotype RS 17 showed the highest anthocyanin content (570 mg/100 g). The RS 17 and RS 54 have high Zn content with higher Fe content. Total tillers per plant exhibit a high phenotypic and genotypic coefficient of variation followed by anthocyanin content, grain yield per plant, grains per panicle, filled grain per panicle, zinc content and plant height. Iron content showed a moderate coefficient of variation. Grain yield per plant was positively and significantly correlated with effective tillers per plant, panicle length, days to maturity and grains per panicle and positively correlated with anthocyanin content. A significant positive correlation was found between Zn and anthocyanin content. Path coefficient analysis showed that a positive direct effect was observed of grains per panicle (4.576), Zn content (1.977), anthocyanin content (0.746) and effective tillers per plant (0.318) on grain yield per plant. **Conclusion:** Among the studied rice genotypes RS 17 and RS 31 have been selected as promising with high grain yield and nutritional quality that would be given positive health effects and also a prerequisite towards improving nutritional enrich rice varieties.

KEYWORDS

Variability, genetic analysis, anthocyanin, micronutrients, correlation, path analysis

Copyright © 2023 Zahir Ullah et al. This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

More than two billion people in the globe suffer from micronutrient deficits for a lack of vital vitamins and minerals in their diet¹. The Fe and Zn deficits are the most worldwide micronutrient malnutrition, predominant in resource-poor countries where there is a substantial dietary reliance on staple food crops². Rice (*Oryza sativa* L.) is the major source of calorie intake for about half of the world's population. Micronutrients are mandatory to convey several metabolic and functional progressions in the human



body. The Zn is necessary as a cofactor in more than 300 enzymes playing critical essential roles but Fe acts as a vital element of haemoglobin and myoglobin^{3,4}. In the past decade, plentiful interest was manifested in evolving varieties of rice with improved micronutrient content of Fe and Zn to increase the nutritional quality of grain. Black rice contains a higher amount of phenolic compounds as compared to white rice⁵. Red and purple pericarp rice varieties exhibited more total phenolics, flavonoids and antioxidant activity as compared to white and brown varieties^{6,7}. Black rice is accumulated of anthocyanin (cyanidin-3-glucoside) which is the highest among all of the studied coloured grains⁸. Cyanidin-3-glucoside showed an inhibitory effect on cancer cell spread⁹ and reduced the risk of atherosclerosis¹⁰. Anthocyanin efficiently reduces the total cholesterol, low-density cholesterol and total triacylglycerol in the body¹¹.

Biologically fortification of nutrients is a genetic methodology to overcome malnutrition and the development of new cultivars with eminent content of micronutrients using conventional breeding and biotechnological approaches¹². Other than agronomical practices, attention has been given to increasing Zn and Fe content in rice grain which includes a selection of genotypes with high content of micronutrients, breeding micronutrient-efficient crop that produces high yields and accumulation of more micronutrients from soil and increasing bio-available Zn and Fe¹³. Knowledge about genotypic variability and genetic relationships among breeding materials could be an invaluable aid in crop improvement strategies.

A four times difference in levels of Fe and Zn content among rice genotypes proposes a possible upsurge in the content of these micronutrients in rice grains with genetic technology⁴. The contents of Fe and Zn in grains of traditional rice cultivars were significantly higher than those in improved cultivars¹⁴. In this situation, the current research was undertaken to carry out, 1: Estimate genetic variability for anthocyanin, Zn and Fe content in grains (brown rice) of different rice genotypes, 2: To analyze the correlation between anthocyanin, Zn, Fe and grain yield and 3: Selection of genotypes for high anthocyanin, Zn and Fe content.

MATERIALS AND METHODS

The experiment was conducted at the research field of Bangladesh Institute of Research and Training on Applied Nutrition (BIRTAN), regional station, Noakhali, Bangladesh during the Aman season of 2021. A total of five Aman rice genotypes were collected from different locations in Bangladesh (Table 1). The experiment was conducted in a randomized complete block design with three replications. A plot consisting of six rows 5 m long by 1.2 m wide (6 m²) with a spacing of 0.2 m between rows and 0.15 m between plants were used. Twenty five days old seedlings were planted in the main field. As recommended, agronomic and plant protection measures were followed during the crop period. Data on grain yield and other important agronomic traits were collected on a plot and individual plant basis. Observations were recorded on the following attributes viz: Days to first flowering, days to 50% flowering, days to maturity, plant height (cm), number of tillers per plant, number of effective tillers per plant, panicle length (cm), grains per panicle, filled grains per panicle, unfilled grain per panicle and grain yield per plant (g) at appropriate stages of crop. The Zn, Fe and anthocyanin content were measured after harvest from brown rice in Waffen Research Laboratories, Tejgaon, Dhaka, Bangladesh. The estimated field data was accumulated through mean values of three replications of each genotype. The OPSTAT¹⁵ research software was used to calculate the analysis of variance, different genetic parameters, character association and path coefficient analysis.

