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Bioavailability of iron, zinc, protein, carbohydrate and phytic acid in selected rice varieties of northern India and their scopes for molecular breeding

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ABSTRACT

The lack of micronutrients such as Fe and Zn in staple food crops is a widespread nutrition and health problem in developing countries. Mineral bioavailability in rice could be improved by the selection of varieties with high Fe and Zn, as well as low phyticacid (PA) content. Therefore, present study was undertaken to investigate the variations of Fe, Zn and PA content in some popular high yielding local varieties. The study also investigated the effect of morphology of rice kernels on these parameters. For this purpose, we selected six varieties (Sarjoo-52, Madhukar, NDR-359, CSR-13, Swarna Sub-1 and Swarna varieties) The highest amount of phytic acid content was found in Swarna (9.12 g/kg) and lowest in Sarjoo-52 (5.61g/k). The highest iron content was found in Sarjoo-52 (24.15 ppm) and lowest in Swarna (12.48 ppm). The zinc content varied greatly from rice to rice. The highest zinc content was found in Sarjoo-52 (35.50 ppm) and lowest in CSR-13 (25.86 ppm). The highest protein was found in Swarna (8.78 %) and lowest in Madhukar (7.47 %). Highest carbohydrate content was found in Madhukar (74.43 g/100g) and lowest in Swarna (70.07 g/100g).
Keywords: *Phytic acid, Iron, Zinc, Protein, Carbohydrates.*

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INTRODUCTION

Rice (*Oryza sativa L.*) which belongs to the family of grasses, Poaceae, is the main food source for more than two-third of the world's population (Sasaki and Burr, 2000), especially in Southeast Asia (Nwugo and Huerta, 2011; Wang *et al.*, 2011). It contains approximately 6-12% protein, 70-80% carbohydrate, 1.2-2.0% mineral matter and significant content of fats and vitamins (Khush*et al.,* 1997). It supplies 23% of global human/ capita energy and it fulfils 16% of per capita protein requirements. Rice has a relatively low protein contents but could be raised. Rice seed storage proteins are generally divided into water-soluble albumins, salt-soluble globulins, alcohol-soluble prolamines, and alkali or acid soluble glutelins. In rice grains glutelin is the major protein of the starchy endosperm and accounting for 60% to 80% of total seed protein (Kawakatsu *et al*. 2008). It is essential that these values must increase to up lift nutritional quality.

Rice is also the major source of intake of micro-nutrients such as zinc, iron, calcium, selenium and vitamins for Indian people specially those who live in the urban countryside (Doesthale*et al.*, 1979). Iron (Fe) is necessary for oxygen transport in the blood that iron is the central atom of the heme group, a metal complex that binds molecular oxygen (O_2) in the lungs and carries it to all of the other cells in the body (*e.g.,* the muscles) that need oxygen to perform their activities (Casiday and Frey, 2000). Like Iron, the micronutrient zinc is essential for all organisms (Andreini*et al*. 2006; Broadley*et al*.2007). Zn deficiency in humans is widespread and is estimated to affect more than 25 % of the world's population (Maret and Sandstead 2006). For Increasing these content including protein in local high yielding variety for this it is important that know their basic content.

Carbohydrates are the most prevalent source of food energy in the world. They play a major role in human diet, comprising about 40-75% of energy intake. In rice carbohydrate content have been reported approximately 87 % in grain (Devindra and Longvah2011). Our aim is to see its status in local varieties and how it is comparable in terms of other nutrients.

In addition, rice contains phytic acid (PA), the most important antinutritional factor impeding availability of divalent minerals (Jianfen *et al*, 2007). It forms complexes with mineral ions, such as Fe, Zn and Ca, and ultimately affects their bio-availability (Gibson *et al*., 2000; Lucca *et al*., 2001; Mendoza, 2002). Phytic acid is also able to form complexes with proteins and thus impairs digestibility and bioavailability of proteins in seeds (Reddy *et al*., 1982).
It is well established that malnutrition is a health concern, but its solution lies more on food based actions rather than on other preventive or curative approaches. To achieve food-based actions to prevent malnutrition, nutrient contents of foods must be known.

MATERIALS AND METHODS

Collection of germplasm

Six varieties of rice (Sarjoo-52, Madhukar, NDR-359, CSR-13, Swarna Sub-1 and Swarna) were collected from seed technology department, NDUAT, Kumarganj, Faizabad.

