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Characterization of Rice Genotypes for Grain Fe, Zn using Energy Dispersive X-Ray Fluorescence Spectrophotometer (ED - XRF)

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Abstract

Iron and zinc are the most essential micronutrients required for growth and development of all organisms including human beings. The deficiency disorders of Fe and Zn constitute a major health concern around the globe. This is majorly affecting rural populations residing in developing countries with minimum purchasing power and access to a diverse diet. Biofortification; the enrichment of staple food crops with bioavailable micronutrients or vitamins in the edible grains, provides a potential sustainable solution towards such issues. Utilization of rice as a platform for the delivery of Fe and Zn through bio fortification could greatly impact the livelihood of people dependent on rice-based agro-food systems globally. In this study, 100 rice genotypes from different mapping populations were analyzed for grain iron and zinc through the non-destructive method, energy-dispersive X-ray fluorescence spectrophotometry for 3 seasons. Considerable variation was observed in the micronutrient density among the germplasm assessed. Iron concentration varied from 1.6 to 15.2 ppm whereas zinc concentration ranged from 6.2 to 33.2 ppm in brown rice samples and 10 genotypes N22, 148S, 61-1B, 70S, 196M, 24K, 105B, 88B, 132Z and 185M with high grain iron and zinc concentration were identified, which have the potential to be used in rice improvement.

Key words: Bio fortification, ED-XRF, micronutrient, Iron, Zinc

Introduction

Rice is one of the most important staple foods that supports the livelihood for nearly half of the world's population and more than 3.5 billion people depends on rice for more than 20% of their daily calories (Global Rice Science Partnership (GRiSP), 2013). An estimated 486.96 million metric tons of rice was consumed globally during 2018/2019 and a further increase is predicted (Rice consumption worldwide, Statista 2019). More than 90% of the rice was produced and consumed by the Asian countries (USDA 2019). India is the second largest producer of rice with an annual production of 116.5 million metric tons for 2018-2019. Globally, Fe and Zn deficiency is widespread particularly in rural and developing regions where people consume cereal-based diets and have less opportunities for diet diversification; however, its presence is also detected in prosperous areas where diets are unbalanced, contributing to 'hidden hunger' (Roohani et. al., 2013). It is estimated that micronutrient deficiencies affect approximately 1.6 billion people globally, particularly children, pregnant and lactating women, in low and middle-income countries. Because of its high consumption, rice constitutes an ideal vehicle for delivering micronutrients such as Fe and Zn, at a large scale. And it is necessary to improve both Fe and Zn concentrations and their bioavailability in rice grain to overcome these deficiency disorders of the populations dependent on rice as their staple food. Hence bio fortification is considered as the most sustainable and cost effective approach to develop micronutrient dense cereals crops.

The pre-requisite for initiating a programme to develop micronutrient rich genotypes, is to screen the available germplasm and to identify the source of the genetic variation for the target trait which can be used further for developing crosses, detecting genetic variation, improved lines, molecular markers and to understand the basic mechanisms of micronutrient enhancement. Selecting genotypes with high efficiency of Fe and 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 (Prom-u-thai et.al, 2006). Cheng et. al., (2009) screened 113 rice landraces from 12 provinces of China. They reported that japonica rice had higher Fe than that of indica rice varieties in brown rice samples. 11,400 rice samples of brown and milled rice were evaluated for Fe and Zn during 2006-2008 by Martinez et. al., (2010). They 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. Anuradha et.al, (2012) screened 126 rice lines including cultivars and wild accessions and showed that wild rice accessions have higher grain Fe and Zn concentration in brown rice samples. Both wild rice and deep water rices are known to be sources of high Fe and Zn (Sarla et. al, 2012).

