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Development, evaluation and release of biofortified rice varieties

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Abstract

Polished rice is a poor source of micronutrients and was reported to be responsible for malnutrition in the developing countries where rice is the major energy source. Biofortification for zinc (Zn) in polished rice is a promising and cost effective approach for the development of Zn-dense rice to alleviate micronutrient malnutrition. Several donors, especially landraces with >50 ppm in brown rice and >35 ppm in polished rice were identified and used in the development of breeding lines with high Zn and yield. The developed lines were evaluated under ICAR - All India Coordinated Rice Improvement Project for their high yield and Zn across the locations and across the years and promising lines were identified and released as varieties. Five varieties since 2015 were released through Central Varietal Release Committee for high Zn in polished rice in India.

Keywords: Biofortified rice varieties, high zinc, polished grains, development and release

Introduction

Rice (*Oryza sativa* L.) is the staple food crop of 50% of the world and a major energy source especially in India. Polished rice, the most preferred form for consumption, is a poor source of micronutrients and the excess dependence on polished rice was reported to be responsible for malnutrition whose daily caloric intake is mainly confined to rice (Juliano, 1993; Bouis and Welch, 2010). Polished grains of most of the rice varieties have 12-14 ppm of Zn, thus providing only one fifth of daily recommended Zn requirement of ~15 mg (though varies across sex and age) (Promo-u-thai *et al.*, 2010).

Dietary deficiency of Zn is a substantial global public health and nutritional problem with one third of the world population at risk due to low dietary intake of Zn (Krishna swamy, 1998; www.zinc.org/health/). A breeding target of 28 ppm was set in polished rice based on the nutrient needs, daily food intake, retention and bioavailability analyses in order to meet at least 25% of the estimated average Zn requirement for overcoming the most severe Zn deficiency (www.harvestplus.org). Enhancing the Zn content of polished rice has lot of potential to address wide spread Zn deficiency problem responsible for malnutrition in developing countries. Different strategies, such as biofortification, foliar or soil application of Zn fertilizers have been suggested and demonstrated to increase the Zn content of cereals (Nestel *et al.*, 2006; Swamy *et al.*, 2016). Of these, biofortification, an approach of the development of micronutrient-dense staple food crops to alleviate micronutrient malnutrition is targeted, sustainable and cost effective, hence most preferred. Since 2000, several attempts are being made in rice for Zn biofortification through conventional breeding approaches across the world. Using the donors for high Zn content, several breeding lines with high Zn are being developed and evaluated under HarvestPlus, international and national programs. As a proof of concept of combining high Zn with high yield, breeding lines with high Zn in polished rice were developed through conventional breeding with funding received from HarvestPlus, Department of Biotechnology (DBT), and Indian Council of Agricultural Research (ICAR), Government of India. These breeding lines were evaluated in India through national trials on Biofortification in All India Coordinated Rice Improvement Project since 2013 (www.harvestplus.org ; www.irri.org ; http://www.icar-iirr.org/). The entries of the trial constitute the biofortified breeding lines developed from all over the country. The present study summarizes the observations and the lessons learnt during the course of development of varieties with high Zn in polished rice and its release since 2013 in the Indian situation.

Materials and Methods

Identification of donors: Around 100 genotypes comprising landraces, breeding lines and farmer varieties were evaluated across four locations *viz*., rice experimental fields at Hyderabad (IIRR), Bengaluru (UAS), Chinsurah (Rice Research Station) and Coimbatore (TNAU) during wet season 2012. The soil properties of the plots of the four locations were given in Table 1.

Development of segregating material and selection of promising lines: The identified promising donors were crossed with rice varieties known for their yield, quality and adoption by the farmers. The favorable recombinants for yield and high Zn in polished rice were selected among the segregants, multiplied and submitted for evaluation.

Evaluation through ICAR - All India Coordinated Rice Improvement Project (AICRIP), ICAR-IIRR, Hyderabad: The nominated promising lines for yield and Zn were evaluated at 10 to 20 locations all over the country with two check varieties (control) *viz*., IR64 and BPT5204 for yield and high Zn *viz*., Kalanamak and Chittimuthyalu in polished rice. The seed samples are being sent to ICAR-IIRR for estimation of Zn in polished rice. The lines screened as Initial varietal trial (IVT), advanced varietal trial 1 (AVT 1) and advanced varietal trial 2 (AVT 2) for three years and the consistent lines were tested for 12 quality parameters for their suitability (http://www.icar-iirr.org/).

