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Evaluation of Iron and Zinc Content in Rice (*Oryza sativa* L.) Germplasm Grown under Aerobic Condition

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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 affecting nearly two billion people worldwide in developing countries. The brown / red rice genotypes have high grain iron and zinc content and attempt was made to study the association between these mineral content with grain yield. Bio-fortification is one of the sustainable approaches, for improving the Fe and Zn content and their bioavailability in rice grain. 50 accessions of rice genotypes were analyzed for Fe and Zn content. These germplasm were tested to estimate phenotypic and genotypic association among grain iron, zinc, yield attributes and grain yield. Iron content in brown rice ranged from 10.6 ppm to 21.43ppm and zinc from 20.60 ppm to 36.15 ppm. Zn content and grain elongation (-0.25) was significantly correlated. It was observed that grain yield was positively correlated with biomass and days to maturity. A positive correlation in Zinc content in brown and polished rice was observed. While there is no correlation between brown rice grain iron and zinc content with grain yield. The germplasms had the highest Fe and Zn are a good source for Bio-fortification of popular rice cultivars using conventional, acceptable, non-transgenic methods.

Keywords: Bio fortification, correlation, grain iron, grain zinc

Rice is a staple food for millions of people and having great importance in food and nutritional security. Rice is the second most widely consumed in the world next to wheat. From poorest to richest people in this world consume rice in one or other form. 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. Bio-fortification is a genetic approach which aims at biological and genetic enrichment of food stuffs with vital nutrients (vitamins, minerals and proteins). Ideally, once rice is bio-fortified with vital nutrients, the farmer can grow indefinitely without any additional input to produce nutrient packed rice grains in a sustainable way. This is also the only feasible way of reaching the malnourished population in India.

In addition to agronomical management, selecting genotypes with high efficiency of Fe & Zn accumulation in the endosperm and their bioavailability from existing germplasm collection may be an efficient and reliable way to deliver Fe nutrition benefits to farmers and local population. Germplasm has been screened for high Fe and Zn in many crops including rice. Cheng *et al.,* screened 113 rice landraces from 12 provinces of China. It is reported that japonica rice had higher Fe than that of indica rice variety. 11,400 rice samples of brown and milled rice were evaluated for Fe and Zn during 2006-2008 by Martinez *et al*. (2010), It is found that brown rice had 10-11 ppm Fe and 20-25 ppm Zn while milled rice had 2-3 ppm Fe and 16-17 ppm Zn. Banerjee *et al*. (2010), Screened 46 rice lines including cultivated and wild accessions and showed that wild rice accessions have higher grain Fe and Zn content.

Micronutrient-dense cultivars can be selected from within existing germplasm, or can be generated *de novo* through genetic modification. Plant breeders involved in breeding staple food crops with more Fe, Zn need to identify donor parents carrying the target traits. Perl's Prussian blue and DTZ staining method are standardized for Fe and Zn estimation, respectively, to conduct the initial screening of genotypes. Although these methods are simple and inexpensive they are qualitative instead of quantitative. Accurate estimation of Fe and Zn content is normally achieved through inductively coupled plasma-optical emission spectrophotometry (ICPOES) or atomic absorption spectroscopy (AAS). Around 75 per cent of total grain Zn was reported to be present in the endosperm of brown rice, while Takahashi *et al.* (2009) revealed that Zn is most abundant in the embryo and in the aleurone layer using X-ray fluorescence imaging. Fe has been localized in the aleurone layer and in the embryo using histo-chemical techniques and in the endosperm by X-ray fluorescence imaging.

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 and Zinc 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; long grains tends to increase the content of Fe and Mn; short grains tend to increase content of Zn and P while selection of single plants with bigger grain weight tends to increase the content of P. The objectives of the present study were to (i) Screen rice germplasm for iron and zinc content in brown rice and white rice. (ii) Analyze the correlation between Fe and Zn content and Grain yield, if any and (iii) Identify lines with high Fe and Zn.

