Assessment of Grain Zinc and Iron Variability in Rice Germplasm using Energy Dispersive X-ray Fluorescence Spectrophotometer (ED - XRF)

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Abstract

Micronutrient and vitamin deficiency diseases are prevalent among the rice eating population and particularly poor people who are unable to afford nutrient rich supplementary food sources. Consequently, biofortification project has been initiated to identify or develop varieties having high iron and zinc. In the present study, rice germplasm was collected from various sources and cultivated at one location. Considering the advantages of grain micronutrient content analysis through non-destructive method, grain iron and zinc content were analyzed with energy dispersive X-ray fluorescence spectrophotometer (ED - XRF). Among the various germplasm studied, promising lines for zinc alone were identified in IRRI and North Eastern Land Races to further enhance zinc through conventional breeding. Effect of sample weight and moisture were also studied and the results indicate that 5 g optimum sample weight is essential for ED - XRF and all the samples should contain similar moisture level to identify the best lines.

Key words: XRF, biofortification, micronutrient, rice, iron and zinc.

Half of the global population consumes rice as staple food and poor people in developing countries solely eat rice and they are rarely accessible to nutrient rich food sources to supplement rice. In fact, rice is consumed in polished form (white rice) and it constitutes starch as chief component followed by proteins, lipids, minerals and negligible levels of vitamins and thus, rice supplies more energy than essential nutrients leading to micronutrient deficiency which is also known as "hidden hunger". The recommended dietary allowance (RDA) of iron and zinc for human population in the age group of 25-50 years are 10-15 and 12-15 mg respectively (FAO/WHO, 2000). In developing countries zinc, iron and vitamin A deficiencies were reported in human population.

Prevalence of micronutrient deficiency diseases among the rice consuming population prompted in initiating biofortification programme

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which has been identified as an efficient means to develop as well as transfer the genetically improved high micronutrient containing rice grains to the poor people who depend on rice for both energy and nutrients. Further, seeds with increased micronutrients also perform better in micronutrient deficient soils by producing longer roots leading to better absorption and yield Graham *et al.* (2001) and improved disease resistance and stress tolerance (Welch, 1999).

Since nutrient quality of rice varies among the varieties, screening of rice germplasm having one or more desired micronutrients at higher concentrations is prerequisite prior proceeding to biofortification. Hence, in this study, rice germplasm was collected from different places and cultivated under the same conditions. Subsequently, grain iron and zinc contents were analyzed and the generated information will be useful for selecting parents/donors for biofortification programme. The effects of grain moisture content and sample weight on grain micronutrient analysis with ED-XRF are also included.

Materials and Methods

Rice germplasm comprising of Landraces collected from North East Land Races (NELR), Aromatic Short Grain (ASG) types from DRR germplasm collection and other germplasm from IRRI were grown at Ramachandrapuram farm of DRR, ICRISAT campus (17.53 ^{0}N latitude and 78.27 ^{0}E longitude, 545 m altitude, with mean temperature of 31.2 ^{0}C and mean annual precipitation of 988.3 mm), Hyderabad, India during *Kharif* 2012 under irrigated rice ecosystem. Before planting, soil pH was in the range of 8.52 to 8.57 and soil iron and zinc in the samples were 2.74 to 3.48 and 3.55 to 3.66 ppm respectively. After harvesting, all the samples were processed using non-iron and zinc husker and miller Ravindra Babu *et al.* (2014).

Micronutrient content can be estimated by both destructive and non-destructive methods. Iron and zinc were estimated using non-destructive ED - XRF machine which is quite useful to select promising lines from large number of samples and further these values may be confirmed with destructive methods. Brown or polished rice grain can be used as sample in ED - XRF. Rice varies in grain size and moisture content and in addition, sample amount is generally less in genetic studies. Therefore, the effects of moisture and sample size on micronutrient estimation were also studied by taking short, medium and long grain rice samples and these parameters were considered while screening the germplasm.

Optimization of sample weight

Thirty rice genotypes of different grain types - short, medium and long grain samples were separately analyzed for grain iron and zinc content in triplicate using ED -XRF with manufacturer recommendations except for sample weight. As the objective of the present study is to standardize sample weight, for each genotype, samples of one to ten grams were made and used for analysis.

Effect of moisture

Grain iron and zinc content were analyzed with ED-XRF and volume of the fixed amount of rice grain was followed using 10 ml measuring jar before and after incubation in hot air oven.

Micronutrient estimation

Grain iron and zinc contents were estimated with ED - XRF in brown and polished rice samples by taking the optimum weight identified during the above experiment. Based on these values, separate equations were also developed to predict the iron and zinc content in the polished samples using values of the brown rice.