The varieties with recommended level of Zn, yield and desirable quality parameters were released as varieties through Central Varietal Release Committee (CVRC). As a case study, the evaluation data of AICRIP- Biofortification 2015-17 was presented.

Estimation of Zn: The seed of each plant was harvested and divided into three parts to be analyzed as three replicates. The seeds were dehusked using JLGJ4.5 testing rice husker (Jingjian Huayuan International Trade Co., Ltd) sponsored by HarvestPlus and polisher (Krishi International India Ltd.) with non-ferrous and non-Zn components. Each sample of brown and polished rice (5 g) was subjected to energy dispersive X-ray fluorescent spectrophotometer (ED-XRF) (OXFORD Instruments X-Supreme 8000) at ICAR-IIRR as per standardized protocols (Sanjeeva Rao *et al.*, 2014).

Results and Discussion

Zn deficiency is a major public health and nutritional issue affecting mostly the developing countries whose staple diet is polished rice (Krishnaswami, 1998; Juliano, 1993). The objective of Zn biofortification is to increase Zn concentration in polished rice and of the various approaches to address Zn deficiency in the human diet, biofortification is the most feasible, sustainable and economical approach. Several biofortified rice varieties have been released in Bangladesh and India and are being evaluated in Philippines and Indonesia (Swamy *et al.*, 2016).

Rice grain Zn concentration is affected by a large number of plant and environmental factors (Welch and Graham, 2002). Zn reported to be poorly available under the irrigated conditions (Fageria, 2013). The Zn content in the grain appears to be significantly affected by the pH, organic matter content and available Zn levels of native soil. However, genotypic variation was observed to be the most significant factor to affect grain Zn content (Chandel *et al.*, 2010). In the present study, the genotypes were evaluated in four locations with differential soil profiles (Table 1). The Zn found to be on higher side in the plots owing to the application of Zn fertilizer as part of the package of practices in rice experimental fields. However, Zn deficiency is reported to be widespread in soils and crops of India and is one of the significantly depleted mineral nutrients under intensively cropping systems (Takkar, 1997). On the basis of the analysis of about 65,000 soil samples, it has been found that about 51.2% of Indian soils are deficient in Zn. The critical levels of DTPA- Zn for cereal crops were found to vary among the soil types and crops (Singh *et al.*, 2015). Thus, farmers need to be sensitized regarding package of practices for growing the biofortified varieties.

Wide genetic variation was observed among the evaluated genotypes for grain Zn content in brown and polished rice. The rice land races or traditional varieties are considered to have high nutritive and therapeutic value (Deb *et al.*, 2015). Extensive genetic variability for grain Zn content has been earlier reported in rice wild accession and landraces (Anandan *et al.*, 2011; Anuradha *et al.*, 2012; Nachimuthu *et al.*, 2014; Huang *et al.*, 2016). Many landraces were identified with >50 ppm in brown rice and >35 ppm in polished rice and have been successfully used or being used in breeding programs (Table 2A and 2B). The screening of landraces for high nutrient content should be a continuous process for identification of new donors for high Zn in polished rice.

Across the locations, the genotypes found highly variable in their grain Zn content and only one genotype was found to be in the top ten genotypes. Surprisingly, the extent of polishing also showed lot of variation across the locations and genotypes (Table 3). The genotypes with high Zn content in brown rice were found not to necessarily contain high Zn content in polished rice, suggesting the effect of quality parameters and filling of the grain on polishing. The rice grain quality is highly influenced by environmental factors like temperature, soil moisture, crop management and post-harvest practices. Before releasing of a biofortified variety, multi-locational evaluation should be compulsorily made.