Material and Methods

Samples : 50 accessions including ten super elite lines are used for the present study. All accessions were grown during *Kharif* season 2015-16 at Aerobic Rice Research laboratory, Department of Plant Biotechnology, University of Agricultural Sciences, GKVK Bengaluru, India located at 120 58' North; longitude770 East and Altitude of 930 meters above mean sea level. Seeds harvested from these lines were used for further analysis.

Iron and zinc content analysis : Fe and Zn content was estimated in the brown and white rice collected from the genotypes grown in field. Grains of individual lines were harvested manually and hand threshed to avoid any contamination. Seeds from all varieties were dehusked gently using a palm dehuskerand cleaned using dilute hydrochloric acid and with double distilled water to remove any surface contaminants and dried in hot air oven at 70 ^{0}C for 72 hours. Brown rice was polished to 5 per cent using non-ferrous miller (Mini lab rice polisher model K-710, Krishi International) rice polisher. Brown and polished rice of 50 accessions were subjected to X-ray fluorescence (XRF) (OXFORD instrument X-supreme 8000), Nicholas *et al*. (2012) at MSSRF, Chennai. Content was expressed in parts per million (ppm). A minimum of two replications from each of the accessions were analyzed for the two micro-nutrients and their average was considered (Fig. 1).

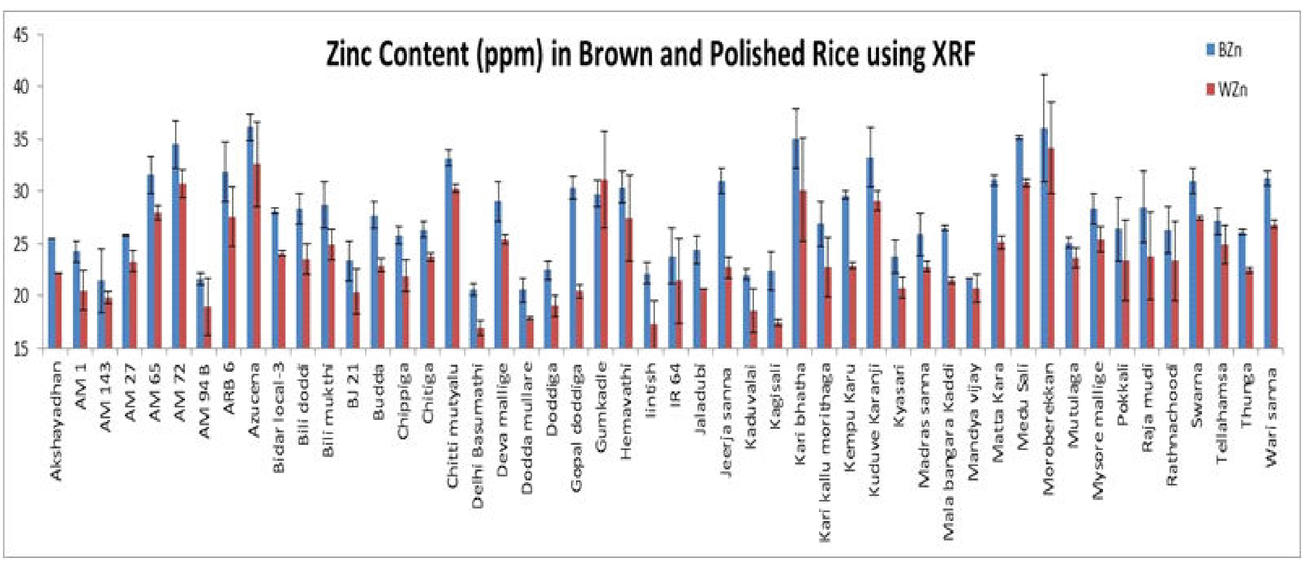

Fig. 1: Mean values of zinc content in brown and polished rice

Statistical analyses : Mean values of five plants used for recording the observations were computed for different plant trait for each of the genotypes. The phenotypic data for all the accessions for each trait were subjected to statistical analyses using 'INDOSTAT 9.1' computer programmer. The level of significance was tested at 5 and 1per cent using F-test.