Table-1. Characteristics of different donor and recurrent rice cultivars taken for cross

S. N.	Rice Cultivar	Year of Release	Parentage	Yield (q/ha)	Maturity duration (days)	Iron Intensity	Special Feature
1.	Sarjoo-52	1980	Tai chung (Native)1 x Kashi	55-60	130 – 135	Medium in endosperm region, periphery embryonic region	Resistance to bacterial leaf blight
2.	Swarna	1982	Vashistha x Mahsuri	60-65	155	Embryonic region	Good performance under low nitrogen land
3.	CSR-13	Not avilable	CSR-1x Basmati 370 x CSR-5	40-50	130-135	Intense in embryo and periphery	Good performance in alkaline land
4.	NDR 359	1993	BG90-2.4/OB-677	60-65	120-125	Low in embryonic region	Resistance to disease and insect
5.	Madhukar	1969	Local selection of Gonda Distric	40-45	135	Intense in embryonic region	Recommended for flood prone area
6.	Swarna *sub 1*	2009	Swarna/IR49830	60-65	155	Intense in embryonic region, periphery region	Recommended for flood prone area

Mineral Quantification for iron and zinc:

The standard for ICP- OES (inductively coupled plasma Optical emission Spectroscopy) was prepared from stock solution of pb obtained from Perkin Elmer USA. Working solution was prepared from stock as necessary calibration standards of different concentrations 0.03mg/l, 0.06 mg /l, 0.2mg /l 0.3mg/l were prepared from working standard solution. All other reagents and solvent used in this study were of analytical grade 0.1 g of finely crushed sample was used for inductively coupled plasma optical emission spectrometry (ICP-OES) 2ml Nitric acid 1 ml percholoric acid, 1ml hydrogen per oxide 1ml milli-Q water were added and made the final volume 8ml.

Easy and Rapid Detection of Iron in Rice Grain:

In this study, we show how a preliminary determination of grain Fe in rice may be made with reaction to Perls' Prussian blue, a stain for Fe (III). Differential localization of Fe in grain parts was indicated by the intensity of reaction of tissue Fe to the dye

Protein Quantification of extracted protein samples:
The concentration of the extracted protein samples was determined using the Lowry's method (1951) at different concentrations of the sample against the control. Relative concentrations of all samples were calculated using BSA standard chart.

Carbohydrate Estimation:
100 mg of the sample was weighted and put into a boiling tube. It was hydrolysed by keeping it in boiling water bath for 3 hours with 5ml of 2.5 N-HCl and the cooled to room temperature. Further, collect the supernatant and takes 0.5 and 1ml aliquots for analysis

Make up the volume to 100mL and centrifuge. Prepare the standards by taking 0, 0.2, 0.4, 0.6, 0.8 and 1mL of the working standard. '0' serves as blank. Make up the volume to 1mL in all the tubes including the sample tubes by adding distilled water. Then add 4mL of anthrone reagent. Heat for eight minutes in a boiling water bath. Cool rapidly and read the green to dark green color at 630nm. Draw a standard graph by plotting concentration of the standard on the *X*-axis versus absorbance on the *Y*-axis. From the graph calculate the amount of carbohydrate present in the sample tube was calculated as follows

$$\text{Amount of carbohydrate present in 100mg of the sample} = \frac{\text{mg of glucose}}{\text{Volume of test sample}} \times 100$$

RESULTS AND DISCUSSION:
Although rice is one of the most predominant cultivated cereals and has a substantial effect on the nutritional status of the Indian people, research on rice in India has until now been focused mainly on yield, macronutrients such as protein and starch, and sensory quality. In India, micronutrient malnutrition, apart from protein energy malnutrition and, is one of the prime concerns along with an increasing trend of diabetes and urban obesity. With objective to understand the level of micronutrients along protein and carbohydrate present studies was taken up.

