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Identification of Iron rich rice genotypes in Bangladesh using chemical analysis

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Abstract

Rice (*Oryza sativa* L.) is the most important food crop of the developing world and the staple food of more than half the global population. An experiment was carried out to identify the iron rich rice genotypes. Total fifty two rice genotypes were used for this purpose. Iron (Fe) content of rice grain samples was determined by using Atomic Absorption Spectrophotometer (AAS). In this process the samples were digested by the application of di-acid mixture which includes nitric acid (HNO_3): and perchloric acid ($HClO_4$) in 2:1 ratio. Iron content was estimated in the aliquot of seed extract by using Atomic Absorption Spectrophotometer (AAS) at 248.33 nm. Iron concentration ranged from 1.32 ppm (Jota Balam) to 100.45 ppm (Lal Gotal). Among the 52 genotypes local landraces had showed the highest Fe content. In this experiment, ANOVA table revealed that significant variation was found among the genotypes for the iron concentration. This suggested that there were inherent genetic differences among the genotypes. Thus, local landraces can be a good source for biofortification of popular rice cultivars using different breeding methods.

Keywords: Iron, Rice genotypes, Atomic Absorption Spectrophotometer (AAS), Biofortification

Introduction

Rice is one of the most important cereal crops in Bangladesh and it is the main staple food for the people. It provides nearly 48% of rural employment, about two-thirds of total calorie supply, and about one-half of the total protein intakes of an average person in the country. Rice sector contributes one-half of the agricultural GDP and one-sixth of the national income in Bangladesh. About 75% of the total cropped area and more than 80% of the total irrigated area is planted to rice. Almost all the 13 million farm families grow rice. Among the large population of this country a large portion suffered in malnutrition and one of the important ways to mitigate this problem by growing nutritional quality improved rice cultivars. In Bangladesh, 11528.51 thousand hectares of land produces 33540.32 thousand metric tons of rice (BBS, 2012).

In the last two decades, new research findings generated by the nutritionists have brought to light the importance of micronutrients, vitamins and proteins in maintaining good health, adequate growth and even acceptable levels of cognitive ability apart from the problem of protein energy malnutrition. (Nagesh *et al.,* 2012).

Iron deficiency is the most common nutritional disorder in the globe affecting between 2 to 5 billion people. In Bangladesh 49% of pregnant woman and 53% of preschool children are anemic due to iron deficiency (Hossain and Hussain, 2004). The severe form of iron deficiency affects about 3.5 billion worldwide (Kracht, 1999; Ahman *et al*., 2000). Overall, 39% of preschool children and 52% of pregnant woman are anemic; more than 90% of them are living in the developing countries. In infant and young children, it impairs immunity, reduces the physical growth and cognitive development; at school age it affects school performance and reduces activity levels; at adulthood, it reduces work capacity and decrease resistance to fatigue. In pregnant women, iron deficiency anemia is associated with an increased risk of pre-mature delivery, retarded growth of the fetus, low birth weight and increased risk that the new born baby die soon after birth. Anemia is the main cause of death during childbirth (Chrispeel and Sadava, 1994).

This study will help in identifying the iron rich rice genotypes among the existing Bangladeshi genotypes. This study will also help in the developing nutritionally improved rice cultivar, training of young scientists in developed laboratories to provide a pool of human resources competent in molecular breeding techniques along with the development of rice varieties with enhanced nutritional quality.

Materials and Methods

Experimental site

This experiment was conducted at the experimental laboratory of Department of Agricultural Chemistry, Bangladesh Agricultural University and Soil Science Division of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, during June 2012 to January 2013.

Experimental materials

A total of fifty two (52) rice genotypes, consisting of forty (40) landraces representing southern and northern part of Bangladesh, five (5) high yielding varieties (HYV), three (3) mega varieties and four (4) advanced lines were collected to carry out the objectives of this research work. List of the rice genotypes are presented in Table 2.