Micronutrient content can be estimated by both destructive and nondestructive methods. Perl's Prussian blue and DTZ staining method are standardized for Fe and Zn estimation to conduct the initial screening of genotypes. Although these methods are simple and inexpensive they are qualitative instead of quantitative in nature (Velu *et al*, 2008). Accurate estimation of Fe and Zn concentration is normally achieved through inductively coupled plasma-optical emission spectrophotometry (ICPOES) or atomic absorption spectroscopy (AAS) (Choi *et al*, 2007). Estimation of grain micronutrients using non-destructive energy dispersive X-ray fluorescence (ED – XRF) machine is also very useful and efficient rapid screening method to select high grain Fe and Zn lines from large number of samples and further these values may be confirmed with destructive methods. Georgia et.al*,* (2017) demonstrated the application of ED-XRF for high throughput screening of Fe and Zn concentration in common bean, maize and cowpea and its advantages in bio fortification breeding programmes. Maganti et. al (2020) studied the variation of grain Fe and Zn concentration in 159 rice germplasm of both brown and polished rice samples using XRF and observed a positive correlation between the two micronutrients. Takahashi et. al, (2009) revealed that Fe is most abundant in the embryo and in the aleurone layer while Zn has been localized in the endosperm of rice by X-ray micro fluorescence imaging. Screening of Fe and Zn concentration using EDXRF is a convenient and cost effective method in bio fortification breeding programs (Paldridge et. al, 2012). The objectives of the present study were to screen rice germplasm for iron and zinc concentration in brown rice using EDXRF method and to identify lines with high Fe and Zn which can be further utilized in bio fortification programmes.

Materials and Methods

Plant material: A set of 100 rice genotypes were grown during 3 seasons - one wet season (Kharif 2015) and two dry seasons (Rabi 2016 and Rabi 2017) at IIRR farm, Hyderabad, India. (17.53^0N latitude and 78.27^0 E longitude, 545 MSL, with mean temperature of 31.2^0C and mean annual precipitation of 988.3 mm). Randomized Complete Block Design (RCBD) with two replications was followed to conduct the experiments. The 100 rice genotypes included N22 and mutants (4), BPT x *O. rufipogon* (WR119) BILs (30), Swarna x *O.nivara* (IRGC818489(S) and 81832(K)) BILs (19) and BCRILs of 233K x 24K (5), KMR3 x *O.rufipogon* (WR120) BILs (29), Madhukar x Swarna RILs (5) and Jalmagna x Swarna RILs (5). At the time of transplanting, soil pH was in the range of 8.52 to 8.57, while soil iron and zinc concentrations were 2.74 to 3.48 ppm and 3.55 to 3.66 ppm respectively.

Iron and Zinc Concentration: Iron and zinc concentration in brown rice samples was estimated using non-destructive, energy-dispersive X-ray fluorescence spectrometry (EDXRF) instrument (model X-Supreme 8000; Oxford Instruments plc, Abingdon, UK) at IIRR, Hyderabad. 10g of well dried paddy sample from each genotype was de husked using non-metallic de-husker (Krishi international 810 de-husker) having roller made of polymer to avoid iron and zinc contamination. De-husked rice was cleaned by removing broken grains and debris and 5g of each sample was weighed and transferred to sample cups. The sample cups were gently shaken for uniform distribution of samples and kept for analysis. Concentration of Fe/ Zn was expressed in microgram/ gram (µg/g) or parts per million (ppm) grains.

Results and Discussion

The mean grain iron concentration of 3 seasons ranged from 1.6 to 15.2 ppm and most of the genotypes (67) showed 10 to 12 ppm Fe. The mean zinc concentration in 3 seasons varied between 6.2 and 33.2 ppm, 50 genotypes showed 20 to 25 ppm Zn (**Table 1**). The mean value of iron in 100 genotypes was 9.7 ppm and

