Importance and advantages of rice biofortification with iron and zinc

V Ravindra Babu

Directorate of Rice Research, Rajendranagar, Hyderabad 500 030, Andhra Pradesh, India

Citation: Ravindra Babu V. 2013. Importance and advantages of rice biofortification with iron and zinc. Journal of SAT Agricultural Research 11.

Rice (*Oryza sativa*) being the staple food for almost two-thirds of the population plays a pivotal role in Indian economy. Moreover, India ranks first in the world in area of rice cultivation with 43.97 million ha and second in production with 104.32 million tons (Anonymous 2013). Almost 31% of calories of Indian diet are supplied through rice. Research efforts focused on development of high-yielding varieties and adoption of modern production technologies resulted in enhanced production leading to self-sufficiency in the country. Along with yield, grain and nutritional quality has also become a primary consideration in rice breeding programs not only in India but also in various rice growing countries across the world. Rice biofortification program aims at biological and genetic enrichment of food products with vital nutrients, vitamins and proteins. Ideally, once rice is biofortified with vital nutrients, the farmer can grow the variety indefinitely without any additional input to produce nutrient packed rice grains in a sustainable way so that the produce reaches the malnourished population in rural India.

Plant breeding is an excellent 'tool' for micronutrient nutritional enhancement in combating the problem of malnutrition. The micronutrient density traits are stable across environments and it is possible to improve the content of several limiting micronutrients together. High nutrient density not only benefits the consumer but also produces more vigorous seedlings in the next generation. Malnutrition is the most common cause of zinc deficiency and 25% of the world's population is at risk of zinc deficiency (Maret and Sandstead 2006). In Asia and Africa, it is estimated that 500 to 600 million people are at risk for low zinc intake (Source: http://www.harvestplus.org/ 2010).

The Directorate of Rice Research (DRR), Rajendranagar, Hyderabad collected various landraces, basmati, non-basmati and high-yielding rice cultivars from different parts of the country. Varieties with relatively high iron and zinc in grains were identified and introduced in the breeding program as donors and some fixed rice lines with high iron (>10 ppm) and zinc (>20 ppm) after 10% polishing are at testing stage under All India Coordinated Rice Improvement Programme (AICRIP) system.

Rice grain

The structure of the rice grain is separated into three parts (Fig. 1). The germ is the heart of the grain, which sprouts when the seed is planted. It is rich in vitamin B, vitamin E, protein, unsaturated fat, minerals, carbohydrates and dietary fiber. The endosperm constitutes the largest part of the grain. It is composed chiefly of carbohydrates in the form of starch, with some incomplete protein and traces of vitamins and minerals. Bran is the covering (Fig. 2) and is composed primarily of carbohydrate cellulose with

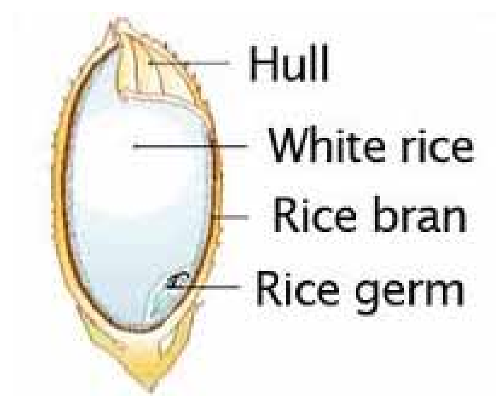

Figure 1. Composition of rice grain (Source: http://www.fortivia-nature.fr/oryzagerm-english.html).

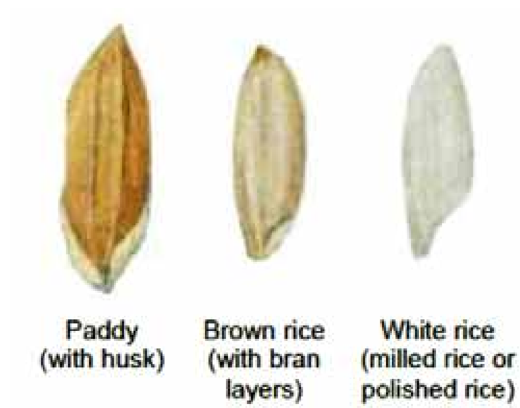

Figure 2. Different forms of rice after processing (Source: Lecture note on paddy cultivation by Tue Kell Nielsen in December 2004).

traces of vitamin B (including thiamin, niacin and B-6), minerals (including iron, phosphorus, magnesium and potassium) and incomplete proteins. The outer husk or hull is inedible but is often used for fuel or fertilizer. Rice grain contains 80% starch, 7.5% protein, 0.5% ash and 12% water. The proportion of amylose and amylopectin in starch determines the cooking and eating qualities of rice. In spite of the fact that rice is a primary source of carbohydrate, it is also a good source of protein, but it is not a complete protein, which means that it does not contain all of the essential amino acids in sufficient amounts for good health, and should be combined with other sources of protein, such as nuts, seeds, beans, fish or meat (Wu et al. 2003) in order to provide a balanced nutrient intake.