Table 1: List of genotypes studied in the experiment

Code	Paddy colour	Pericarp colour
RS17	Black	Black
RS31	White	Red
RS45	White	Red
RS47	White	Red
RS 54	Black	Red

Anthocyanin content: The anthocyanin content was measured through the High-Pressure Liquid Chromatographic (HPLC) method. The calculated anthocyanin pigment concentration was expressed as cyanidin-3-glucoside equivalents. The total anthocyanin content is calculated using the following formula¹⁶:

$$\text{Total anthocyanin (mg kg}^{-1}\text{)} = \frac{A \times MW \times DF \times 10^3}{\epsilon \times l}$$

Where:

- A = (A520 nm-A700 nm), pH 1.0-4.5
- MW = Molecular weight (cyanidin-3-glucoside)
- DF = Dilution factor
- l = Path length in cm
- ϵ = Molar extinction coefficients
- 1000 = Factor for conversion from g to mg

Fe and Zn content analysis: Micronutrients (Fe and Zn) content from all studied genotypes was measured through atomic absorption spectrophotometer (AAS) at Waffen Research Laboratories, Tejgaon, Dhaka, Bangladesh. Rice seeds were dehusked quietly by using a palm dehusker. Zinc and iron content measurement was denoted in mg/100 g. A minimum of two replications from each of the cultivars were analyzed for the two micronutrients. The average value of the two replicates is calculated.

Statistical analysis: The analysis of variance (mean sum of a square), mean performance, genetic variability, correlation and path analysis were analysed through computer software OPSTAT and Microsoft Excel 2010. The means were compared with Duncan's Multiple Range Test (DMRT) at a 5% level of probability.

RESULTS AND DISCUSSION

Morphological mean performance: A wide range of variability was observed among the studied five rice genotypes for ten morphological and three nutritional traits (Table 2). Significant genetic variation was found for all the characters except effective tillers per plant and unfilled grains per panicle revealed by the genotypes designated that the effectiveness of the selection of these traits. The highest effective tillers per plant were observed in genotype RS 31 (34.33) which was statistically dissimilar and followed by RS 47 (20.00) (Table 3). Grains per panicle are an important trait of rice. The highest grains per panicle and filled grains per panicle were observed in the genotype RS 31 (302 and 288.33) which was statistically similar to RS 45 (272 and 270). The highest grain yield per plant was observed in RS 31 (60 g) which was statistically dissimilar and followed by RS 17 (39 g).

Nutritional parameter: The level of anthocyanin in rice genotypes was measured through the HPLC method. Previously, there are observed many research findings on anthocyanin content in rice^{17,18}. Anthocyanin levels were effect by a colour difference of rice and stated that black rice is concentrated of higher anthocyanin levels¹⁹.

The highest level of anthocyanin was observed in genotype RS 17 (570 mg/100 g) as significantly compared to other genotypes (Table 3). This was in harmony with the findings of the statement of the rice colour which demonstrate blackish-dark purple as the result of high anthocyanin concentration. The minimum anthocyanin content value was revealed in RS 47 (97.3 mg/100 g). Anthocyanin found in black rice exists in aleuron and endosperm layers. Anthocyanin produces comparatively large amounts in these layers, to facilitate the morphology of the rice appears blackish purple. The anthocyanin colorants in black rice have an inhibitor effect that has enormous benefits on human health which are useful for preventing

Table 2: Analysis of variance (mean sum of square) for different traits in rice genotypes

Variant	DF	DFF	D50F	DM	pH (cm)	TPP	ETP	PL (cm)	GPP	FGP	UFGP	Zn (mg)	Fe (mg)	An (mg)	GYP (g)
Replication	2	1.40	4.06	12.86	43.40	63.29	38.4000	5.26	695.40	794.06	43.40	0.04	0.24	118.29	1.26
Genotypes	4	699.00**	682.40**	307.76**	3,391.73**	5,153.25**	151.76	24.50*	18,423.10**	18,286.50**	62.43	0.34**	0.59**	122,193.90**	520.76**
Error	8	0.90	8.65	0.36	70.98	39.85	42.56	5.60	541.40	362.90	23.98	0.01	0.02	25.61	4.26

** *Significant at 1 and 5%, respectively, DFF: Days to 50% flowering, D50F: Days to first flowering, DM: Days to maturity, PH (cm): Plant height, TPP: Tillers per plant, ETP: Effective tillers per plant, PL (cm): Panicle length, GPP: Grains per panicle, FGP: Filled grains per panicle, UFGP: Unfilled grains per panicle, Zn (mg/100 g): Zinc, Fe (mg/100 g): Iron, An (mg/100g): Anthocyanin and GYP (g): Grain yield per plant.