Table 1: Mean grain Fe and Zn concentration in 100 rice genotypes for 3 seasons

S No.	Genotype	Zn (ppm)	Fe (ppm)	S no.	Genotype	Zn (ppm)	Fe (ppm)
1	**N-22**	30.3	14.1	51	14K	21.3	12.5
2	NH787	13.4	7.2	52	7K	20.8	11.2
3	NH59	16.8	2.6	53	233K	14.2	3.8
4	NH686	15.2	12	54	24K	24.8	9.2
5	**BPT5204**	17.3	7.6	55	132Z	24.3	13
6	105B	24.43	12.63	56	127Z	23.4	11.2
7	88B	24.40	10.70	57	133Z	21.3	10
8	27B	23.87	12.47	58	314Z	21.3	9.7
9	161B	23.17	10.60	59	515Z	12.8	2.7
10	16-1B	22.90	12.40	60	KMR3	13.4	7.7
11	61-1B	26.73	12.17	61	50-7	6.2	1.8
12	34B	22.63	11.43	62	50-13	11.3	1.6
13	26B	22.57	11.87	63	86-18	14.2	6.8
14	76B	22.53	11.17	64	363-12	24.2	10.3
15	55B	22.17	9.83	65	90-5	17.8	6.8
16	69B	22.17	11.77	66	12-38	18.8	9.8
17	33B	22.17	11.83	67	377-24	10.5	8.3
18	71B	22.13	12.23	68	363-5	13	7.3
19	83B	21.87	10.60	69	13-5	18.5	11.3
20	77B	21.80	10.97	70	10-3	17.6	11
21	4B	20.53	11.67	71	86-1	21.4	10.3

S No.	Genotype	Zn (ppm)	Fe (ppm)	S no.	Genotype	Zn (ppm)	Fe (ppm)
22	78B	20.50	10.63	72	50	20.2	8.3
23	41B	20.50	11.70	73	381	16	8.4
24	32B	20.47	10.63	74	14	23.2	12.2
25	30B	20.43	11.57	75	495	18	8.8
26	16B	20.43	11.63	76	463	19.5	10.2
27	28B	20.40	11.47	77	407	18.8	9
28	98B	17.73	9.83	78	473	18	8.8
29	122B	17.77	10.20	79	410	11.8	9.7
30	51B	19.80	10.23	80	213	21.2	8.8
31	21B	17.80	12.00	81	198	23.6	11.8
32	112B	17.93	10.10	82	117	17.3	8.3
33	14B	17.93	12.47	83	109	16.8	8
34	148B	18.00	10.87	84	458	22.1	10.3
35	154B	17.60	9.73	85	431	21.1	15.2
36	**Swarna**	18.2	8.8	86	501	21.8	9.4
37	14S	20	11.5	87	40	18.3	9.9
38	166S	10.8	4.9	88	194	22.6	11.1
39	75S	23.2	11.8	89	**Madhukar**	24.3	11.2
40	70S	25.4	12.3	90	185M	33.2	3.5
41	148S	27.5	12.2	91	140M	21.3	7.2
42	228S	15.6	8.7	92	166M	21	4.9
43	166-30S	14.2	6.8	93	176M	23.2	7.7
44	166S	11.5	5.2	94	196M	25.2	10.1
45	14-3S	12.6	9.1	95	**Jalmagna**	20.1	9.3
46	248S	14.8	8.8	96	421J	22.3	8.3
47	65S	15.2	10	97	287J	20.9	10.2
48	250K	23.2	10.2	98	43J	19.7	9.7
49	3K	22.2	10.8	99	284J	18.5	10.1
50	236K	20.2	13.2	100	281J	18.4	10

*N22 mutants-1to 4; BPT BILs – 5 to 35; Swarna BILs -36 to 54; 233K x 24K BCRILs-55 to 59; KMR3 BILs -60 to 88; Madhukar x Swarna RILs-89 to 94; Jalmagna x Swarna RILs- 95 to 100;

that of zinc was 19.6 ppm (**Table 2 & Figure 1**). The lowest concentration of iron was observed in IL 50-13 with 1.6 ppm and that of zinc in IL 50-7 with 6.2 ppm while the highest grain Fe and Zn concentrations were observed in IL 431 with 15.2 ppm and 185M with 33.2 ppm respectively. The results showed a significant genetic diversity or variation in the existing rice germplasm. Ten genotypes - N22, 148S, 61-1B, 70S, 196M, 24K, 105B, 88B, 132Z and 185M had high grain zinc concentration of > 24 ppm. Among these 10 genotypes except 185M and 24K, all the other lines also showed high grain Fe concentration (> 10 ppm). Most of the lines with high Fe (> 10 ppm) also had high Zn (> 20 ppm) concentration, however high Zn lines did not always have high Fe. Gautami *et al* (unpublished) observed similar results in in 136 Backcross Introgression Lines (BILs) from the cross of BPT5204 x *O. rufipogon*.