Table 1. Soil properties of experimental plots of the four locations during wet season 2012

Trait	Hyderabad (HYD)		Chinsurah (CHN)		Coimbatore (CBT)		Bengaluru (BLR)	
	Before Planting	After Harvesting	Before Planting	After Harvesting	Before Planting	After Harvesting	Before Planting	After Harvesting
pH	8.52	8.47	6.04	7.12	7.82	7.87	6.02	5.99
E.C (dS m^{-1})	0.92	0.71	0.14	0.19	0.64	0.47	0.25	0.27
O.C (%)	0.56 (M)	0.70 (M)	0.75 (M)	0.72 (M)	0.73 (M)	0.67 (M)	0.24 (L)	0.23 (L)
N (kg/ha)	230 (L)	187 (L)	164 (L)	183 (L)	195 (L)	227 (L)	181 (L)	183 (L)
P_2O_5 (kg/ha)	81 (H)	107 (H)	39 (M)	41 (M)	100 (H)	84 (H)	9 (L)	14 (L)
K_2O (kg/ha)	616 (H)	641 (H)	458 (H)	410 (H)	957 (H)	926 (H)	359 (H)	336 (H)
Fe (ppm)	3.48 (L)	2.30 (L)	56.50 (H)	31.92 (H)	0.74 (L)	0.94 (L)	28.82 (H)	30.50 (H)
Zn (ppm)	3.66 (H)	3.61 (H)	1.61 (H)	1.28 (H)	3.61 (H)	3.50 (H)	1.55 (H)	1.00 (H)

Around 20% average loss of Zn is observed in the polished rice samples in comparison to brown rice across the locations, the percentage loss of Zn content on polishing found to be broadly varying from 1.2 to 59.5. Among the genotypes, the range of percentage reduction was too wide, only two genotypes showed <10 % across the locations.

Combining the yield and high Zn in polished rice is a major challenge owing to the dilution effect of the micronutrient content (Impa *et al.*, 2013). Earlier focus of the rice breeding programs was the production of high yielding varieties to ensure the food security, thus breeding for high micronutrient contents was not a priority objective (Graham and Welch, 1996). With growing awareness of importance of biofortification, especially of Zn, breeding for high Zn in polished rice is one of the important objectives in global rice breeding programs.

Table 2A. Zn content in the brown rice of the top ten genotypes in the four locations estimated through ED-XRF

Location	HYD		BLR		CHN		CBT
Name	Zn (ppm)	Name	Zn (ppm)	Name	Zn (ppm)	Name	Zn (ppm)
GMP 22*	51.9	GMP 33	39.5	GMP 23*	39.4	GMP 14*	24.5
GMP 23*	48.5	GMP 45	39.3	GMP 24**	37.9	GMP 24**	22.2
GMP 35*	45.2	GMP 31	38.9	GMP 20*	30.0	GMP 12*	21.0
GMP 24**	45.1	GMP 43*	38.2	GMP 22*	29.9	GMP 22*	21.0
GMP 25	44.8	GMP 24**	35.6	GMP 38	29.6	GMP 11*	19.3
GMP 39*	43.3	GMP 40*	34.3	GMP 36*	28.9	GMP 43*	19.1
GMP 28*	41.8	GMP 28*	34.1	GMP 8	26.3	GMP 44	20.9
GMP 32	41.6	GMP 29	33.9	GMP 39*	26.2	GMP 13	16.8
GMP 40*	40.0	GMP 5	33.8	GMP 11*	25.8	GMP 27	16.4
GMP 14*	39.6	GMP 1	32.7	GMP 35*	25.6	GMP 23*	16.3

* top genotype in more than one location, ** top genotype in four locations

Table 2B. Zn content in the polished rice of the top ten genotypes in the four locations estimated through ED-XRF

Location	HYD		BLR		CHN		CBT
Name	Zn (ppm)	Name	Zn (ppm)	Name	Zn (ppm)	Name	Zn (ppm)
GMP 24*	42.7	GMP 45*	36.1	GMP 20	28.7	GMP 24*	21.3
GMP 23*	42.0	GMP 33	35.1	GMP 24*	27.9	GMP 44*	18.1
GMP 25	38.5	GMP 31	32.9	GMP 23*	27.0	GMP 14	17.3
GMP 28*	38.3	GMP 40	32.2	GMP 39*	24.7	GMP 43*	17.1
GMP 22*	36.6	GMP 43*	32.1	GMP 43*	23.7	GMP 27	14.5
GMP 39*	34.5	GMP 28*	32.0	GMP 45*	23.0	GMP 12	14.1
GMP 44*	34.1	GMP 29	31.7	GMP 35*	22.6	GMP 23*	13.9
GMP 35*	34.1	GMP 1	31.6	GMP 21	22.5	GMP 22*	13.6
GMP 37	33.6	GMP 26	30.8	GMP 41	22.5	GMP 6	13.3