Table 3: Mean performance of agronomic and nutritional traits in five rice genotypes

Genotype	DFF	D50F	DM	PH (cm)	TPP	ETP	PL (cm)	GPP	FGP	UFGP	Zn (mg/100 g)	Fe (mg/100 g)	An (mg/100 g)	GYP (g)
RS17	115 ^b	120 ^b	160.33 ^a	179 ^a	19 ^c	19 ^b	25 ^d	244.33 ^{bc}	243.33 ^{bc}	1 ^b	1.45 ^b	2.66 ^b	570 ^a	49 ^b
RS31	116 ^b	119.33 ^b	145.33 ^b	154.33 ^b	34.33 ^b	34.33 ^a	28.67 ^a	302 ^a	288.33 ^a	13.67 ^a	1.13 ^c	2.28 ^c	151.05 ^b	60 ^a
RS45	120 ^a	129.33 ^a	142 ^c	113.33 ^c	17 ^c	16.67 ^b	25 ^{ab}	272 ^{ab}	270 ^{ab}	6.67 ^{ab}	0.88 ^d	3.38 ^a	102.93 ^d	28 ^c
RS47	120 ^a	128 ^a	142.33 ^c	93.67 ^d	113.69 ^a	20 ^b	24 ^{ab}	219.33 ^c	214 ^c	5.33 ^{ab}	0.87 ^d	2.38 ^c	97.3 ^d	47 ^b
RS 54	84 ^c	92 ^c	132.33 ^d	141.67 ^b	19 ^c	19 ^b	20.67 ^b	98.33 ^d	91 ^d	7.33 ^{ab}	1.63 ^a	2.41 ^c	134.55 ^c	31.67 ^c
Min	84.00	92.00	132.33	93.67	17.00	16.67	20.67	98.33	91.00	1.00	0.87	2.28	97.30	28.00
Max	120.00	129.33	160.33	179.00	113.69	34.33	28.67	302.00	288.33	13.67	1.63	3.38	570.00	60.00
Mean	111.00	117.73	144.47	136.40	40.60	21.80	24.67	227.20	221.33	6.80	1.19	2.62	211.17	43.13
SE	0.36	1.11	0.23	3.18	2.39	2.47	0.89	8.79	7.20	1.85	0.02	0.05	1.91	1.91
F test	**	**	**	**	**	NS	*	**	**	NS	**	**	**	**
CV (%)	0.85	2.50	0.42	6.18	15.55	29.93	9.59	10.24	8.61	72.02	3.65	4.91	2.40	4.79

The same letter(s) in a column did not differ significantly at p<0.05 by DMRT, **, *Significant at 1 and 5% respectively. DFF: Days to first flowering, D50F: Days to 50% flowering, DM: Days to maturity, PH (cm): Plant height, TPP: Tillers per plant, ETP: Effective tillers per plant, PL (cm): Panicle length, GPP: Grains per panicle, FGP: Filled grains per panicle, UFGP: Unfilled grains per panicle, Zn (mg/100 g): Zinc, Fe (mg/100 g): Iron, An (mg/100 g): Anthocyanin, GYP (g): Grain yield per plant, SE: Standard error and CV: Coefficient of variation

Table 4: Assessment of genetic parameters for different quantitative and qualitative traits in rice genotypes

Parameters	PCV	GCV	ECV	h ² b	GA (5%)	GAP (mean (%))
DFF	13.77	13.74	0.85	99.61	31.36	28.26
D50F	12.97	12.73	2.50	96.29	30.29	25.73
DM	7.02	7.01	0.42	99.64	20.82	14.41
PH (cm)	25.16	24.39	6.18	93.97	66.44	48.71
TPP	102.86	101.68	15.55	97.72	84.07	207.05
ETP	40.76	27.68	29.93	46.10	8.44	38.71
PL (cm)	13.99	10.18	9.59	52.94	3.76	15.25
GPP	35.49	33.98	10.24	91.67	152.28	67.02
FGP	35.97	34.92	8.61	94.27	154.60	69.85
UFGP	89.21	52.65	72.02	34.83	4.35	64.00
Zn (mg/100 g)	28.60	28.37	3.65	98.37	0.69	57.96
Fe (mg/100 g)	17.45	16.74	4.91	92.09	0.87	33.10
An (mg/100 g)	95.59	95.56	2.40	99.94	415.58	196.80
GYP (g)	30.79	30.42	4.79	97.58	26.70	61.90

PCV: Phenotypic coefficients of variations, GCV: Genotypic coefficients of variations, ECV: Environmental coefficients of variations, h²b: Heritability in a broad sense, GA (5%): Genetic advance in five percent, GAP (mean (%)): Genetic advance in percent of the mean, DFF: Days to first flowering, D50F: Days to 50% flowering, DM: Days to maturity, PH (cm): Plant height, TPP: Tillers per plant, ETP: Effective tillers per plant, PL (cm): Panicle length, GPP: Grains per panicle, FGP: Filled grains per panicle, UFGP: Unfilled grains per panicle, Zn (mg/100 g): Zinc, Fe (mg/100 g): Iron, An (mg/100 g): Anthocyanin and GYP (g): Grain yield per plant

the pulverization of arteries²⁰. Black rice contains more active flavonoids compound compared to white rice. Consequently, the colour alteration of rice can be used as a reference to the projection of the anthocyanin content in rice^{21,22}. It is recognized that the pigment can decrease the risk of oxidative damage from low-density lipoprotein in humans. In addition, the pigment in black rice can reduce the construction of nitric oxide synthase in macrophage cells which serves to prevent DNA damage.