Anuradha et.al, (2012) screened 126 accessions of rice germplasm in brown rice samples for grain Fe and Zn using Atomic Absorption Spectrophotometer (AAS). Two popular varieties BPT5204, Swarna, the drought and heat tolerant line Nagina22 and deep water rice varieties Madhukar and Jalmagna were common with this study. When compared, these lines had different grain Fe and Zn concentration in the two studies. In our study, the grain Fe and Zn concentration in these five lines BPT5204, Swarna, Nagina22, Madhukar and Jalmagna was 7.6ppm, 17.6ppm; 8.8ppm, 18.2ppm; 14.1ppm, 30.3ppm; 11.2ppm, 24.3 ppm; and 9.3ppm, 20.1 ppm respectively while the grain Fe and Zn concentration of these lines reported in their study was 13.4ppm, 47.8ppm; 32.1ppm, 58.2ppm; 30.3ppm, 43.2ppm; 12.4ppm, 51ppm and 11.5ppm, 42.2ppm respectively. Analysis of grain Fe and Zn concentration using AAS which is a destructive method requires extensive preanalysis preparation of

Table 2 Descriptive statistics of grain Fe and Zn in 100 rice genotypes for 3 seasons

Variable	Samples	Min	Max	Mean	Range	Variance	Std_ Dev	SE_ Mean	Skewness	Kurtosis
Fe-Kharif_2015	100	1.4	15.0	9.4	13.6	6.5	2.56	0.25	-1.13	1.69
Zn-Kharif_2015	100	5.6	33.2	18.8	27.6	20.6	4.53	0.45	-0.01	0.61
Fe-Rabi_2016	100	1.6	15.3	9.8	13.7	7.5	2.74	0.27	-1.02	1.17
Zn-Rabi_2016	100	6.2	33.3	19.8	27.1	20.7	4.55	0.45	-0.21	0.68
Fe-Rabi_2017	100	1.8	15.3	9.8	13.5	6.9	2.64	0.26	-1.06	1.47
Zn-Rabi_2017	100	6.8	33.1	20.4	26.3	21.5	4.64	0.46	-0.28	0.40

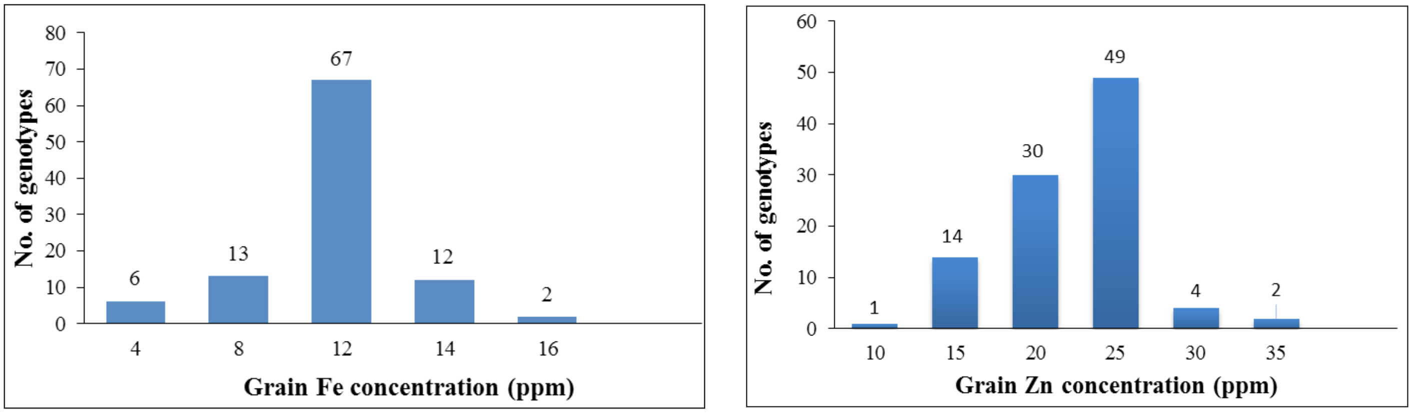

Figure1: Frequency distribution of grain Fe and Zn concentration in 100 rice genotypes