* top genotype in more than one location

Table 3. Percentage reduction of Zn content in polished rice to brown rice

Location	HYD	BLR	CHN	CBT		
Genotype						
Range	3.7 - 39.6	1.2 - 56.5	2.2 – 59.5	2.8 – 43.2	Mean	Range
GMP 1	16.4	3.4	25.0	43.2	22.0	3.4 – 43.2
GMP 2	18.1	11.2	24.0	15.1	17.1	11.2 – 24.2
GMP 3	21.9	3.5	14.3	20.3	15.0	3.5 – 21.9
GMP 4	7.8	14.0	18.8	22.9	15.9	7.8 - 22.9
GMP 5	14.8	15.4	6.9	16.3	13.3	6.9 – 16.3
GMP 6	22.8	6.1	4.8	7.0	10.2	7.0 - 22.8
GMP 7	33.7	3.3	33.8	21.4	22.3	3.3 – 33.8
GMP8	35.2	3.4	23.6	28.7	22.7	3.4 – 35.2
GMP 9	15.3	12.1	22.8	13.8	16.0	12.1 – 22.8
GMP 10	27.7	10.0	8.7	14.1	15.1	8.7 – 27.7
GMP 11	21.0	13.7	17.4	34.2	21.6	13.7 – 34.2
GMP 12	27.4	27.2	24.4	32.9	28.0	24.4 – 32.9
GMP 13	7.2	12.7	31.6	38.7	22.5	7.2 – 38.7
GMP 14	39.6	12.5	21.7	29.4	25.8	12.5 – 39.6
GMP 15	9.7	4.8	7.1	2.8	6.1	2.8 – 9.7
GMP 16	7.3	1.2	14.7	31.3	13.6	1.2 – 31.3
GMP 17	19.3	5.3	5.1	25.2	13.8	5.1 – 25.2
GMP 18	19.2	2.6	4.5	30.4	14.2	2.6 – 30.4
GMP 19	3.7	3.1	29.5	28.4	16.2	3.1 – 29.5
GMP 20	22.4	10.7	4.3	26.4	16.0	4.3 – 26.4
GMP 21	27.5	9.6	2.2	36.7	19.0	2.2 – 36.7
GMP 22	29.5	6.2	49.5	35.2	30.1	6.2 – 49.5
GMP 23	13.4	9.5	31.5	14.7	17.3	9.5 – 31.5
GMP 24	5.3	16.0	26.4	4.1	12.9	4.1 – 26.4
GMP 25	14.1	12.4	18.4	3.2	12.0	3.2 – 18.4
GMP 26	16.1	5.5	26.9	7.0	13.9	5.5 – 26.9
GMP 27	21.6	6.7	28.9	11.6	17.2	6.7 – 28.9
GMP 28	8.4	6.2	9.0	6.7	7.5	6.2 – 9.0

Location	HYD	BLR	CHN	CBT		
Genotype						
Range	**3.7 - 39.6**	**1.2 - 56.5**	**2.2 – 59.5**	**2.8 – 43.2**	**Mean**	**Range**
GMP 29	13.1	6.5	36.4	19.1	18.8	6.5 – 36.4
GMP 30	10.5	11.7	40.7	24.1	21.8	10.5 – 40.7
GMP 31	23.5	15.4	26.7	14.7	20.0	15.4 – 26.7
GMP 32	23.8	1.4	13.6	20.0	14.4	1.4 – 23.8
GMP 33	15.1	11.1	11.5	35.1	18.2	11.1 – 35.1
GMP 34	26.8	4.1	25.6	26.7	20.8	4.1 – 26.8
GMP 35	24.6	56.5	11.7	23.0	28.9	11.7 – 56.5
GMP 36	25.8	11.5	57.8	20.8	29.0	11.5 – 578
GMP 37	14.1	20.8	27.8	18.8	20.4	14.1 – 27.8
GMP 38	15.6	3.8	59.5	6.0	21.2	3.8 – 59.5
Mean	**20.0**	**15.0**	**30.5**	**21.0**	**21.6**	

Several combination of crosses were developed using promising donors for Zn in polished rice and popular rice varieties. In a cross combination of a land race donor and a popular variety, out of the selections made in fixed lines, only one line could show high yield (>20 g single plant yield) and high Zn (> 28 ppm) (Figure 1). Similarly, though a few, promising lines were selected from all cross combinations and are being evaluated.

