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Characterization of Rice [*Oryza sativa* L.] Germplasm Based on Iron and Zinc Content

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Abstract
Hidden hunger is caused by the consumption of food grains (rice) which are deficient in mineral micronutrients specifically iron and zinc. This hunger affects more than one-half of the world's population especially woman and children in developing countries causing anemia and child blindness because 60 per cent of the world's population is dependent on rice as their staple food. Considering this biofortification as one of the suitable approaches was considered for improving the Fe and Zn content and their bioavailability in rice grain. Local landraces of rice were collected and Fe and Zn content were quantified for their genotypic characterization. Iron (Fe) and Zinc (Zn) content of 112 local landraces were estimated according to Lindsey and Norwell by using Atomic Absorption Spectrophotometer Varian Speetra AA 50B. Iron concentration ranged from 0.25µg/g to 3.25µg/g and zinc from 0.85µg/g to195.3µg/g in the landraces. Highest iron containing rice was Swetonunia with 34.8µg/g and highest Zn was found in Nepali Kalam which was 195.3µg/g. Zn content is quite comparable to other HYVs and can be used as donor parent in breeding program for biofortification of micronutrient Zn.

Keywords: Rice landraces, Minerals Iron, Zinc, and Genotyping.

Introduction

Rice is a major staple food and energy source of more than half of the world population, being the major source of carbohydrate and even protein. However, rice is a poor source of essential micronutrients such as Fe and Zn (Bouis and Welch 2010). In countries where rice is used as staple food, the per capita consumption is very high ranging from 62 to 190 kg/year. Thus, even a small increase in the nutritive value of rice can be highly significant for human nutrition (Grahama *et al.* 1999). Micronutrient malnutrition, and particularly Fe and Zn deficiency affect over three billion people worldwide, mostly in developing countries (Sperotto *et al.* 2010). Diet deficient in minerals such as Fe and Zn in staple food crops causes 'hidden hunger' or micronutrient malnutrition in developing countries (Welch *et al* 2004). It causes several diseases (anemia, endemic goiter, child blindness, *etc.*); the affected people are more prone to infection to other diseases resulting in further deterioration in quality of life. Of these, iron deficiency is the most common nutritional disorder in the world affecting over 4 billion people, with more than 2 billion woman, mainly in developing countries (WHO; http://www.who.int/nut/ida.htm). Zinc deficiency in humans reduces growth, sexual maturity and the immune defense system (Parsad 1993). The human body requires more than 22 mineral elements that can be supplied by an appropriate diet (Philip and Martin 2005). Trace minerals are important not only for human nutrition, but for plant nutrition as well, plant breeding holds great promise for making a significant, low-cost, and sustainable contribution to reducing micronutrient, particularly mineral deficiencies in humans, and may have important by-product effects for increasing farm productivity in developing countries in a way that is environmentally-beneficial (Cary *et al.* 1994, Kannenberg *et al.* 1995).

Several groups have examined the probability of "Biofortification" approach for improving the micronutrient (iron and zinc) content of staple crops including rice. It is observed that substantial useful genetic variation exists in key staple crops. Nutritional quality traits are highly heritable in some crops, mineral rich traits are sufficiently stable across a wide range of growing environments, and

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traits for high micronutrient content can be combined with superior agronomic and high yield characteristics.

Most of the commercially cultivated indica and japonica rice cultivars are deficient in iron and zinc compared to the other staple food crops such as wheat and maize (Gregorio *et al.* 2000). Zinc deficiency is probably the most widespread micronutrient deficiency in cereals. Sillanpaa (1990) found that 49 percent of the global sample of 190 soils in 25 countries was low in zinc. Unlike other micronutrients, zinc deficiency is a common feature of both cold and warm climates, drained and flooded soils, acid and alkaline soils, and both heavy and light soils (Rahman *et. al.* 1993).