Need for biofortification in rice

In comparison with other cereals, rice contains low nutritional value (Table 1). Therefore, rice alone cannot meet the recommended daily allowance (RDA). Healthy and productive populations require adequate amounts of essential vitamins and minerals. As staple foods are eaten in large quantities everyday by malnourished poor, addition of even small quantities of micronutrients is beneficial. High zinc seeds are more vigorous and better able to withstand weed competition, and pathogen and pest attack (Gregorio et al. 2000). Deficiencies of zinc, iron and vitamin A in human population of developing countries were noticed and particularly, zinc deficiency is the fifth major cause of diseases and deaths in these countries. Health problems caused by zinc deficiency include anorexia, dwarfism, weak immune system, skin lesions, hypogonadism and diarrhea (McClain et al. 1985). Males aged 15 to 74 years need about 12 to 15 mg of zinc daily while females aged 12 to 74 years need about 68 mg of zinc daily (Sandstead 1985). Iron dependent anemia in turn leads to maternal mortality, preterm births (Scholl et al. 1992), decreases immunity (Kandoi et al. 1991) and increases placental weight (Wingerd et al. 1976) during pregnancy. Further, the iron requirement is highest during 7–9 months of pregnancy (O'Brien et al. 1999). Crops bred for increased uptake and utilization of trace minerals (eg, zinc and iron) could be harnessed to simultaneously improve crop productivity and human nutrition (Graham and Welch 1996).

Table 1. Micronutrient status of rice vis-à-vis other cereals.

Crop	Protein (%)	Iron (ppm)	Zinc (ppm)
Rice	6–7	2–34	10–33
Wheat	13–14	25–55	25–65
Maize	8–11	10–63	13–58
Sorghum	10–15	10–65	14–55
Pearl millet	6–21	30–146	25–85
Small millets (finger millet, foxtail millet)	8–20	37–142	5–60

Biofortification and its need

Biofortification is defined as the enhancement of micronutrient levels of staple crops through biological processes, such as plant breeding and genetic engineering (Bouis 2002). It could be effective in reducing the problem of malnutrition as part of a strategy that includes dietary diversification, supplementation, commercial fortification and other aspects. HarvestPlus is a CGIAR initiative which started "biofortification" umbrella through which international agricultural and research centers have made efforts to develop new breeds of staple foods that are rich in vitamins and minerals.

Biofortification has multiple advantages, including the fact that it capitalizes on the regular daily intake of a consistent amount of staple food by all family members. Rice is one of the global staple foods being cultivated since 10,000 years and provides 70–80% or more daily calorie intake for 3 billion people, which is almost half of the world's population. The grain has large genetic variability in micronutrient concentration. Hence, rice was included in biofortification program (Graham et al. 1999), which implicitly targets low income households who have limited access to commercially marketed fortified foods that are more readily available in urban areas. In all crops studied, it is possible to combine the high micronutrient density trait with high yield economically. Predictive benefit-cost analyses show biofortification to be important for controlling micronutrient deficiencies. Getting consumers to accept biofortified crops will be a challenge, but with the advent of good seed systems, the development of markets and products, and demand creation, this can become a reality (Nestel et al. 2006).

Rice is a major source of dietary carbohydrate for more than half of the world's population (Zimmermann and Hurrell 2002). Modern agriculture had reasonable success in meeting the energy needs of developing countries. In the past 40 years, agricultural research in developing countries has met Malthus' challenge by placing increased cereal production at its center. However, agriculture must focus on a new paradigm that will not only produce more food but also better quality food. Biofortification of staple food crops for enhanced micronutrient content through genetic manipulation is the best option available to alleviate hidden hunger with little recurring costs (Welch and Graham 2004, Monasterio et al. 2007).

Breeding strategies

With the help of molecular markers, the loci associated with nutrient content in grains can be identified and used for marker-assisted selection in regular breeding programs. Rice breeders are expected to concentrate on increasing the total nutrient content in the endosperm of the grain, the part that remains after milling. The range of iron and zinc concentrations in brown rice is 6.3–24.4 µg g^{-1} and 13.5–28.4 µg g^{-1} respectively. There was approximately fourfold difference in iron and zinc concentrations, suggesting vast genetic potential to increase the concentration of these micronutrients in rice grains (Gregorio 2002).