The variation in grain Zn content ranged from 0.87 mg/100 g (RS 470) to 1.63 mg/100 g (RS 54) with an average value of 1.19. Variations in grain Fe content ranged from 2.28 mg/100 g (RS 310) to 3.38 mg/100 g (RS 45) with an average value of 2.62. The RS 17 and RS 54 have comparatively high Zn content with higher Fe content of rice.

Genetic variation: The phenotypic coefficient of variation (PCV) was higher than the corresponding genotypic coefficient of variation (GCV) for all the studied characters and was presented in Table 4. The environmental coefficient of variation (ECV) was less for the characters like days to first flowering, days to maturity and anthocyanin signifying less effect of environment on these characters. Comparable judgments were testified earlier^{23,24}. Among all the characters, tillers per plant (102.86 and 101.68) displayed the highest PCV and GCV (>20%) followed by anthocyanin content (95.59 and 95.56), unfilled grain per panicle (89.21 and 52.65), effective tillers per plant (40.76 and 27.68), filled grains per panicle (35.97 and 34.92), grains per panicle (35.49 and 33.98), grain yield per plant (30.79 and 30.42) and Zn content (28.60 and 28.37). The results were designated that the opportunity of genetic enhancement through selection for these traits. Parallel outcomes were documented for grain yield per plant, total tillers and the number of productive tillers per plant²⁵. Moderate variations of PCV and GCV (11-20%) were recorded for grain Fe content, panicle length and days to flowering in studied rice genotypes. These outcomes confirm the outcomes of Govindraj *et al.*²⁶ for grain Fe content. The low magnitude of PCV and GCV (<10%) was presented by days to maturity, reported beforehand²⁷.

The measurement of difference does not give the full assessment of the heritable variant. It can be originated with a better degree of correctness when heritability is coupled with a genetic advance. Great heritability assessments alongside great genetic advances are more supportive in foretelling the achievement beneath assortment than heritability appraisals only. Nonetheless, it is not essential that a character presenting high heritability will also demonstrate high genetic advance²⁸. High heritability

(>70%) was presented in anthocyanin content, days to maturity, days to flowering, Zn content, tillers per plant, Fe content, filled grains per panicle and grains per panicle under this experiment. These outcomes were dependable with the outcomes of moderate heritability (50-70%) was revealed by effective tillers per plant, panicle length and unfilled grain per panicle. These outcomes were consistency with earlier outcomes²⁹ for panicle length.

The high genetic advance as a percent of the mean (>20%) was observed for tillers per plant (207.06) followed by anthocyanin percent (196.80), filled grains per panicle (69.85), grains per panicle (67.02), unfilled grains per panicle (64.00), grain yield per plant (61.90), zinc content (57.96), plant height (48.71), effective tiller per plant (38.71), iron content (33.10), days to first flowering (28.26) and days to first fruiting (25.73). Modest genetic advance (10-20%) was noted for days to maturity (14.41) and panicle length (15.25). These outcomes were in validation with outcomes examined by Tuwar *et al.*³⁰ and for Zn and Fe content³¹. High heritability joined with high genetic advance as a percent of mean was documented for Zn and Fe content.

Correlation studies: The relationship of yield with its constituent traits reproduces the nature of the association between them. The association analysis assistances in inspecting the opportunity of improving yield through an indirect selection of its component traits which are highly correlated. The highest positive significant correlation was observed for grain yield per plant with effective tiller per plant (0.936** and 0.637**) followed by panicle length (0.804** and 0.607**), days to maturity (0.519* and 0.513*) and grains per panicle (0.524* and 0.501*) at both genotypic and phenotypic levels (Table 5). These results were in confirmation with results for tillers per plant and grains per panicle³². Effective tillers per plant had a significant positive correlation with panicle length (0.900** and 0.576*), related to the outcome testified researchers^{33,34}. Therefore, selection for grain yield contributing traits like effective tillers per plant, panicle length and grains per panicle is effective in the accumulation of the grain yield.