Processing of seeds

Processing of seeds of fifty two rice genotypes for chemical analysis was done by dehusking and grinding. Before analyzing the rice samples for iron estimation, the seeds of all the 52 accessions were subjected to dehusking and grinding. The collected rice seeds were oven dried at 55^0 C temperatures for 48 hours to facilitate the dehusking process. The oven dried seeds were then placed in winnower and rubbed across it for the dehusking purpose. The dehusked seeds were then grinded to make it powder with the help of a mechanical grinder. The rice grain powder of each genotypes was then stored in a polythene bag. From there one gram (1g) of the rice grain powder for each genotypes was weighed separately for the further use.

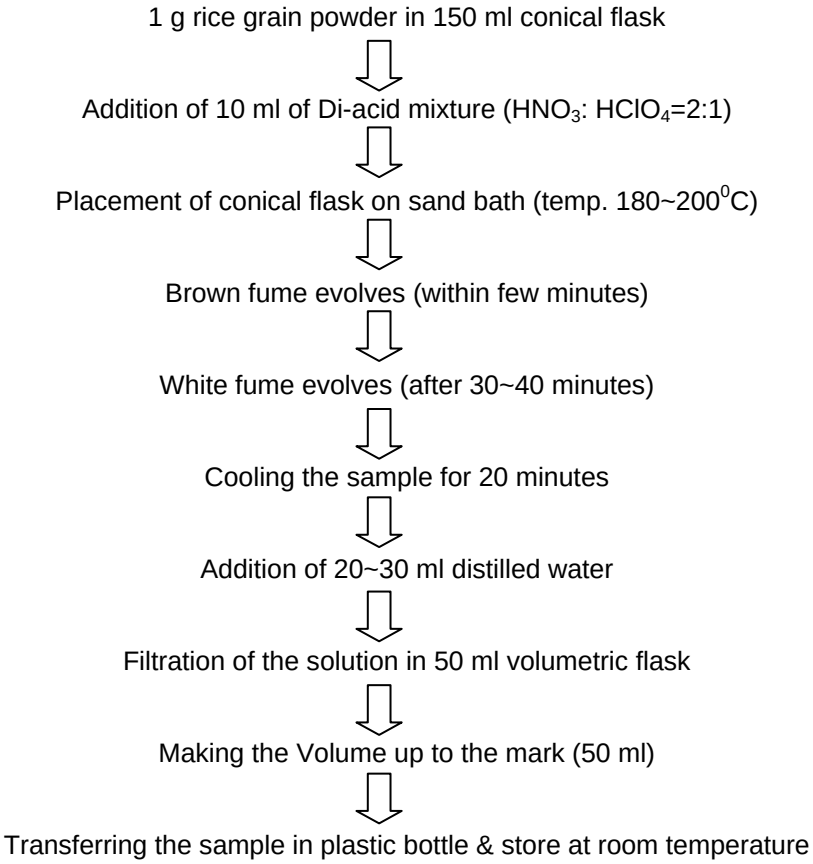
Determination of iron content from the rice grain sample

Iron (Fe) content of rice grain samples was determined by using Atomic Absorption Spectrophotometer (AAS) as stated by Lindsay and Novell (1978). In this process the samples were digested by the application of di-acid mixture (Bhatia and Khetarpaul, 2012; Shaibur *et al.*, 2010) which includes nitric acid (HNO_3): and perchloric acid ($HClO_4$) in 2:1 ratio. The detailed procedure of this process described below.

Procedure of Digestion of rice grain for Iron (Fe) estimation

From the each genotypes one gram (1g) amount of rice grain powder was taken in 150 ml conical flask and 10 ml of di-acid mixture (HNO_3: $HClO_4$=2:1) added to it. It was kept overnight at room temperature (2.00 PM to 11.00 AM). Then the conical flask was placed on sand bath at temperature 180~200^0C for 30~40 minutes. After a few minutes brown fume was evolved. This indicated the starting of digestion process. Finally white fume was seen by clearing the solution. At the bottom of the conical flask about 2-3 ml solution was noticed. After that heating was stopped and the digested sample was cooled for 20 minutes. Then about 20~30 ml distilled water was added to each conical flask. Then this solution was filtered into a 50 ml volumetric flask and the volume was made up to the mark (50 ml) by adding distilled water. The 50 ml solution was then transferred into a plastic bottle for each genotype for the further use in future. The plastic bottle was stored at a room temperature. Total procedure is outlined as a flow diagram as follows:

Flow diagram for the estimation of Iron (Fe) content from rice grain



Iron (Fe) content determination by AAS

It is based on the principle that atoms of iron (Fe) which is normally remain in ground state, under flame condition absorb energy when subjected to radiation is proportional to the specific wavelength. The absorption of radiation is proportional to the concentration of iron. Iron content was estimated in the aliquot of seed extract by using Atomic Absorption Spectrophotometer (AAS) at 248.33 nm.