Results and Discussion

Sample weight optimization

Manufacturer recommended filling rice sample in ED - XRF sample cups to a mark which is roughly 3/4th volume of the total space and around 20 g of sample is required to fill up to this mark. As per the results obtained (Figure 1), a minimum of 3 and 5 g sample is required for iron and zinc respectively in all the samples tested and therefore, 5 g sample is necessary for simultaneous estimation of both iron and zinc. Nicholas *et al.* (2012) reported 4 g sample mass is required for both rice and pearl millet. The determined minimum sample weight will enhance the life of specially designed sample processing non-contaminating machines as well as significantly reduces the sample processing time.

Effect of moisture

In general, dried samples exhibited more iron and zinc values (Table 1) than samples with moisture. This was due to decrease in the size (Figure-2) of individual grains and thus more number of dried grains was distributed in the inner space of the sample cup leading to increase in the values. Therefore, it is important to store all the samples in uniform conditions to identify the best lines while screening with ED - XRF. Improper moisture content increases grain breakage which inturn affect micronutrient analysis with ED – XRF.

Grain iron and zinc content

Iron content is less in soft rices whereas the range and means of iron are similar in the other collections (Table 2). Highest zinc content was found in IRRI collection followed by NELR germplasm while it was similar in soft and ASG collections.

Among the analyzed, ASG group is smaller in size with more grain surface area, however, the present results doesnot indicate any advantage due to variation in surface area. Range of both iron and zinc is well within the reported Graham *et al.* (1999) range of iron (7.5 to 24.4 ppm) and zinc (13.5 to 58.4 ppm).

Correlation between iron and zinc

In this study, significant positive correlation between iron and zinc was observed in IRRI (Table 3) and soft rice whereas negligible positive association was observed in ASG and NELR germplasm. Correlation analysis between the estimated and predicted values indicates that equation for zinc alone is useful to predict the values in polished rice from the estimated values of brown rice samples. Graham *et al.* (1999) reported positive correlation between iron and zinc in rice, wheat and beans and Stangoulis *et al.* (2007) reported significant positive correlation in double haploid rice population indicating co-segregation of concerned factors. In contrast, Vijay *et al.* (2009) could not find significant correlation in recombinant inbred line populations of wheat except Xgwm473-Xbarc29 and also opined that correlation might be possible with some loci.

Effect of polishing

After polishing, large variation in iron and zinc levels was observed among the varieties. Compared with zinc (~20 to 40%), loss of iron (~60 to 80%) is nearly twice after 10% polishing across the grain shapes. Gregorio (2002) also observed more loss of iron than zinc during polishing. This could be due to partial or complete loss of both embryo and aleurone regions during polishing, more iron is distributed in the embryo followed by aleurone layer and endosperm (Gregorio, 2002), variation in the thickness of the aleurone layer or embryo size or both, etc. In addition to the loss of iron during polishing, another 10 per cent is lost during washing before cooking and ultimately around 20 per cent remains in the cooked food. Whereas, Sanjeeva Rao *et al*. (2014) reported that maximum loss of zinc during polishing is around 40 % and loss during washing before cooking is almost negligible and this indicates that around 60 per cent zinc remains in the cooked food.

Apart from genotypic differences, grain micronutrient content is also dependent on location (Ravindra Babu *et al.,* 2014). Considering this, losses during polishing as well as washing and international threshold values of 7 ppm for iron and 24 ppm for zinc, varieties having ≥ 30.0 ppm zinc / iron in brown rice can be considered as potential donors for breeding programme for enhancing zinc/iron. Promising high zinc containing lines are more in IRRI germplasm (Table 4) than NELR (Table 5). Using the brown and polished values, regression equations were developed separately for iron and zinc to predict the values in polished samples from brown rice values.

However, world average per capita rice consumption is 65.0 Kg, European Union-27 is least with 5.7 Kg, Cambodia is highest with 292 Kg and India with 76.7 Kg (Eric and Eddie, 2012). Hence, in India the average daily intake of rice is around 220 g and polished rice having 45.5 to 68.2 ppm (mg Kg^{-1}) iron and 54.5 to 68.2 ppm zinc can only meet the RDA (FAO/WHO, 2000) without considering their assimilation (bioavailability) from the digested food in the alimentary tract. These values are much higher than the present international threshold values considered for grain iron and zinc. Bioavailability is a complex phenomenon governed by various dietary components. Anti-nutrient like phytic acid binds to these ions and makes them unavailable for absorption and in contrary, citric acid being a pro-nutrient promotes iron absorption. The composition and availability of these components varies among the varieties and thus, a part of the available iron and zinc in the cooked food enter the blood stream. Hence, bioavailability studies on high zinc lines are prerequisite to plan for further enhancement of zinc in rice varieties.