samples such as drying, cleaning, grinding, weighing, digestion and dilution compared to EDXRF method. Previous reports also suggest that the micronutrient concentration values obtained from AAS analysis were higher compared to EDXRF analysis (Singh et. al., 2017). ILs 185M (IET23814), 51B (IET24775), 24K (IET22624), 233K (IET25459), 515Z (IET27998), 61-1B (IET28715), 83B (IET28695), 88B (28706) were entered in AICRIP Bio fortification trials and their respective overall mean Fe and Zn values were 3.3, 31.69 ppm; 4.0, 19.9 ppm; 2.29, 18.7 ppm; 1.6, 18.13 ppm; 3.7, 18.7 ppm; 4.0, 20.19 ppm; 3.2, 19.6 ppm in polished samples across different locations of India (**Figure 2**). IL185M showed the highest overall mean value of 31.69 ppm zinc in polished samples out of 45 entries across 17 locations in AICRIP 2014. Bioavailability studies using the in vitro Caco-2 cell system at National Institute of Nutrition, India showed that in the presence of ascorbic acid, Fe was two times and Zn was three times more bioavailable in 185M than in its parent Swarna (Raghu et.al, 2019). Recently, 185M was reported to show 22.6 to 40.07 ppm Zn in brown rice and showed the highest mean of 30.62 ppm Zn among 68 entries studied at 6 locations in 3 years (Naik et.al, 2020). IL51B with high Zn in unpolished rice was evaluated under AICRIP- Bio fortification trial for 4 years 2014-2017. In these trials, 51B showed high grain zinc (19.1ppm) in polished rice consistently compared to the two check rice varieties Kalanamak and Chittimuthyalu measured using XRF.

Figure 2: Paddy and brown rice of elite lines 185M [IET 23814] and 51B [IET24475] tested in AICRIP Bio fortification trials

It was observed from the previous work and our results that Fe and Zn content values were not consistent. The values can vary with the sample lots even from same accession and also with the analytical method used for the estimation of grain Fe and Zn. The position of grain on the panicle may also significantly affect its Fe and Zn levels. Su *et al*., (2014) reported that the heavy-weight grains, located on primary rachis and top rachis had higher mineral concentrations compared to the small-weight grains located on secondary rachis and bottom rachis, regardless of rice genotypes. Variations in Fe and Zn values in different samples of the same accession can also arise due to presence or absence of embryo in grains, variations in time of harvest or different digestion or analytical methods. This variation in iron and zinc values is also due to homeostasis regulating their absorption, translocation, and transport within the plant system (Welch et.al, 2004). The moisture content of grain samples also influences grain iron and zinc concentration in EDXRF analysis as the instrument underestimates the grain micronutrient concentration of the samples with high moisture and hence the samples should be well dried before analysis (Rao et.al, 2014). Another factor contributing to difference in iron and zinc values is the phloem sap loading and unloading rates within the reproductive organs. Different seed lots of the same accession had different Fe and Zn concentration even though they were harvested from the same plot. Thus, there is a wide range of variation in Fe and Zn concentration and are not consistent quite akin to the yield trait.

Soil properties also influence the grain Fe and Zn concentration. The pH, organic matter content and Fe/Zn levels of native soil showed significant effects on grain Fe and Zn content (Chandel et.al, 2010). Low soil pH and anaerobic conditions, as found in lowland rice fields, trigger the reduction of Fe^{3+} to Fe^{2+}, which ultimately enhances Fe absorption (Zhai et. al, 2014). A relatively low concentration of $ZnSO_4 \cdot 7H_2O$ added to Fe-AA (Amino acid) significantly increased Fe and Zn accumulation in rice grain. Foliar sprays of iron and zinc fertilizers are known to be an important agronomic practice to improve Fe and Zn concentrations in rice grain (Yuan et. al, 2013; Ram et. al., 2016). Younes et. al, (2016) studied the effect of bio fertilizers and foliar application of zinc on nutrient content of cultivated variety of wheat. Inoculation of plants with bio fertilizers and zinc, improved zinc content and yield in wheat under water-limited condition as well as normal irrigation.. Fe concentration is known to vary with location but Zn values appear to be more consistent (Chandel et. al, 2010). Also, the range of variation is much more for Fe concentration than for Zn. Environment, genotype and genotype × environment interaction significantly affected Fe concentration in rice grains (Suwarto, 2011). While grain Fe content showed significant genotype × environment interaction effect, Zn content of brown rice was significantly influenced more by native soil properties (Chandel et. al, 2010; Suwarto, 2011). Thus, in general grain zinc appears to be more consistent than grain Fe content. Sellappan et. al, (2009) suggested that the number of aleurone layers, size of the embryo and size of the caryopsis determine the quantity of important micronutrients such as iron, zinc in the grains. The high genetic correlation between grain characteristics and some mineral element contents can be used for indirect selection of grain characteristics for mineral element content in a breeding program (Zang et. al, 2005). All these factors influence the grain iron and zinc concentration in rice and might be responsible for the variations in the same genotype under different environmental conditions in different studies.