Since rice is the principal food of the Asian continent (Developing world), a lot of efforts are being made to develop nutritionally improved genotypes of rice (Sasaki 1998). The first pre-requisite for initiating a breeding program to develop micronutrient-rich genotypes, is to screen the available germplasm and identify the source of genetic variation for the target trait, which can be used in crosses, genetic studies, molecular marker development and to understand the basis of enhanced micronutrient accumulation. Available literature do show existence of variability for grain iron and zinc contents in rice, but to date only a small portion of the existing genetic diversity has been assayed for micronutrients (Gregorio *et al.*1999).

A distinction has to be made between content and concentration. The content of iron and zinc in rice depends on the grain size. Aromatic long grain basmati lines are known to be high in iron content. The high or low content of mineral elements in grain largely determine the nutrient value of rice. Zhang *et al* (2005) showed that single grain selection of narrow grains tends to increase the content of Zn, Mn and P, while selection of single plants with bigger grain weight tends to increase the content of P.

Considering all the above references, objectives of the present investigation is to screen rice germplasm for iron and zinc content.

Materials and Methods

Rice cultivars

Total of 112 rice cultivars were collected from West Bengal and adjoining area (during kharif 2010 & 2011) and maintained at the Plant Genetics & Tissue Culture Laboratory, University of North Bengal (NBU), India.

Fe and Zn concentration analysis

Iron (Fe) and Zinc (Zn) content of 112 local cultivars were estimated according to standard method (Lindsey and Norwell 1969) by Atomic Absorption Spectrophotometer (Varian Spectra AA50B). Seeds from all varieties were dehusked gently using a palm dehusker. Concentration was expressed in µg/g. One gram oven dried ground dehusked seed samples were collected in a 150 ml conical flask. To this 25-30 ml diacidic mixture (HNO3:HClO4; 5:1 v/v) was added and kept overnight. Next day, it was digested by heating till clear white precipitates settled down at the bottom. The crystals were dissolved by diluting in double distilled water. The contents were filtered through Whatman No. 42 filter paper. The filtrate was made to 50 ml with double distilled water. The acid digested samples were used for the determination of iron and zinc contents.

Results and Discussion

Iron content ranged between 0.25µg/g to 3.25µg/g and Zinc ranged between 0.85µg/g to 195.3µg/g (Table 1). Local cultivar Swetonunia had highest iron content of 34.8µg/g followed by the other cultivars Chamormoni 3.25µg/g, Bunkulon 3.15µg/g, Govindobhog 3.1µg/g, and Addey 2.05µg/g (Table 1). Nepali Kalam had the highest Zinc content 195.3µg/g followed by Govindobhog 138.6 µg/g, Begunbeej 20.4µg/g and Ghiosh16.15µg/g. Iron content in all the local landraces were very poor but Zinc content in some of the landraces was promising containing 195.3µg/g in Nepali Kalam and 138.6µg/g in Gobindobhog).

On the basis of iron contents, rice cultivars could be grouped in two categories, low (0-10µg/g); moderate (>10µg/g). Similarly, rice cultivars were placed into two groups on the basis of zinc contents low (<10μg/g); moderate (>10μg/g). Most of the rice cultivars studied here was placed in the low iron and zinc containing categories. Least amount iron content cultivars are Birohi (0.4μg/g), Kabiraj (0.35μg/g), and Tulaipanji (0.45μg/g). Similarly least amount zinc content cultivars are Birohi (0.85), Chamormoni (0.85μg/g) and Thulo Addey (1.4μg/g). These results indicated that there is significant genetic diversity in the rice germplasm.

A plant breeding strategy has been formulated to improve the mineral nutrition in rice grain which includes selecting for germplasm with greater quantities of essential minerals (such as Fe, Zn etc). For this the breeding lines that are with high yields and accumulate minerals from infertile soils are selected and enhancing bio-available minerals in edible portions through increasing the concentrations of metal-binding proteins (Fumiyuki *et al.* 1999; Lucca *et al.* 2001; Holm *et al.* 2002; Zhang *et al.* 2004; Heinemann *etal.* 2005; Philip and Martin 2005).