Association between zinc and iron contents with grain yield: There was a significant negative correlation of grain yield with Fe content (-0.687** and -0.623**) and a negative correlation with Zn (-0.061 and -0.057) at both genotypic and phenotypic levels, respectively (Table 5). Positively correlation of grain yield with anthocyanin was observed (0.299 and 0.296) at both genotypic and phenotypic levels. Zn content had a negative correlation with Fe (-0.362 and -0.344) while a positive significant correlation with anthocyanin (0.489* and 0.485*) at both genotypic and phenotypic levels, respectively. If we increased the Zn content in grain it also increased the anthocyanin content in rice grain. These outcomes highlight the significance of genetic variation extant in existing genotypes to the escalation of anthocyanin content in prevailing rice variations. The Fe content has a minor negative correlation with anthocyanin (-0.012 and -0.011) at both phenotypic and genotypic levels. Additionally, there is essential to monitor more genotypes obtainable as present outcomes are in confirmation with prior intelligence³⁵ that some traditional/local landraces often demonstrate great genetic variation for maximum micronutrients than improved varieties.

Path-coefficient analysis: Path-coefficient analysis was conceded to recognize the direct and indirect effects of unlike quantitative and nutritional traits on grain yield per plant (Table 6). Grains per panicle (4.576) exhibited a maximum positive direct effect on grain yield per plant followed by Zn content (1.977). Moderate to low positive direct effects estimated by anthocyanin content (0.746), effective tillers per plant (0.318) and panicle length (0.167) on grain yield per plant. These outcomes are supported by prior reports^{36,37} demonstrating that grains per panicle, grain Zn content, anthocyanin content and effective tillers per plant can act as selection criteria simultaneously to escalate grain yield per plant. So, selection based on other characteristics such as days to maturity and filled grain per panicle would be evidence active to boost grain yield per plant and nutritional quality. These outcomes are supported by the outcomes of Yuan *et al.*³⁸ and Yaqoob *et al.*³⁹.

Table 5: Genotypic (rg) and phenotypic (rp) correlation coefficients of yield and yield related traits in T. Aman rice genotypes

Parameters		DFF	D50F	DM	PH (cm)	TPP	ETP	PL (cm)	GPP	FGP	Zn	Fe	An
D50F	rg	0.964**											
	rp	0.934**											
DM	rg	-0.674**	-0.676**										
	rp	-0.673**	-0.675**										
PH (cm)	rg	0.082	0.076	0.577*									
	rp	0.079	0.076	0.554*									
TPP	rg	-0.289	-0.288	-0.110	-0.682**								
	rp	-0.286	-0.285	-0.109	-0.641**								
ETP	rg	-0.264	-0.269	0.067	0.323	-0.020							
	rp	-0.177	-0.183	0.050	0.278	0.093							
PL (cm)	rg	-0.893**	-0.902**	0.570*	0.196	-0.021	0.900**						
	rp	-0.650**	-0.645**	0.414	0.267	0.018	0.576*						
GPP	rg	-0.933**	-0.935**	0.575*	0.069	0.022	0.537*	1.065**					
	rp	-0.895**	-0.896**	0.558*	0.061	0.028	0.384	0.783**					
FGP	rg	-0.943**	-0.944**	0.600*	0.063	0.015	0.465	1.035**	0.999**				
	rp	-0.917**	-0.917**	0.588*	0.058	0.024	0.348	0.771**	0.997**				
Zn	rg	0.714**	-0.712**	0.016	0.739**	-0.547*	-0.027	-0.522*	-0.647**	-0.648**			
	rp	0.709**	-0.705**	0.017	0.703**	-0.552*	-0.098	-0.386	-0.615**	-0.626**			
Fe	rg	-0.260	-0.256	0.085	0.234	-0.412	-0.684**	-0.078	0.286	0.328	-0.362		
	rp	-0.249	-0.245	0.073	0.173	-0.388	-0.453	0.049	0.232	0.279	-0.344		
An	rg	-0.218	-0.220	0.866**	0.789**	-0.345	-0.161	0.108	0.120	0.150	0.489*	-0.012	
	rp	-0.217	-0.220	0.865**	0.763**	-0.340	-0.108	0.086	0.119	0.148	0.485*	-0.011	
GYP (g)	rg	-0.493*	-0.497*	0.519*	0.382	0.302	0.936**	0.804**	0.524*	0.489*	-0.061	-0.687**	0.299
	rp	-0.486*	-0.493*	0.513*	0.381	0.297	0.637**	0.607**	0.501*	0.472	-0.057	-0.623**	0.296

*Significance at a 5% level of significance, **Significance at a 1% level of significance, DFF: Days to first flowering, D50F: Days to 50% flowering, DM: Days to maturity, PH (cm): Plant height, TPP: Tillers per plant, ETP: Effective tillers per plant, PL (cm): Panicle length, GPP: Grains per panicle, FGP: Filled grains per panicle, UFGP: Unfilled grains per panicle, Zn (mg/100 g): Zinc, Fe (mg/100 g): Iron, An (mg/100 g): Anthocyanin, GYP (g): Grain yield per plant

Table 6: Path coefficient analysis showing the direct (bold) and indirect effect of yield contributing traits on grain yield