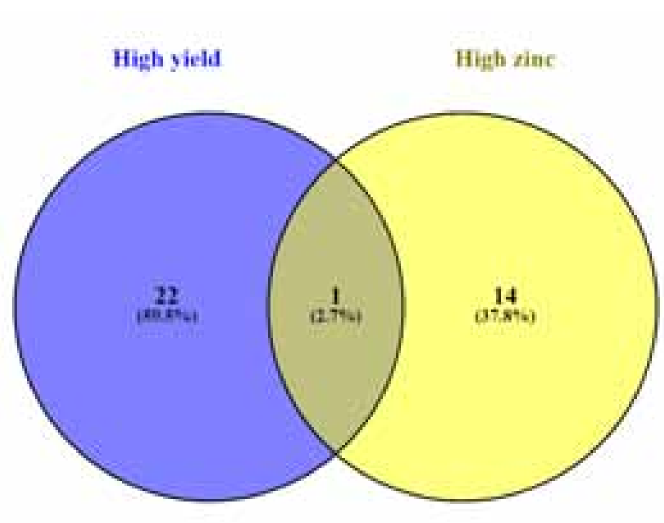

Figure 1.Common breeding line between the RILs for high yield and high Zn

The evaluation constitutes the comparison of biofortified lines with existing varieties for yield (IR64) and Zn content (landraces with high zinc) as check varieties. Initially, a concentration of 20 ppm was set as threshold value in AICRIP during 2013; however the threshold value has been raised to 24 ppm. The recommended dietary allowance (RDA) of Zn for human population in the age group of 25-50 years is 12-15 mg, respectively (FAO/WHO, 2000). In India, the average daily intake of rice is around 220 g and the polished rice with 28 ppm Zn would give 6.16 ppm Zn which is just 50% of RDA (Sanjeeva Rao *et al.*, 2014).

With funding from HarvestPlus, DBT and ICAR, several institutions have developed breeding lines for high Zn in rice. Based on the need for the evaluation of the breeding lines, a trial was constituted for biofortification during 2013 in ICAR – AICRIP and till date, several lines have been screened at multi-locations. Around 50 % of the nominated lines are advanced to AVT 1 based on their yield and Zn content. Approximately half of the AVT 1 lines were advanced to AVT 2 and based on the stringent yield, Zn and quality parameters, and the varieties are released (Table 5 and Table 6a & 6b).

Table 5. The number of breeding lines nominated under AICRIP

Year	IVT	AVT1	AVT2
2013	29/9		
2014	45/11	6/3	
2015	45/26	30/14	4/2
2016	32/10	12/11	12/3
2017	31/3	12/2	11/1
	182/59	60/30	27/6

Table 6a.An example data set for yield and Zn content

Entry details	2017		2016		2015	
	Mean yield (20)	Zn (ppm)	Mean yield (17)	Zn (ppm)	Mean yield (14)	Zn (ppm)
IET 25477	4791	25.5	4360	28.3	3620	28.4
IET 25475	5102	25.3	4478	27.7	3881	27.6
IET 25443	4728	25.0	4660	26.5	3629	24.4
IR 64	5941	18.5	5044	19.3	3933	17.2
Chittimuthyalu	4597	24	3185	24.8	2804	21.5

Table 6b.An example data set for quality parameters

Entry details	2017			2016		
	HRR	AC	GT	HRR	AC	GC
IET 25477	51.4	24.2	71	52	24.58	59
IET 25475	57	27.57	23	50.8	25.93	53
IET 25443	61.5	25.63	22	56.3	25.96	22
IR 64	na	na	na	52	23.29	55
Chittimuthyalu	65.7	23.52	22	60.8	23.4	22