Of 112 rice genotypes evaluated, one genotype, Swetonunia was placed under high iron category 34.8μg/g and Nepali Kalam under high Zinc 195.3μg/g category. Grahem *et al* (1999) and Gregorio *et al* (2000) reported wide range of Fe (6.3-24.4μg/g) and Zn (13.5-58.4μg/g) concentration in brown rice. Notably, highest grain-Fe (18-22μg/g) and grain-Zn (24-35μg/g) concentration were found in several aromatic rice varieties such as Jalmagna, Zuchen and Xua Bue Nuo. Notably, there was about many fold difference in Fe and Zn content suggesting the existence of genetic potential to increase the concentration of these micronutrients in rice grain. This type of large genotypic variation especially for iron content in rice has not been reported earlier. Iron and Zinc contents in edible portions also depend on the efficiency of translocation of minerals from root tissues to edible plant organs and accumulation thereof. Mineral-rich and mineral poor rice genotypes identified in this study may be used in breeding program for introgression of high Fe and Zn content gene or QTLs in the improved varieties.

Table 1. Iron and Zinc content in the rice germplasm

Sl. No.	Rice landraces	Fe (μg/g)	Zn (μg/g)
1	Agundhepi	2.05±0.011	9.77±0.011
2	Aichung	4.3±0.003	3.8±0.029
3	Anandi	0.75±0.025	2.55±0.003
4	Ashami	0.7±0.029	3.4±0.003
5	Attey-1	7.9±0.006	4.1±0.002
6	Attey-2	6.9±0.002	1.0±0.011
7	Badsabhog	6.2±0.001	2.1±0.008
8	Banni	6.9±0.002	1.4±0.001
9	Begunbeej	7.0±0.002	2.1±0.008
10	Bhadaore	1.6±0.021	20.4±0.020
11	Bunkulon	7.3±0.001	2.8±0.005
12	Chamormoni	3.15±0.008	10.62±0.58
13	Champa	3.25±0.068	0.85±0.28
14	Champasali	0.6±0.024	2.55±0.21
15	Champasari	5.2±0.004	2.4±0.003
16	Chanachur	0.96±0.015	8.8±0.104
17	Charinagrey	6.8±0.004	1.5±0.001
18	China Boro	4.7±0.005	2.6±0.001
19	Chiniatop	0.33±0.032	3.4±0.24
20	Chinisakkar	13.4±0.008	4.4±0.003
21	Chirakhe	6.0±0.000	2.3±0.001
22	Chulthey	4.5±0.007	2.12±0.097
23	Chunakathi	7.0±0.000	3.4±0.004
24	Chunia	7.1±0.003	2.1±0.003
25	Dangimarua	7.3±0.001	2.7±0.002
26	Desi nunia	6.7±0.002	2.5±0.009
27	Dhankutte	7.0±0.004	3.2±0.004
28	Dhanraj	4.7±0.011	2.7±0.000
29	Dhepi	4.9±0.002	2.2±0.008
30	Dhusuri dhan	7.0±0.002	2.3±0.13
31	Kalamkathi	4.6±0.002	1.7±0.13
32	Kalampanati	4.9±0.001	2.4±0.002
33	Kalobhog	7.1±0.001	2.4±0.12
34	Kalojera	6.9±0.001	1.6±0.003
35	Kalokure	10.7±0.002	1.9±0.16
36	Kalonunia	6.0±0.005	2.55±0.266
37	Kakuriya	7.0±0.004	4.1±0.14
38	Kanta Rangi	4.9±0.002	2.6±0.009
39	Kantajinghasal	3.8±0.003	3.9±0.003
40	Kataribhog	5.5±0.001	2.6±0.009
41	Kattaka	5.1±0.003	2.5±0.006
42	Khalkhajara	6.9±0.001	2.5±0.009
43	Khasa dhan	6.9±0.004	2.2±0.001
44	Khasdhan	4.0±0.002	2.1±0.11
45	Kholako Dhan	0.25±0.07	2.12±0.385
46	Khechri	1.73±0.014	4.67±0.137
47	Koshia Binni	4.3±0.001	2.1±0.017
48	Kumrogore	7.2±0.001	1.6±0.007
49	Ladua	8.0±0.001	2.9±0.006