	DFF	D50F	DM	PH (cm)	TPP	ETP	PL (cm)	GPP	FGP	Zn	Fe	An	Genotypic correlation with grain yield
DFF	-0.640	-3.393	1.309	-0.026	-0.004	-0.084	-0.149	-4.272	5.502	1.412	0.017	-0.162	-0.493*
D50F	-0.640	-3.392	1.312	-0.024	-0.004	-0.085	-0.150	-4.278	5.508	1.406	0.016	-0.164	-0.497*
DM	0.432	2.294	-1.941	-0.187	-0.001	0.021	0.095	2.630	-3.498	0.032	-0.005	0.646	0.519*
PH (cm)	-0.052	-0.257	-1.120	-0.324	-0.011	0.102	0.032	0.314	-0.367	1.461	0.015	0.588	0.382
TPP	0.185	0.976	0.213	0.221	0.016	-0.006	-0.003	0.101	-0.090	-1.082	0.027	-0.257	0.302
ETP	0.169	0.912	-0.129	-0.104	-0.001	0.318	0.150	2.459	-2.710	-0.053	0.045	-0.120	0.936**
PL (cm)	0.572	3.059	-1.106	-0.063	0.001	0.286	0.167	4.873	-6.039	-1.031	0.005	0.080	0.804**
GPP	0.598	3.171	-1.115	-0.022	0.001	0.171	0.177	4.576	-5.826	-1.278	-0.018	0.089	0.524*
FGP	0.604	3.203	-1.163	-0.020	-0.001	0.148	0.173	4.570	-5.834	-1.281	-0.021	0.112	0.489*
Zn	-0.457	-2.414	-0.031	-0.239	-0.008	-0.008	-0.087	-2.959	3.781	1.977	0.023	0.365	-0.061
Fe	0.166	0.869	-0.165	-0.075	-0.006	-0.217	-0.013	1.307	-1.912	-0.716	-0.066	-0.008	-0.687**
An	0.139	0.746	-1.681	-0.255	-0.005	-0.051	0.018	0.550	-0.875	0.966	0.001	0.746	0.299

*Significance at a 5% level of significance, **Significance at a 1% level of significance, Residual effect, R = 0.347, DFF: Days to first flowering, D50F: Days to 50% flowering, DM: Days to maturity, PH (cm): Plant height, TPP: Tillers per plant, ETP: Effective tillers per plant, PL (cm): Panicle length, GPP: Grains per panicle, FGP: Filled grains per panicle, UFGP: Unfilled grains per panicle, Zn (mg/100 g): Zinc, Fe (mg/100 g): Iron and An (mg/100 g): Anthocyanin

Genetic variation was found among the studied genotypes for morphological traits including grain yield. A significant variation of the genotypes for anthocyanin content in rice grain. A genetic variation was also observed in rice grains for Zn and Fe content. Anthocyanin has a great impact on human health as an antioxidant and prevents cell damage as a reference of many research findings. The Zn and Fe are micronutrients that prevent our bodies from diseases. Anthocyanin, Zn and Fe content have high heritable characteristics. So, can improve in the next filial generation after conventional breeding through segregation. Anthocyanin percent can be increased with the increase of grain yield in rice because of the positive correlation found. The Zn and anthocyanin can be increased simultaneously through genetic improvement like breeding in grain due to a significant positive correlation among them.

CONCLUSION

We can increase anthocyanin content in rice grain with increased Zn content because they are positively correlated. Correlation and path coefficient analysis revealed that filled grains per panicle, effective tillers per plant, Zn content and anthocyanin content were the most effective variables indicating the importance of these traits as a selection criterion in Aman rice grain yield as well as nutritional enhancement. Genotypes RS 17 and RS 31 have been selected as more promising with high grain yield and nutritional quality.

SIGNIFICANCE STATEMENT

Due to a proper understanding of the genetic analysis of colored pericarp rice genotypes to formulate and accelerate the conventional breeding programs, this study aimed to evaluate the extent of genetic variability existing for agronomic and nutritional traits in five colored pericarp rice genotypes in Bangladesh. Quantitative, qualitative and nutritional traits of plants and grains were assessed. Results showed genetic variability agronomic and nutritional content of colored rice which assisted in classifying the genotypes based on high grain yield with high anthocyanin as well as high Zn and Fe content in grain. The variations recorded would assist the breeders in efficient selections for their breeding program.

ACKNOWLEDGMENT

The authors are highly thankful to the Bangladesh Institute of Research and Training on Applied Nutrition (BIRTAN), Arayhazar, Narayanganj for providing technical and financial assistance during the research program.