Conclusion

Optimum sample weight of 5 g is required to analyze grain micronutrient content in ED - XRF. This is the first report which indicates that ED - XRF underestimates grain micronutrient content in samples with grain moisture content and therefore, moisture should be either uniform across the samples or it should be removed before analysis while selecting the best lines among the germplasm. ED - XRF is quite useful to screen large number of samples and to select promising lines. Variability observed in the present study offers scope for further enhancement of zinc through conventional breeding whereas transgenic approach appears inevitable for iron.

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Table 1: Effect of grain moisture on grain micronutrient content (in ppm) with ED - XRF

S. No.	Before oven treatment (A)		After oven treatment (B)		Difference (A-B)	
	Fe	Zn	Fe	Zn	Fe	Zn
1	13.5	16.7	12.7	18.7	0.8	-2
2	19.4	23.8	15.4	26.6	4.0	-2.8
3	18.5	18.1	19	19.6	-0.5	-1.5
4	12	25.8	11	25.4	1.0	0.4
5	39.6	36	42.5	43.6	-2.9	-7.6
6	48	22.7	50.1	23.5	-2.1	-0.8
7	19.8	21.8	16.9	25	2.9	-3.2
8	15.4	26.4	15.6	26.4	-0.2	0
9	12.8	24.2	13.7	24.5	-0.9	-0.3
10	19.2	38.6	18.9	42	0.3	-3.4
11	20.3	28.9	20.4	30.9	-0.1	-2
12	17.8	44.4	19.8	40.5	-2.0	3.9
13	19.4	37.5	18.1	39.7	1.3	-2.2
14	18.9	46.1	18.9	42.8	0	3.3
15	27.1	35.2	27.9	36	-0.8	-0.8
16	22.6	48	20.7	49.1	1.9	-1.1
17	30.1	36.6	31.9	36.3	-1.8	0.3
18	56.9	29.6	60.1	33.1	-3.2	-3.5
19	38.3	32.8	35.5	36.2	2.8	-3.4

Table 2: Micronutrient contents in the brown rice of some germplasm collections

Germplasm (number)	Range of micronutrient in ppm (mean ± 1.0)		No. of potential donors/entries	
	Iron	Zinc	Iron	Zinc
NELR (230)	7.4-22.7 (10.67)	16.5-33.0 (22.67)	Nil	06
Soft rices (30)	2.7-6.9 (3.98)	17.3-26.9 (21.44)	Nil	Nil
ASG (236)	6.1-14.2 (10.01)	12.7-27.4 (19.12)	Nil	Nil
IRRI (306)	7.2-16.6 (10.29)	18.6-50.0 (31.74)	Nil	164

Table 3: Correlation between iron and zinc in IRRI rice germplasm

	Iron brown	Iron Polished	Zinc brown	Zinc Polished
Iron brown	1			
Iron Polished	**0.25***	1		
Zinc brown	**0.46***	0.12	1	
Zinc Polished	0.22	**0.45***	**0.43***	1