The association of yield and its component traits reflects the nature and degree of relationship between them. The correlation analysis helps in examining the possibility of improving yield through indirect selection of its component traits which are highly correlated.

Results and Discussion

Brown rice fe content ranged from 10.6 ppm to 21.43ppm and Zn content ranged from 20.60 ppm to 36.15ppm. Both Fe and Zn were high in germplasm accessions. The mean value of brown Fe content in all accessions was 15.29±2.14 ppm ranging from 10.64 ppm in Kaduvalai to 21.46ppm in Gopaldoddiga. The mean value of Zn was 27.59±1.70 ppm ranging from 20.66ppm in Doddamullare to 36.15 ppm in Azucena. Mean values of Zn and Fe content in Brown and polished rice (Fig. 1 and 2).

In super elite genotypes the highest Fe and Zn content for brown rice was 13.02 ppm for Fe and 36.15 ppm for Zn, polished rice was 10.11 ppm for Fe and 32.60 ppm for Zn. The brown rice Fe content in super elite genotypes ranged from 13.01ppm in Am 65 to 9.85 ppm in AM143 and Zn content ranged from 21.41 ppm in AM143 to 36.15ppm in Azucena. The Fe content in polished rice in super elite genotypes ranged from 4.61 ppmin AM27 to 10.15 ppm in AM72 and Zn content ranged from 19.94ppm in AM143 to 32.63ppm in Azucena. Among 10 super elite lines AM65, AM72, ARB6, Azucena had highest brown zinc (>30ppm) and AM27, AM65, AM72, ARB6, Azucena had highest polished Zinc (>20ppm). AM1, AM143, AM72, Azucena had highest brown iron (>15ppm) and AM143, AM72, AM94B, ARB6 had highest polished iron (>8ppm) content. Out of 50 accessions only 13 lines had >30 ppm brown Zn and only 7 lines had >30ppm in polishedZn rice. Only 23 lines had >15 ppm brown Fe and only 14 lines had >10ppm in polished rice Fe content (Figu. 1 and 2).

Different genetic parameters were studied *viz.,* phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), broad sense heritability (h^2) and genetic advance as percent mean (GAM) for all traits grown in aerobic condition are depicted in Table I.

Grain yield depicted high GCV, PCV, and h^2 (43.64, 44.63 and 95.60) with moderate GAM (12.98). Biomass depicted high GCV, PCV, and h^2 (24.92, 34.50 and 90.01) with moderate GAM (17.72). Zinc content in Brown rice depicted moderate GCV and PCV (14.24 and 16.6) with high h^2 (72.80) and low GAM