HRR: head rice recovery, AC: amylose content, GC: Gel consistency

Table 7.Summary of five released varieties with high Zn

Variety	III year		II year		I year	
	Mean yield (20)	Zn (ppm)	Mean yield (17)	Zn (ppm)	Mean yield (14)	Zn (ppm)
IET 25477 (Zinco Rice)	4791	25.5	4360	28.3	3620	28.4
West Bengal, Chattisgarh and Odisha IGKV Raipur						
IET 24760 Surabhi (NSL)	4804	22.84	4693	20.0	4214	24.0
Maharashtra and Gujarat Nuziveedu Seeds Limited						
IET 24555 (DRR Dhan 48)	5008	20.91	4989	19.8	6202	na
Andhra Pradesh, Telangana, Tamil Nadu, Karnataka and Kerala.						
(IET 24557 (DRR Dhan 49)	4562	26.13	5079	20.6	6373	na
Gujarat, Maharashtra and Kerala						
IET 23832 (DRR Dhan 45)	4068	22.9	4222	22.68	4356	20.23
Andhra Pradesh, Tamil Nadu and Karnataka						

Till now, five varieties were released through central release varietal committee for high Zn in polished rice in India through ICAR - All India Coordinated Rice Improvement Project. One of the varieties released was a contribution from private sector, Nuziveedu Seeds Limited (Table 7). Many studies reported a significant negative association between the yield and grain Zn content (Jiang *et al.*, 2008; Norton *et al.*, 2010; Wissuwa *et al.*, 2008). Non-significant correlations were also reported between yield and grain Zn (Gangashetty *et al.*, 2013; Sathisha, 2013) in a set of landraces. However, from our experience in developing the breeding lines for high Zn and yield and from the nominations to AICRP-rice, we suggest the feasibility of combining the yield and high Zn.

Invitro bioavailability studies of a released variety *viz.*, DRR Dhan 45 showed that the Zn content and bioavailability was 50% more than the control IR64 variety. The coupled *in vitro* digestion/Caco-2 cell model studies with extrinsic ^{65}Zn isotopic labeling demonstrated higher absorption of Zn from DRR Dhan 45 in intestinal cells (Neeraja and Voleti, 2019).

Thus, with consorted efforts of various disciplines of plant breeding, soil science, biochemistry and plant physiology, the biofortified rice varieties have been developed. An evaluation system for biofortified varieties has been evolved by ICAR- IIRR through AICRIP for their release. However, the impact of the biofortified rice varieties in addressing malnutrition of the nation appears to be a long way to go, owing to its adoption by the growers and consumers.

Policy recommendations

- Proof of concept for the development of rice biofortified varieties with high yield and Zn
- Combination of genetic and agronomic strategies for biofortified varieties
- Multi-location evaluation is compulsory for stable biofortified varieties
- Clinical trials as proof of concept for inclusion of high Zn rice in public distribution system and mid-day meal schemes.

References

Anandan A, Rajiv G, Eswaran R, and Prakash M. 2011. Genotypic variation and relationships between quality traits and trace elements in traditional and improved rice (*Oryza sativa* L.) genotypes. *J Food Sci.*76: 122-125. doi:10.1111/j.1750-3841.2011.02135.

Anuradha K, Agarwal S, Batchu AK, Babu AP, Swamy BPM, Longva T, and Sarla N. 2012. Evaluating rice germplasm for iron and zinc concentration in brown rice and seed dimensions. *J Geophys Res*. 4: 19–25.

Bouis HE. and Welch RM. 2010. Biofortification - A sustainable agricultural strategy for reducing micronutrient malnutrition in the global south.

Crop Science. 50: S-20-S-32. doi:10.2135/cropsci2009.09.0531.

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.* 3: 213-227.

Deb D. 2015. A profile of heavy metals in rice (*Oryza sativa* ssp. indica) landraces. *Current Sci.* 23: 407-409.

Fageria NK. 2013. Mineral Nutrition of Rice. Boca Raton, FL: CRCPress. doi: 10.1201/b15392.

Gangashetty P, Salimath PM, and Hanamaratti NG. 2013. Association analysis in genetically diverse non-basmati local aromatic genotypes of rice (*Oryza sativa* L). *Mol. Plant Breed*. 4: 31–37.

Graham RD. and Welch RM. 1996. Breeding for staple-food crops with high micronutrient density.Washington DC: International Food Policy Institute.