REFERENCES

1. Moreno-Moyano, L.T., J.P. Bonneau, J.T. Sánchez-Palacios, J. Tohme and A.A.T. Johnson, 2016. Association of increased grain iron and zinc concentrations with agro-morphological traits of biofortified rice. *Front. Plant Sci.*, Vol. 7. 10.3389/fpls.2016.01463.
2. Sands, D.C., C.E. Morris, E.A. Dratz and A.L. Pilgeram, 2009. Elevating optimal human nutrition to a central goal of plant breeding and production of plant-based foods. *Plant Sci.*, 177: 377-389.
3. Sperotto, R.A., T. Boff, G.L. Duarte, L.S. Santos, M.A. Grusak and J.P. Fett, 2010. Identification of putative target genes to manipulate Fe and Zn concentrations in rice grains. *J. Plant Physiol.*, 167: 1500-1506.
4. Welch, R.M. and R.D. Graham, 2004. Breeding for micronutrients in staple food crops from a human nutrition perspective. *J. Exp. Bot.*, 55: 353-364.
5. de Mira, N.V.M., I.L. Massaretto, C. de Simone Carlos Iglesias Pascual and U.M.L. Marquez, 2009. Comparative study of phenolic compounds in different Brazilian rice (*Oryza sativa* L.) genotypes. *J. Food Compos. Anal.*, 22: 405-409.
6. Walter, M., E. Marchesan, P.F.S. Massoni, L.P. da Silva, G.M.S. Sartori and R.B. Ferreira, 2013. Antioxidant properties of rice grains with light brown, red and black pericarp colors and the effect of processing. *Food Res. Int.*, 50: 698-703.
7. Shao, Y., F. Xu, X. Sun, J. Bao and T. Beta, 2014. Phenolic acids, anthocyanins, and antioxidant capacity in rice (*Oryza sativa* L.) grains at four stages of development after flowering. *Food Chem.*, 143: 90-96.
8. Abdel-Aal, E.S.M., J.C. Young and I. Rabalski, 2006. Anthocyanin composition in black, blue, pink, purple, and red cereal grains. *J. Agric. Food Chem.*, 54: 4696-4704.
9. Chen, P.N., S.C. Chu, H.L. Chiou, W.H. Kuo, C.L. Chiang and Y.S. Hsieh, 2006. Mulberry anthocyanins, cyanidin 3-rutinoside and cyanidin 3-glucoside, exhibited an inhibitory effect on the migration and invasion of a human lung cancer cell line. *Cancer Lett.*, 235: 248-259.
10. Xiaodong, X., L. Wenhua, M. Jing, X. Min and H. Mengjun *et al.*, 2006. An anthocyanin-rich extract from black rice enhances atherosclerotic plaque stabilization in apolipoprotein E-deficient mice. *J. Nutr.*, 136: 2220-2225.

11. Zawistowski, J., A. Kopec and D.D. Kitts, 2009. Effects of a black rice extract (*Oryza sativa* L. indica) on cholesterol levels and plasma lipid parameters in Wistar Kyoto rats. *J. Funct. Foods*, 1: 50-56.
12. Graham, R., D. Senadhira, S. Beebe, C. Iglesias and I. Monasterio, 1999. Breeding for micronutrient density in edible portions of staple food crops: Conventional approaches. *Field Crops Res.*, 60: 57-80.
13. Chandel, G., S. Banerjee, S. See, R. Meena, D.J. Sharma and S.B. Verulkar, 2010. Effects of different nitrogen fertilizer levels and native soil properties on rice grain Fe, Zn and protein contents. *Rice Sci.*, 17: 213-227.
14. Anandan, A., G. Rajiv, R. Eswaran and M. Prakash, 2011. Genotypic variation and relationships between quality traits and trace elements in traditional and improved rice (*Oryza sativa* L.) genotypes. *J. Food Sci.*, 76: H122-H130.
15. Lal, N., A. Singh, A. Kumar, E.S. Marboh, A.K. Gupta, S.D. Pandey and V. Nath, 2022. Genetic variability, correlation and path-coefficient studies in litchi (*Litchi chinensis* Sonn.) for plant growth, panicle and yield attributes. *Int. J. Bio-resource Stress Manage.*, 13: 29-36.
16. Bouis, H.E. and R.M. Welch, 2010. Biofortification-a sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. *Crop Sci.*, 50: S20-S32.
17. Pratiwi, R., W.A.S. Tunjung, R. Rumiati and A.R. Amalia, 2015. Black rice bran extracts and fractions containing cyanidin 3-glucoside and peonidin 3-glucoside induce apoptosis in human cervical cancer cells. *Indonesian J. Biotechnol.*, 20: 69-76.
18. Sui, X., Y. Zhang and W. Zhou, 2016. Bread fortified with anthocyanin-rich extract from black rice as nutraceutical sources: Its quality attributes and *in vitro* digestibility. *Food Chem.*, 196: 910-916.
19. Min, B., L. Gu, A.M. McClung, C.J. Bergman and M.H. Chen, 2012. Free and bound total phenolic concentrations, antioxidant capacities, and profiles of proanthocyanidins and anthocyanins in whole grain rice (*Oryza sativa* L.) of different bran colours. *Food Chem.*, 133: 715-722.
20. Sholikhah, U., Parjanto, T. Handoyo and A. Yunus, 2021. Anthocyanin content in some black rice cultivars. *IOP Conf. Ser.: Earth Environ. Sci.*, Vol. 709. 10.1088/1755-1315/709/1/012076.
21. Hu, C. and D.D. Kitts, 2001. Evaluation of antioxidant activity of epigallocatechin gallate in biphasic model systems *in vitro*. *Mol. Cell. Biochem.*, 218: 147-155.
22. Hu, C., Y. Zhang and D.D. Kitts, 2000. Evaluation of antioxidant and prooxidant activities of bamboo *Phyllostachys nigra* var. henonis leaf extract *in vitro*. *J. Agric. Food Chem.*, 48: 3170-3176.
23. Hasan-Ud-Daula and U. Sarker, 2020. Variability, heritability, character association, and path coefficient analysis in advanced breeding lines of rice (*Oryza sativa* L.). *Genetika*, 52: 711-726.
24. Prajapati, M.K., C.M. Singh, G.S. Babu, G.R. Lavanya and P. Jadhav, 2011. Genetic parameters for grain yield and its component characters in rice. *Elect. J. Plant Breed.*, 2: 235-238.
25. Bastia, D., T.K. Mishra and S.R. Das, 2008. Genetic variability and selection indices for grain yield in upland rice. *ORYZA Int. J. Rice*, 45: 72-75.
26. Govindaraj, M., B. Selvi, S. Rajarathinam and P. Sumathi, 2011. Genetic variability and heritability of grain yield components and grain mineral concentration in India's pearl millet (*Pennisetum glaucum* (L) R. Br.) accessions. *Afr. J. Food Agric. Nutr. Dev.*, 11: 4758-4771.
27. Oladosu, Y., M.Y. Rafii, N. Abdullah, M. Abdul Malek and H.A. Rahim *et al.*, 2014. Genetic variability and selection criteria in rice mutant lines as revealed by quantitative traits. *Sci. World J.*, Vol. 2014. 10.1155/2014/190531.
28. Johnson, H.W., H.F. Robinson and R.E. Comstock, 1955. Estimates of genetic and environmental variability in soybeans. *Agron. J.*, 47: 314-318.
29. Khodadadi, M., H. Dehghani and M.H. Fotokian, 2014. Heritability and genetic diversity of iron, zinc and some morphological and physiological traits in some spring wheat genotypes (*Triticum aestivum* L.). *Int. J. Biosci.*, Vol. 4. 10.12692/ijb/4.2.1-9.
30. Tuwar, A.K., S.K. Singh, A. Sharma and P.K. Bhati, 2013. Appraisal of genetic variability for yield and its component characters in rice (*Oryza sativa* L.). *Biolife*, 1: 84-89.