*Significant at 95.0 %

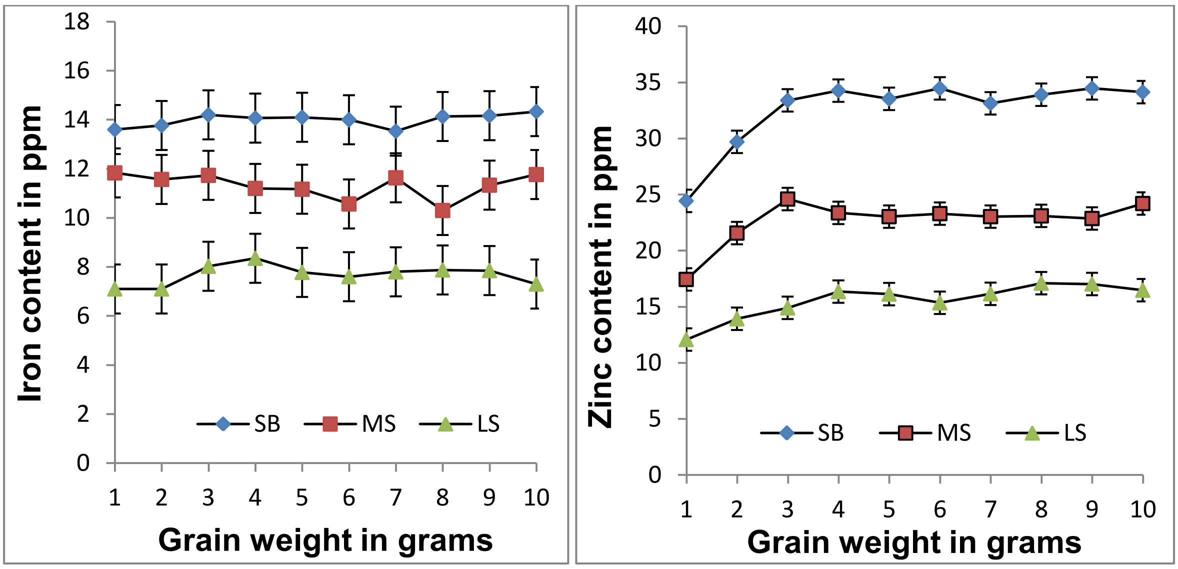

Figure 1: Relationship between iron and zinc content with sample weight. SB - short bold, MS - medium slender and LS - long slender

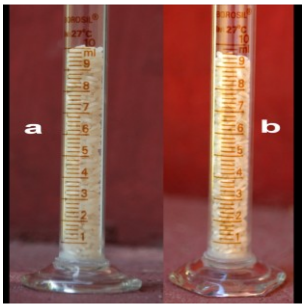

Figure 2: **Decrease (0.5 mm) in the height of rice sample a) before and b) after the removal of grain moisture content**

Table 4: List of promising rice varieties from IRRI germplasm for zinc (in ppm)