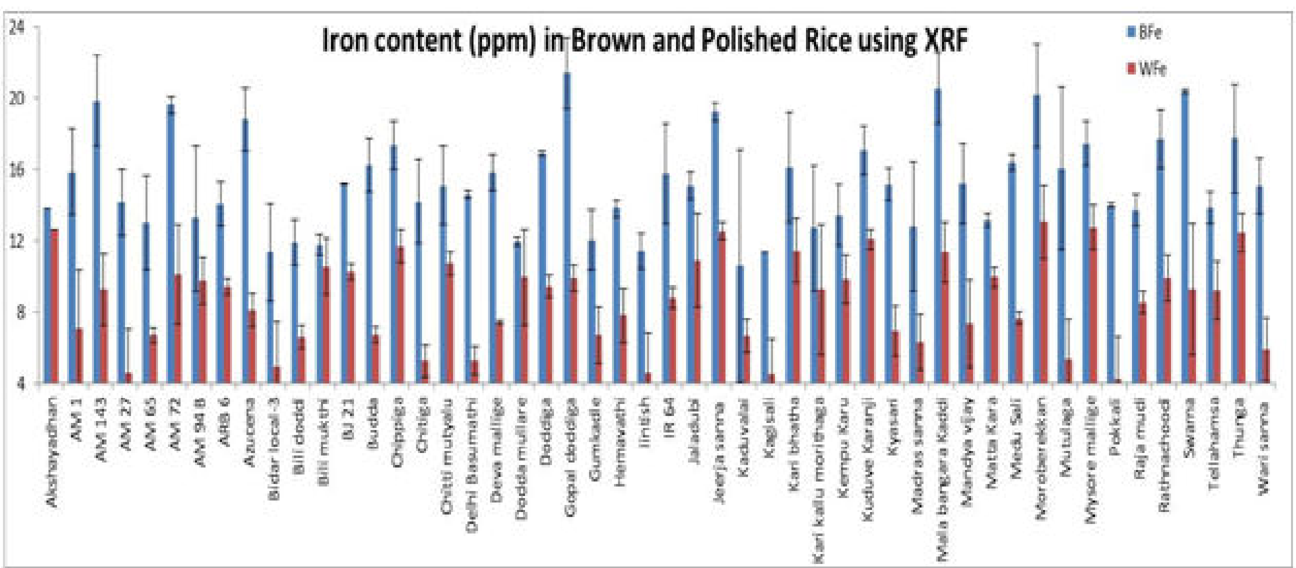

Fig. 2: Mean values of Iron content in Brown and Polished rice.

TABLE I

Descriptive statistics and genetic parameters among grain yield and micro nutrient content of various rice germplasm.

Trait	Min	Genotype	Max	Genotype	Mean ± SE	GCV%	PCV%	h^2 %	GAM %
PH	49.90	Swarna	156.10	Azucena	107.78 ± 2.31	22.08	22.29	98.25	45.06
NOPT	6.50	AM1	24.70	Swarna	13.37 ± 2.19	26.02	34.80	55.91	40.07
PL	11.20	Swarna	29.47	Azucena	20.59 ± 0.96	11.75	13.47	76.12	21.11
GY	0.51	Raja Mudi	19.61	Delhi Basumathi	11.53 ± 0.77	43.65	44.64	95.60	12.99
BIO	16.98	Matta Kara	68.93	Jaladubi	37.29 ± 6.30	24.92	34.50	52.27	17.72
DM	114.00	Mysore Mallige	149.00	Deva mallige	132.57 ± 1.77	5.67	5.97	90.01	11.08
BZn	20.60	Dodda mullare	36.15	Azucena	27.59 ± 1.70	14.24	16.69	72.80	8.85
WZn	16.95	Delhi Basumathi	34.15	Moroberekan	23.9 ± 2.22	14.90	19.85	56.33	7.06
BFe	10.60	Kaduvalai	21.40	Gopal doddiga	15.29 ± 2.14	11.41	22.80	25.12	11.77
Wfe	4.25	Pokkali	13.05	Moroberekkan	8.64 ± 1.73	21.08	35.18	35.90	26.02

PH: Plant Height (cms); NOPT: Number of Productive Tillers; PL: Panicle Length (cms); GY: Grain yield per plant (g), BIO: Biomass (g); DM: Days to Maturity, BZn: Zinc content in Brown Rice (ppm); WZn: Zinc content in Polished rice (ppm), BFe: Iron content in Brown rice (ppm); WFe: Iron content in Polished Rice (ppm); GCV: Genotypic Co-efficient Variance; PCV: Phenotypic Co-efficient Variance; h^2: Heritability at broad sense; GAM: Genetic Advance as per cent of mean

TABLE II

Estimates of phenotypic correlation coefficients for 10 characters in selected rice germplasm.