Sl. No.	Rice landraces	Fe (μg/g)	Zn (μg/g)
50	Lalmala	5.4±0.008	2.4±0.004
51	Ravan	12.1±0.001	6.7±0.001
52	Sadanunia	7.8±0.002	35.2±0.002
53	sanu addey	6.9±0.002	2.2±0.004
54	Sikkimey	6.7±0.005	2.7±0.18
55	Sitabhog	9.7±0.002	6.3±0.14
56	Sorulalat	11.9±0.001	3.2±0.006
57	Bhadoi	0.5±0.038	1.7±0.002
58	Bhale musuri	3.0±0.004	1.7±0.002
59	Bhangeri	6.7±0.000	1.7±0.001
60	Bharlang	6.7±0.001	1.8±0.003
61	Borni	6.9±0.004	2.3±0.008
62	Borisal	4.2±0.011	2.5±0.016
63	Birohi	0.70±0.05	7.6±0.105
64	Birimphole	0.4±0.026	0.85±0.70
65	Banni	7.1±0.003	3.3±0.004
66	Buchi	7.0±0.002	2.1±0.008
67	Dos nunia	6.9±0.004	3.8±0.001
68	Dudhekalam	4.4±0.001	2.0±0.008
69	Dudheswar	5.8±0.005	2.4±0.005
70	Dudhey	65.4±0.055	8.5±0.008
71	Enda	0.4±0.021	0.85±0.35
72	Ghiosh	0.55±0.13	8.5±0.177
73	Gokhraj	8.6±0.002	2.0±0.006
74	Govindobhog	6.2±0.002	138.6±0.071
75	Harintore	12.8±0.005	1.4±0.001
76	Hipsa nunia	6.9±0.001	2.9±0.009
77	IR64	5.00±0.003	2.0±0.12
78	Jaldhepa	0.5±0.029	1.27±0.199
79	Jamaisal	7.0±0.002	3.2±0.006
80	Jeerasare	4.4±0.001	2.0±0.006
81	Jetti dhan	4.4±0.004	1.9±0.11
82	Jhapka	5.2±0.003	2.1±0.002
83	Jhulur	6.5±0.002	4.6±0.14
84	Jungli	1.8±0.023	15.4±0.077
85	Kaberi	7.1±0.002	1.8±0.11
86	Kabiraj	0.35±.049	0.85±0.99
87	lalpanati	8.3±0.009	3.2±0.008
88	Laxmansal	6.8±0.004	1.3±0.003
89	Laxmikajal	7.3±0.001	2.5±0.008
90	Magursali	7.0±0.003	2.9±0.007
91	Mala	6.6±0.003	4.2±0.007
92	Malsiara	7.0±0.003	1.7±0.004
93	Minjurijal	7.1±0.003	2.9±0.003
94	Murshi	9.8±0.003	1.5±0.006
95	Nageswari	6.6±0.004	2.2±0.11
96	Nagra	6.3±0.003	2.4±0.003
97	Nav dhan	6.6±00.03	1.7±0.006
98	Nazarius Ekka	6.8±0.000	2.5±0.14
99	Nepali Kalam	12.3±0.001	195.3±0.3
100	Pahal man	6.7±0.001	2.0±0.005
101	Paijam	5.2±0.001	1.7±0.007
102	Pari	4.8±0.006	2.8±0.009
103	Phoolpakri	7.4±0.003	3.4±0.001
104	Puasabasmoti	6.2±0.004	1.9.009
105	Radhunipagal	6.2±0.003	2.0±0.005
106	Rasna	6.9±0.003	1.7±0.007
107	Swetonunia	34.8±0.003	35.2±0.002
108	Tangrey	0.43±0.06	1.27±0.34
109	Thakurbinni	6.4±0.008	3.0±0.001
110	Thulo addey	5.1±0.001	1.4±0.006
111	Timbure	13.8±0.001	4.2±0.001
112	Tulaipanji	0.45±0.04	2.21±0.14

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