31. Al-Daej, M.I., 2022. Genetic studies for grain quality traits and correlation analysis of mineral element contents on Al-Ahsa rice and some different varieties (*Oryza sativa* L.). Saudi J. Biol. Sci., 29: 1893-1899.
32. Debsharma, S.K., R.F. Disha, M.M.E. Ahmed, M. Khatun, M. Ibrahim and T.L. Aditya, 2020. Assessment of genetic variability and correlation of yield components of elite rice genotypes (*Oryza sativa* L.). Bangladesh Rice J., 24: 21-29.
33. Laxuman, P.M. Salimath, H.E. Shashidhar, H.D. Mohankumar, S.S. Patil, H.M. Vamadevaiah and B.S. Janagoudar, 2011. Character association and path coefficient analysis among the backcross inbred lines derived from Indica X NERICA cross for productivity traits in rice (*Oryza sativa* L.). Karnataka J. Agric. Sci., 24: 626-628.
34. Ullah, M.Z., M.K. Bashar, M.S.R. Bhuiyan, M. Khalequzzaman and M.J. Hasan, 2011. Interrelationship and cause-effect analysis among morpho-physiological traits in birain rice of Bangladesh. Int. J. Plant Breed. Genet., 5: 246-254.
35. Anuradha, K., S. Agarwal, A.K. Batchu, A.P. Babu, B.P.M. Swamy, T. Longvah and N. Sarla, 2012. Evaluating rice germplasm for iron and zinc concentration in brown rice and seed dimensions. J. Phytology, 4: 19-25.
36. Nagesh, V. Ravindrababu, G. Usharani and T.D. Reddy, 2012. Grain iron and zinc association studies in rice (*Oryza sativa* L.) F₁ progenies. Arch. Appl. Sci. Res., 4: 696-702.
37. Nath, S. and P.C. Kole, 2021. Genetic variability and yield analysis in rice. Electron. J. Plant Breed., 12: 253-258.
38. Yuan, W., S. Peng, C. Cao, P. Virk and D. Xing *et al.*, 2011. Agronomic performance of rice breeding lines selected based on plant traits or grain yield. Field Crops Res., 121: 168-174.
39. Yaqoob, M., R. Anjum, M. Hussain and M.J. Shah, 2012. Genetic diversity analysis and character association in some Chinese hybrid rice under dry conditions. Pak. J. Agric. Res., 25: 249-256.