Results and Discussion

Fe concentration analysis

Fe concentration in all the genotypes was analyzed using Atomic Absorption Spectrophotometer at Soil Science Division, Bangladesh Institute of Nuclear Agriclture (BINA), Mymensingh. Seeds from all varieties were dehusked gently. Concentration was expressed in parts per million (ppm). Calibration graph was constructed (Fig. 1) from iron standards containing the concentrations given in the Table 1.

Table 1. Data on Iron concentration & absorbance for calibration graph

Sl. No.	Fe Conc.(ppm)	Absorbance
1	0	0.00
2	0.5	0.06
3	1.0	0.12
4	2.0	0.22
5	3.0	0.31
6	4.0	0.39
7	5.0	0.45

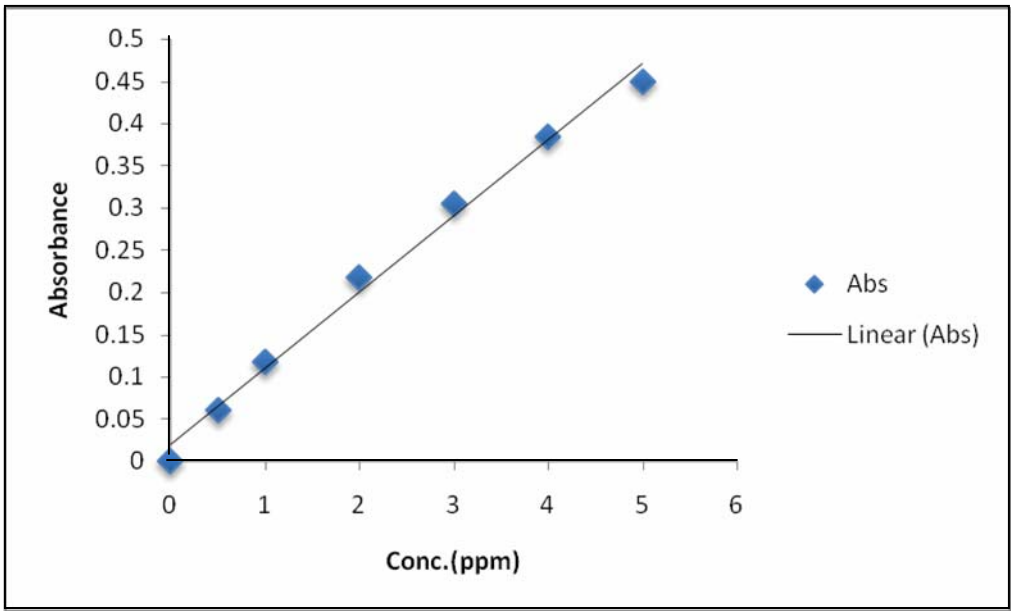


Fig. 1. Calibration graph by plotting iron concentration & absorbance

A minimum of two replications from each of the cultivars and local genotypes were analyzed for Fe. The di-acid method of digestion was followed. The variation in replications for each sample did not exceed ± 2ppm for Fe. The mean of the two replicates was presented in results (Table 2).

Table 2. Mean performance of 52 rice genotypes based on Iron (Fe) Concentration

Sl. No.	Name of the variety	Iron Concentration (mean) (ppm)
1	Dudh Kalam	16.69
2	Enghi	55.91
3	Kajol Shail	11.98
4	Jamai Naru	14.94
5	Hari	12.97
6	Dakh Shail	37.15
7	Moina Moti	73.61
8	Patnai	5.57
9	Kute Patnai	62.29
10	Mohini Shalot	23.75
11	Moghai Balam	16.58
12	Khak Shail	88.52
13	Holde Gotal	11.80
14	**Jota Balam**	1.32
15	Durga Bhog	18.15
16	Khainol	11.29
17	Ghunshi	12.56
18	Chinikani	13.44
19	Hamai	92.21
20	Mura Bajal	9.79
21	**Lal Gotal**	100.45
22	Sylhet Balam	20.09
23	Mota Aman	15.64
24	Ghochi	11.49
25	Tal Mugur	3.45
26	Tor Balam	7.94

Table 2 Contd.