Conclusions

Ten high grain Fe and Zn lines - N22, 148S, 61-1B, 70S, 196M, 24K, 105B, 88B, 132Z and 185M were identified among the germplasm screened using the nondestructive ED-XRF method. Lines with high Fe invariably had high zinc but not vice versa and is supported by our subsequent unpublished work on BILs derived from BPT5204 x *O. rufipogon.* Variability in grain Fe and Zn concentrations in the introgression lines derived from wild species and crosses with deep water rices offers scope for further enhancement of Fe and Zn.

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References

Anuradha K, Agarwal S, Batchu AK, Babu AP, Swamy M, Longvah T, *et al*. 2012. Evaluating rice germplasm for iron and zinc concentration in brown rice and seed dimensions. *Journal of Phytology*, 4:19–25.

Velu G, Bhattacharjee R, Rai KN, Sahrawat KL and Longvah T. 2008. A simple and rapid screening method for grain zinc content in pearl millet. *Journal of SAT Agricultural Research,* 6.

Chandel G, Banerjee S, See S, Meena R, Sharma DJ and Verulkar SB. 2010. Effects of different nitrogen fertilizer levels and native soil properties on rice grain Fe, Zn and protein contents. *Rice Science* 17(3): 213−227.

Cheng CJ, Lan CJ, Ping SA, Qiang JZ and Zhi HL. 2009. Difference of Iron contents in rice landraces. *Journal of Plant Genetic Resources* DOI: CNKI: SUN: ZWYC.0.2009-01-012.

Choi EY, Graham R and Stangoulis J. 2007. Semi quantitative analysis for selecting Fe- and Zn-dense genotypes of staple food crops. *Journal of Food Composition and Analysis,* 20: 496–505.

Ghasemian V, Ghalavand A, Soroosh Zadeh A and Pirzad A. 2010. The effect of Iron, Zinc and Manganese on Quality and Quantity of Soybean Seed. *Journal of Phytology,* 2: 73-79.

Global Rice Science Partnership (GRiSP). 2013. Rice almanac. Incomplete?

Maganti S, Swaminathan R and Parida A. 2020. Variation in iron and zinc content in traditional rice genotypes. *Agricultural Research*, 9: 316-328. https://doi.org/10.1007/s40003-019-00429-3 .

Martínez, C.P., J. Borrero, R. Taboada, J.L. Viana, P. Neves,L. Narvaez, V. Puldon, A. Adames and A. Vargas. 2010. Rice cultivars with enhanced iron and zinc content to improve human nutrition. 28th International Rice Research Conference, 8-12 November 2010.

Naik SM, Raman AK, Nagamallika M, Venkateshwarlu C, Singh SP, Kumar S, Singh SK, Ahmed HU, Das SP, Prasad K, Izhar, Mandal NP, Singh NK, Yadav S, Reinke R, Swamy BPM, Virk P, Kumar A. 2020. Genotype × Environment interactions for grain iron and zinc content in rice. *Journal of the Science of Food and Agriculture*. https://doi.org/10.1002/jsfa.10454.

Paltridge, N.G., Palmer, L.J., Milham, P.J. *et al*., 2012. Energy-dispersive X-ray fluorescence analysis of zinc and iron concentration in rice and pearl millet grain. *Plant Soil,* 361: 251–260.