All six rice varieties were analyzed for phytic acid and mineral content. Table-1 presents the iron, zinc, protein and carbohydrate content in all rice varieties. The mean value of Iron content in Sarjoo-52 was 24.15 ppm, in Madhukar was 17.38 ppm, in NDR-359 was 15.39 ppm, in CSR-13 was 21.13 ppm, in Swarna Sub-1 was 18.20 ppm and in Swarna was 12.48 ppm. Maximum iron content was recorded in the Sarjoo-52 (24.15 ppm) followed by CSR-13 (21.13 ppm) and minimum iron content was noticed in the swarna (12.48 ppm). Zinc content in Sarjoo-52 was 35.50 ppm, in Madhukar was 31.02 ppm, in NDR-359 was 32.13 ppm, in CSR-13 was 25.86 ppm, in Swarna Sub-1 was 28.71 ppm and in Swarna was 30.86 ppm. Maximum zinc content was recorded in the Sarjoo-52 (35.50 ppm) followed by NDR-359 (32.13 ppm) and minimum zinc content was noticed in the CSR-13 (25.86 ppm). Protein content in Sarjoo-52 was 8.52, in Madhukar was 7.47, in NDR-359 was 7.81, in CSR-13 was 8.17, in Swarna Sub-1 was 7.62 and in Swarna was 8.78. Highest protein in swarna was 8.78 % and lowest in madhukar was 7.47 %. Carbohydrates content in the Sarjoo-52 was 70.52, in Madhukar was 74.43, in NDR-359 was 73.44, in CSR-13 was 73.08, in Swarna Sub-1 was 71.63 and in Swarna was 70.07. Highest carbohydrate in Madhukar was 74.43 g/100g and lowest carbohydrate in swarna was 70.07 g/100g. Phytic acid content in Sarjoo- 52 was 5.61 g/kg, in Madhukar was 6.39 g/kg, in NDR-359 was 8.05 g/kg, in CSR-13 was 6.72 g/kg, in Swarna Sub-1 was 7.40 g/kg, and in Swarna was 9.12 g/kg. Highest phytic acid was in Swarna and was 9.12 g/kg and lowest in Sarjoo-52 and was 9.12 g/kg.

Table:2 Phytic acid, iron and zinc content of the rice varieties

Rice Varieties	Iron content (ppm)	Zinc content (ppm)	Protein content (%)	Carbohydrate content (g/100g)	Phytic acid content (g/kg)
Sarjoo-52	24.15	35.50	8.52	70.52	5.61
Madhukar	17.38	31.02	7.47	74.43	6.39
NDR-359	15.39	32.13	7.81	73.44	8.05
CSR-13	21.13	25.86	8.17	73.08	6.72
Swarna Sub-1	18.20	28.71	7.62	71.63	7.4
Swarna	12.48	30.86	8.78	70.07	9.12

Data are presented as mean ± SEM (standard error of mean)

Perls' Prussian blue within the rice grain and across genotypes that clearly indicates differential localization of Fe in Fig. 1. Fig. (1a) represents iron distribution in Sarjoo-52. Sarjoo-52 localized iron in whole region of seed but high in embryonic region. Fig. 1(b) represents iron distribution in Madhukar.

Madhukar showed Low iron content in embryonic region. Fig. 1(c) represents iron distribution in NDR-359. NDR-359 localized medium iron content embryonic region. Fig. 1(d) show. CSR-13 localized high iron in embryonic region. Fig. 1(e) represents iron distribution in Swarna Sub-1. Swarna sub-1 localized high iron in embryonic region, Fig. 1(f) represents iron distribution in Swarna. This figure showed high iron content in embryonic region of Swarna .

Plant breeding programs in biofortification of staple food crops such as rice and wheat require screening of germplasms, varieties and elite lines having Fe and Zn-dense grains to be used as donor parents (Stangoulis 2010). An increase in concentration of Fe and Zn in grain is a high-priority research area. Exploitation of large genetic variation for Fe and Zn existing in cereal germplasm is an important approach to minimize the extent of Fe and Zn deficiencies in developing world (Brar, Brar*et al*. 2011).

In this study six local rice genotypes were analysed for iron and zinc content among the germplasm screened for Fe and Zn concentration, the sarjoo-52 was found to be high for both iron and zinc. Among the germplasm swarna was found to be low iron and CSR-13 was found to be low zinc.

Figure-1

Fig. 1(a) Sarjoo-52

Fig. 2(b) Madhukar

Fig. 1(c) NDR-359

Fig. 1(d) CSR-13

Fig. 1(e) Swarna Sub-1

Fig. 1(f) Swarna

CONCLUSIONS

From this study we conclude that the levels of Fe and Zn in rice are very diverse. In principle, rice has the potential to provide an adequate intake of Fe and Zn. However, the bio-availability of Fe and Zn is very low because of the presence of phytic acid, even in rice varieties with the lowest PA levels and highest levels of Fe and Zn. In this research Sarjoo-52 had the high iron, high zinc and low phytic acid. Swarna had the low iron and high phytic acid. This information will be useful for breeder in selecting donors for biofortification programme.

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