Sl. No.	Name of the variety	Iron Concentration (mean) (ppm)
27	Fulkainja	49.60
28	Piarjat	9.42
29	Koicha Binni	8.25
30	Lal Biroi	5.11
31	Kakua Binni	60.72
32	Nona Bokhra	14.37
33	Sada Binni	30.25
34	Kalo Binni	18.85
35	Jongli Boro	6.88
36	Kashrail	9.76
37	Ledra Binni	11.57
38	Roti Shail	13.85
39	Lal Binni	18.83
40	Kali Boro	16.13
41	BR 11	63.53
42	BRRI dhan 28	35.81
43	BRRI dhan 29	69.12
44	BRRI dhan 52	26.82
45	BRRI dhan 55	14.10
46	BRRI dhan 57	66.52
47	BINA dhan-7	9.02
48	BINA dhan-8	21.38
49	GPB-F-1	17.71
50	GPB-F-2	31.47
51	Samba Mashuri	20.13
52	Cheheran	11.07
	CV%	2.29
	Minimum	1.32
	Maximum	100.45
	Mean	27.11
	LSD at 5%	1.73

Analysis of variance (ANOVA) was constructed to determine the variations among the concerned genotypes (Table 3).

Table 3. Analysis of variance for screening of iron rich rice genotypes

Source of variation	Degrees of freedom	Sum of Squares	Mean square	F-value
Between	51	64558.942	1265.862	3014.390**
Within	52	21.837	0.420	
Total	103	64580.778		

** indicates significant at 0.01 probability

A wide range of variation was observed among fifty two rice (*Oryza sativa* L.) genotypes for iron concentration. The perusal of data revealed that variance due to genotypes was highly significant for the iron concentration (Table 2). This suggested that there were inherent genetic differences among the genotypes. Significant genetic variation for iron content exhibited by the genotypes indicated this character might be effective for further crop improvement. Total 52 accessions of rice genotypes were analyzed for iron (Fe) concentration. Iron concentration ranged from 1.32 ppm to 100.45 ppm. Among the 52 genotypes local landraces i.e. Lal Gotal, Hamai, Khak Shail, Moina Moti, Kute Patnai, Kakua Binni, Enghi and commercially cultivated varieties i.e. BRRI dhan 29, BRRI dhan 57, BR 11 had showed the highest Fe content. The iron content of local landraces is higher than the others which is supported by the findings that were earlier reported by Ilango and Sarla (2010), Banerjee *et al.,* (2010) and Anuradha *et al.,* (2012). Banerjee *et al.,* (2010) screened 46 rice lines including cultivated and wild accessions and

showed that wild rice accessions have higher grain Fe concentration. Anuradha *et al.* (2012) reported that they analyzed brown rice of 126 accessions of rice genotypes for Fe concentration. Iron concentration ranged from 6.2 ppm to 71.6 ppm and the local accessions had the highest Fe. In this study few genotypes (local landraces) had showed higher iron concentration than the previously reported. Thus, local landraces are a good source for biofortification of popular rice cultivars using different breeding methods.

Conclusion

Screening of germplasm for Fe content is the initial step of biofortification. In this study 52 genotypes were screened to identify iron rich rice in Bangladesh. The local landrace, Lal Gotal had the highest (100.45 ppm) iron concentration where as Jota Balam had the lowest amount of iron concentration (1.32 ppm). Among the commercial cultivated varieties; BR 11, BRRI dhan 29 and BRRI dhan 57 have the considerably higher amount of iron content, so it can be proposed to eat these rice varieties to cope with iron deficiencies. Furthermore, among the screened materials, the genotypes having higher iron content can be used as a breeding material for biofortification process in future.

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