Huang Y, Tong C, Xu F, Chen Y, Zhang C, and Bao J. 2016. Variation in mineral elements in grains of 20 brown rice accessions in two environments. *Food Chem.* 192: 873–878. doi:10.1016/j.foodchem.2015.07.087.

Impa SM, Morete MJ, Ismail AM, Schulin R. and Johnson-Beebout SE. 2013. Zn uptake, translocation and grain Zn loading in rice (*Oryza sativa* L.) genotypes selected for Zn deficiency tolerance and high grain Zn. *J Exp Bot.*64: 2739–2751. doi:10.1093/jxb/ert118.

Jiang W, Struik PC, Van Keulen H, Zhao M, Jin LN, and Stomph TJ. 2008. Does increased zinc uptake enhance grain zinc mass concentration in rice? *Ann Appl Biol.* 153: 135–147. doi:10.1111/j.1744-7348.2008.00243.x.

Juliano B. 1993. Rice in Human Nutrition. Food and Agriculture Organization of the United Nations and International Rice Research Institute.

Krishnaswami K. 1998. Country profile: India. Nutritional disorders–old and changing. *Lancet.* 351: 1268–1269.

Kuldeep S. 2009. The critical zinc deficiency levels in Indian soils and cereal crops. UC Davis: Department of Plant Sciences, UC Davis. Retrieved from https://escholarship.org/ uc/item/4h0788h5.

Nachimuthu VV, Robin S, Sudhakar D, Rajeswari S, Raveendran M, Subramanian KS, *et al*. 2014. Genotypic variation for micronutrient content in traditional and improved rice lines and its role in biofortification programme. *Indian J Sci Technol.*7: 1414–1425. doi: http://dx.doi.org/10.17485/ijst%2F2014%2Fv7i9%2F51512.

Neeraja CN. and Voleti SR. 2019. Consortia Research Platform (CRP) – Biofortification in selected crops for nutritional security – status. Technical Bulletin No. 104/2019.ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad – 5000300, India. p.96

Nestel P, Bouis HE, Meenakshi JV. and Pfeiffer W. 2006. Biofortification of staple food crops. *J Nutr.* 136: 1064–7. doi:022-3166/06

Norton GJ, Deacon CM, Xiong L, Huang S, Meharg AA, and Price AH. 2010. Genetic mapping of the rice ionome in leaves and grain: identification of QTLs for 17 elements including arsenic cadmium iron and selenium. *Plant and Soil*. 329: 139–153.

Prom-u-thai C, Rerkasem B, Cakmak I, and Huang L. 2010. Zinc fortification of whole rice grain through parboiling process. *Food Chem.* 120: 858–863. doi:10.1016/j.foodchem.2009.11.027.

Sanjeeva Rao D, Madhu BP, Swarnalatha P, Suneetha K, Bhadana VP, Varaprasad GS, Surekha K, Neeraja CN, and Ravindra BV. 2014. Assessment of grain zinc and iron variability in rice germplasm using Energy Dispersive X-ray Fluorescence Spectrophotometer (ED-XRF). *Journal of Rice Research*. 7: 45-52.

Sathisha TN. 2013.Genetic variation among traditional landraces of rice with specific reference to nutritional quality. *Karnataka J Agric Sci.* 26: 474.

Swamy BPM, Rahman MA, Inabangan-Asilo MA, Amparado A, Manito C, Chadha-Mohanty P. *et al*. 2016. Advances in breeding for high grain zinc in rice. *Rice*. 9:49. doi:10.1186/s12284-016-0122-5.

Takkar PN, Singh MV, and Ganeshmurthy AN. 1997. In. Plant nutrient needs, supply, efficiency and policy issues, 2000- 2025. *National Academy of Agricultural Sciences*, New Delhi. P. 238-264.

Welch RM, and Graham RD. 2002. Breeding crops for enhanced micronutrient content. *Plant Soil.* 245, 205–214.doi:10.1023/A:10206681 00330.

Wissuwa M, Ismail AM, and Graham RD. 2008. Rice grain zinc concentrations as affected by genotype, native soil-zinc availability, and zinc fertilization. *Plant Soil.* 306: 37–48. doi:10.1007/s11104-007-9368-4.