Variety designation	Zinc (ppm)	Variety designation	Zinc (ppm)	Variety designation	Zinc (ppm)	Variety designation	Zinc (ppm)
IR91158-133-2-1-2-3	50.0	IR 91184-219-3-3	36.9	IR 92937-237-3-3	34.5	IR 92963-85-1-3	32.4
IR91159-65-2-2-3-3	48.2	IR 91159-11-3-3-2-3	36.8	IR 92943-58-3-3	34.4	IR 91949-111-1-1-3	32.3
IR 91916-3-3-1-3	47.5	IR 91167-157-1-1-3-3	36.7	IR 92966-95-1-3	34.2	IR 92960-87-3-3	32.3
IR 91158-85-3-2-3-3	45.2	IR 92977-170-1-3	36.7	IR 91167-99-1-1-1-3	34.1	IR 91909-53-3-1-3	32.2
IR 90210-123-2-1-1-3	45.1	IR 92937-163-2-3	36.6	IR 91169-14-2-2-2-3	34.1	IR 91949-14-3-3-3	32.2
IR 92937-119-3-3	44.2	IR 91173-81-2-3-1-3	36.5	IR 92937-168-2-6	34.0	IR 92947-140-1-3	32.2
IR 92966-86-1-3	43.8	IR 92971-70-3-3	36.4	IR 92947-26-1-3	34.0	IR 92972-9-2-3	32.2
IR 90210-106-2-1-2-3	42.8	IR 91158-16-1-1-2-3	36.3	IR 90241-72-1-1-3-3	33.8	IR 91166-113-2-3-3-3	32.1
IR 91175-27-1-3-1-3	42.5	IR 91917-6-1-1-3	36.1	IR 92966-30-3-3	33.8	IR 92938-73-1-3	32
IR 92945-68-2-3	42.0	IR 91957-44-2-2-3	36.1	IR 92969-127-2-3	33.8	IR 91173-35-2-3-1-3	31.9
IR 91173-104-1-1-3-3	41.7	IR 92957-147-1-3	36.0	IR 91949-87-1-3-3	33.7	IR 91189-10-1-1-1-3	31.9
IR 91175-50-1-2-1-3	41.6	IR 90210-199-2-1-1-3	35.9	IR 92937-189-1-3	33.7	IR 91896-83-3-1-3	31.9
IR 92956-97-2-3	41.5	IR 92965-82-2-3	35.9	IR 92966-106-2-6	33.6	IR 92969-40-3-3	31.8
IR 91188-63-3-3-1-3	41.2	IR 91175-65-1-2-2-3	35.8	IR 91949-78-2-3-3	33.5	IR 92978-131-2-3	31.8
IR 92957-99-2-3	41.2	IR 91964-143-2-2-3	35.8	IR 92960-75-1-3	33.5	IR 92963-110-2-3	31.7
IR 92967-101-1-3	41.2	IR 91937-213-2-3	35.8	IR 92963-23-1-6	33.5	IR 91189-39-1-2-1-3	31.6
IR 92934-39-1-6	40.7	IR 91184-162-1-3	35.8	IR 91167-133-1-1-2-3	33.4	IR 91922-9-3-1-3	31.6
IR 92937-235-2-3	40.7	IR 91967-25-1-1-3	35.7	IR 92953-60-2-3	33.4	IR 92934-79-1-3	31.6
IR 92947-88-2-3	40.4	IR 92937-166-1-3	35.7	IR 92969-229-1-3	33.4	IR 91949-21-1-1-3	31.5
IR 92937-168-2-3	39.6	IR 92935-29-3-3	35.6	IR 90240-31-2-2-2-3	33.3	IR 91967-30-1-1-3	31.5
IR 91167-106-2-1-3-3	39.3	IR 92937-178-2-3	35.6	IR 91906-4-3-1-3	33.3	IR 90241-197-2-2-1-3	31.4
IR 91171-104-1-2-1-3	39.0	IR 92963-19-2-3	35.5	IR 92947-43-1-3	33.3	IR 9191182-3-2-3	31.4
IR 91182-36-3-3-3-3	38.9	IR 91167-31-3-1-3-3	35.3	IR 91167-46-2-1-2-3	33.2	IR 91949-52-3-1-3	31.4
IR 92937-215-3-3	38.8	IR 91949-48-3-1-8	35.3	IR 91967-88-1-1-3	33.2	IR 92937-116-2-3	31.4
IR 92953-3-2-3	38.7	IR 91175-61-1-2-2-3	35.2	IR 92972-85-3-3	33.2	IR 91184-129-3-6	31.4
IR 91184-122-3-3	38.6	IR 90241-7-1-1-2-3	35.1	IR 92978-5-1-3	33.2	IR 92968-88-1-3	31.4
IR 92966-106-2-3	38.5	IR 92937-205-1-3	35.1	IR 91917-12-1-1-3	33.1	IR 92971-109-1-3	31.4
IR 91149-26-1-3	38.5	IR 92969-162-2-3	35.1	IR 91953-141-2-1-3	33.0	IR 90240-81-3-3-1-3	31.3
IR 91159-99-1-3-1-3	38.4	IR 92937-226-1-3	35.0	IR 92970-111-1-3	33.0	IR 92942-57-3-3	31.2
IR 92937-85-2-3	38.4	IR 92969-227-3-3	35.0	IR 92969-61-2-3	32.9	IR 91184-3-3-3-2-3	31.1
IR 91149-23-3-3	38.2	IR 92963-49-1-3	34.9	IR 91186-39-3-3-2-3	32.8	IR 92969-201-1-3	31.1
IR 92978-79-2-3	37.7	IR 91913-115-3-1-3	34.8	IR 91967-64-1-1-3	32.8	IR 92947-12-1-3	31
IR 92956-129-1-3	37.6	IR 92956-146-3-3	34.8	IR 92934-39-1-3	32.8	IR 92957-123-1-3	31
IR 91180-80-3-2-1-3	37.5	IR 92957-32-3-3	34.8	IR 91172-75-1-2-3-3	32.7	IR 92972-170-1-3	31
IR 92952-144-1-3	37.5	IR 91899-145-1-2-3	34.7	IR 92977-145-1-3	32.6	IR 92978-74-1-3	31
IR 91181-96-1-1-1-3	37.4	IR 91916-35-2-1-3	34.7	IR 90241-2-1-2-1-3	32.5	IR 92978-90-2-3	30.9
IR 91949-97-1-2-3	37.4	IR 91184-159-2-3	34.7	IR 92965-23-2-3	32.5	IR 91962-54-2-1-3	30.8
IR 91166-195-2-1-2-3	37.2	IR 91184-198-1-6	34.6	IR 92969-24-2-3	32.5	IR 91964-172-3-2-3	30.8
IR 91171-103-1-1-1-3	37.0	IR 90210-123-2-1-1-6	34.5	IR 91184-32-2-3-2-3	32.4	IR 91906-99-3-1-3	30.7
IR 92947-57-2-3	36.9	IR 91171-66-3-2-1-3	34.5	IR 92957-88-1-3	32.4	IR 91184-198-1-3	30.7
IR 92972-92-1-3	30.7	IR 92937-56-2-3	30.6	IR 91184-158-2-3	30.6	IR 92958-39-3-3	30.5

Table 5: List of promising rice varieties from NELR germplasm for zinc

Entry name	Zinc (ppm)
VPB/GP 61	33.0
VPB/GP 73	32.5
VPB/GP 229	31.6
VPB/GP-114	29.6
VPB/GP 204	29.5
VPB/GP 50	29.3
VPB/GP 105	29.1