Prom-u-thai, C., L. Huang, R.P. Glahn, R.M. Welch, S. Fukai and B. Rerkasem. 2006. Iron (Fe) bioavailability and the distribution of anti-Fe nutrition biochemicals in the unpolished, polished grain and bran fraction of five rice genotypes. *Journal of the Science of Food and Agriculture*. 86:1209–1215.

Raghu P, Agarwal S, Tripura Venkata VGN, Ratnavathi CV, Madhavan Nair K, and Neelamraju S. 2019. Iron and zinc bioavailability from Madhukar x Swarna derived bio fortifed rice lines. *Current Science,* 118 (3), 455-461**.**

Ram, H., Rashid, A., Zhang, W., Duarte, A. P., Phattarakul, N., Simunji, and Mahmood, K. 2016. Biofortification of wheat, rice and common bean by applying foliar zinc fertilizer along with pesticides in seven countries. Plant and Soil, 403(1-2): 389-401.

Rao, D. S., Babu, P. M., Swarnalatha, P., Kota, S., Bhadana, V. P., Varaprasad, G. S., & Babu, V. R. 2014. Assessment of grain zinc and iron variability in rice germplasm using Energy Dispersive X-ray Fluorescence Spectrophotometer. *Journal of Rice Research*, 7(1): 45-52

Roohani N, Hurrell R, Kelishadi R, Schulin R (2013) Zinc and its importance for human health: an integrative review. *Journal of Research in Medical Sciences*, 18(2):144–157.

Sarla N, Mallikarjuna Swamy BP, Kaladhar K, Anuradha K, YV Rao, Batchu AK, Surekha A, Babu AP, Sudhakar T, Sreenu K, Longvah T, Surekha K, Rao KV, Ashoka Reddy G, Roja TV, Kiranmayi SL, Radhika K, Manorama K, Cheralu C, Viraktamath BC. 2012. Increasing iron and zinc in rice grains using deep water rices and wild species–identifying genomic segments and candidate genes. *Quality Assurance and Safety of Crops and Foods,* 4 (3), 138-138.

Sellappan, K., K. Datta, V. Parkhi and S.K. Datta. 2009. Rice caryopsis structure in relation to distribution of micronutrients (iron, zinc, and β-carotene) of rice cultivars including transgenic indica rice. *Plant Science*, 177: 557–562.

Singh, V., Padalia, D., & Devlal, K. 2017. Determination of Cu, Zn, Mn & Fe metals in soil employing the EDXRF & FAAS techniques and comparative study of results. *Journal of Nuclear Physics, Material Sciences, Radiation and Applications*, 4 (2): 1–9.

Suwarto and Nasrullah. 2011. Genotype×Environment Interaction for iron concentration of rice in central Java of Indonesia. *Rice Science*, 18(1): 75−78.

Su, D., Sultan, F., Zhao, N.C., Lei, B.T., Wang, F.B., Pan, G. and Cheng, F.M., 2014. Positional variation in grain mineral nutrients within a rice panicle and its relation to phytic acid concentration. *Journal of Zhejiang University Science* B, 15(11): 986-996.

Takahashi, M., T. Nozoye, N. Kitajima, N. Fukuda, A. Hokura and Y. Terada. 2009. In vivo analysis of metal distribution and expression of metal transporters in rice seed during germination process by microarray and X-ray Fluorescence Imaging of Fe, Zn, Mn, and Cu. *Plant Soil*, 325:39–51.

United States Department of Agriculture (USDA). World Agricultural Production. (2019) Available online at: https://apps.fas.usda.gov/psdonline/circulars/production.pdf (accessed September 2020)

Welch, R.M. and R.D. Graham. 2004. Breeding for micronutrients in staple food crops from a human nutrition perspective. *Journal of Experimental Botany*, 55:353–64.

Yuan, L., Wu, L., Yang, C., & Lv, Q. (2013). Effects of iron and zinc foliar applications on rice plants and their grain accumulation and grain nutritional quality. *Journal of the Science of Food and Agriculture,* 93(2), 254-261.