	NOPT	PL	GY	BIO	DM	BZn	WZn	BFe	WFe
PH	-0.33 ***	0.63 ***	-0.08	0.38 ***	0.26 **	0.15	0.14	-0.03	-0.10
NOPT	1.00	-0.18	0.30	0.31 **	-0.14	-0.07	-0.03	-0.07	0.04
PL		1.00	0.23	0.28 **	0.29 **	0.03	0.01	0.01	-0.11
GY			1.00	0.34 ***	0.21 *	-0.30 ***	-0.27 **	0.12	0.00
BIO				1.00	0.12	0.30 **	0.26 **	0.35 ***	1.00 ***
DM					1.00	-0.08	-0.05	-0.01	-0.20 *
BZn						1.00	0.86 ***	0.33 ***	0.30
WZn							1.00	0.28 **	0.26 **
BFe								1.00	0.35 ***
WFe									1.00

*Significant at .05%; **Significant at .01%; ***Significant at .001%; PH: Plant Height (cms); NOPT: Number of Productive Tillers; PL: Panicle Length (cms); GY: Grain yield per plant (g); BIO: Biomass (g); DM: Days to Maturity; BZn: Zinc content in Brown Rice (ppm); WZn: Zinc content in Polished rice (ppm); BFe: Iron content in Brown rice (ppm); WFe: Iron content in Polished Rice (ppm)

(8.85). Zinc in polished rice recorded moderate GCV, PCV, and h^2 (14.90, 198.85 and 56.33) with low GAM (7.05). Iron in Brown rice depicted moderate GCV and PCV (11.41 and 22.80) with low h^2 (25.12) and moderate GAM (11.77). Iron content in Polished Rice showed high GCV and PCV (21.09 and 35.18) with moderate h^2 (35.90) and high GAM (26.01) respectively (Table 1).

Association between grain yield and its component characters

The results of the correlation analysis are presented in Table II, respectively, for phenotype in selected germplasm. Highly significant positive correlation was observed for grain yield per plant with bio mass (0.34) followed by days to maturity (0.21). Grain yield per plant displayed significant negative effect with brown zinc (-0.30) and polished zinc content (-0.27) at phenotypic level (Table II).

These results are in corroboration with Shashidhar *et al*. (2005), Girish *et al*. (2006), Monalisa *et al*. (2006) for number of productive tillers per plant; Choudhury and Das (1998), Yogameenakshi *et al*. (2004), Shashidhar *et al*. (2005), Monalisa *et al*. (2006), Gholipoor *et al*. (1998), for test-weight.However, grain yield with plant height (-0.08), panicle length (0.23), grain brown zinc (0.12) and polished iron content (0.01) had non-significant correlation. The yield contributing traits like, biomass, days to maturity are useful in increasing the grain yield.

Association between micronutrient content with grain yield and yield attributing traits

There is a positive correlation between grain brown iron and zinc content and results are in accordance with Stangoulis *et al*. (2007), Jeom Ho *et al*. (2008) and Patil (2008). Zinc content in polished rice displayed significant negative correlation with grain yield per plant (-0.27) and significant positive correlation with zinc content in brown rice (0.86) at phenotypic level. There is no correlation between grain mineral content with grain yield, hence we can take up separate breeding producer to enhancement of grain mineral content and grain yield. Further, Iron content in Brown rice shows significant positive correlation with zinc content in brown rice (0.33) and polished rice (0.28). In addition to this iron content in polished rice showed significant positive correlation with zinc content in polished rice (0.26) and iron content in brown rice (0.35) (Table II).

There is no much direct effect of grain yield; simultaneous selection has to be made to get higher yield and higher grain iron and zinc content. In plant breeding, it is very difficult to have complete knowledge of all component traits of yield. The residual effect determines how best the casual factors account for the variability of the dependent factor, the yield in this case. Its estimate being 0.7545, the traits (Plant height, number of productive tillers, panicle length, grain yield, biomass, days to maturity Zinc and Iron content in brown and polished rice) explains about 24.30 per cent of the variability in the yield. The traits included in the study account fully for the variation in brown or polished zinc and iron content.

It was found that Medusali, Karibhatta, Moroberekan, Azucena and AM 72 had the highest Zinc content in Brown and Polished rice. Malbangarakaddi, Moroberekan, Gopal Doddiga, AM 143 and Swarna also had more than 20 ppm of Iron in Brown Rice. The germplasms are the good source of high Fe and Zn in Brown and Polished rice.

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