Targeting these traits, DRR in collaboration with National Institute of Nutrition (NIN), Hyderabad started a biofortification program. Several rice varieties and landraces collected from different parts of the country were grown at RC Puram Farm, DRR and iron and zinc contents were estimated at both DRR and NIN. Iron and zinc contents ranged from 6.9 ppm (DL 163) to 37.5 ppm (Varsha) and from 11.3 ppm (Karjat 3, IR 64) to 37.2 ppm (Phou Dum) in brown rice respectively. Among the genotypes tested, 10 genotypes having high iron and zinc contents were identified (Tables 2 and 3) and introduced in the breeding program of DRR to develop high nutritional genotypes. When the same samples were polished (5% and 10%), iron and zinc contents reduced due to polishing (Table 4). In general, Basmati genotypes, deepwater rices and landraces were found to have high iron and zinc contents in the grains (Table 5).

Variation in iron and zinc contents in brown rice as well as polished rice (5% and 10%) was studied. The top 5 entries with high iron are Kalanamak (34.4), Karjat 4 (30.6), Chittimutyalu (24.9), MSE 9 (24.4) and Kanchana (20.4) and top 5 entries with less loss after polishing are ADT 43, Manoharshali, Karjat 4, Swarna and Seshadri. The top 5 entries having high zinc are Poornima (31.3), Ranbir Basmati (30.9), ADT 43 (30.9), Chittimutyalu (30.5) and Type 3 (30.3) and top 5 entries with less loss after polishing are White Ponni, Bas 386, Kanishk, Giri and Karjat 4. Statistical analysis (SAS) of the 168 genotypes grown at four different locations revealed that there is no significant correlation between yield and iron content and yield and zinc content in brown rice.

X-Ray Fluorescence Spectrometry and its potential at DRR

Energy dispersive X-Ray Fluorescence Spectrometry (XRF) was donated by HarvestPlus program to DRR. In XRF the preselected wavelength of incident X-rays expel an electron from the innermost orbit followed by the transfer of one of the electrons from the outermost orbit to innermost orbit leading to release of specific wavelength of X-rays. The energy of the emitted radiation is specific for a particular atom. Therefore, it is simultaneously identified and quantified by the detector. This instrument is quite useful in non-destructive determination of relative iron and zinc concentrations in rice samples with more ease in comparison with Atomic Absorption Spectrometry (AAS) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS). As with AAS and ICP-MS, XRF also requires certain maintenance guidelines like dust free, air-conditioned room, etc. Due to these advantages, iron and zinc contents in ten thousand samples were analyzed in one year. However, among these three machines, more variation was observed in the values of iron content and

Table 2. Rice varieties with high iron content in grain.

Variety	Grain type[1]	Fe (ppm) content in polished rice		
		0%	5%	10%
MSE 9	LB	34.4	12.4	10.8
Kalanamak	SB	34	12.1	10.9
Kanchana	MS	20.4	12.8	6.6
Karjat 4	MS	25.6	20.6	19
Chittimutyalu	SB	24.9	14	9.8
Udayagiri	SB	30.1	9.5	9
Jyothi	LB	19.8	14.9	4
VRM 7	SB	22.8	7.9	7.8
Metta Triveni	SB	26.1	7	7
Varsha	SB	37.5	11.2	8.1

1. LB = Long bold; SB = Short bold; and MS = Medium slender.

Table 3. Rice varieties with high zinc content in grain.

Variety	Grain type[1]	Zn (ppm) content in polished rice		
		0%	5%	10%
Chittimutyalu	SB	30.5	25.7	24.4
Poornima	SS	31.3	27.8	27
ADT 43	MS	30.9	26.6	20.9
Ranbir Basmati	LS	30.9	28.3	27.4
Type 3	LS	30.3	28.3	26.5
Udayagiri	SB	30.1	19.5	11.3
Ratna	LS	32.7	25.2	23
Jyothi	LB	31.3	22.4	20.6
Pant Sugandh 17	LS	32.5	24.7	20.6
Kesari	MS	31.5	19.9	19.3

1. LB = Long bold; SB = Short bold; LS = Long slender; MS = Medium slender; and SS = Short slender.

Table 4. Loss of iron and zinc in rice after 5% and 10% polishing.

	Iron		Zinc	
Form of rice	Content (ppm)	Loss (%)	Content (ppm)	Loss (%)
Brown rice	4.9–22.5		17.4–33.1	
5% polished rice	2.4–17.2	10.9–82.2	11.0–28.3	4.1–40.8
10% polished rice	1.1–11.2	26.9–90.7	11.6–28.4	14.2–44.4

Table 5. Elite rice genotypes with high iron (Fe) and zinc (Zn) contents and reduction in Fe and Zn after 5% and 10% polishing.

		Fe (ppm)			Zn (ppm)		
Genotype	Grain type[1]	0%	5%	10%	0%	5%	10%
Basmati types							
Basmati 386	LS	14.8	13.1	9.5	30.3	27.7	25.9
Ranbir Basmati (R3)	LS	14.2	10.4	7.8	33.8	30.9	30.0
Type 3 (R3)	LS	15.3	9.7	7.1	33.7	31.4	29.4
Kasturi	LS	11.3	8.3	5.8	34.3	25.4	24.9
Pusa Basmati	LS	12.1	6.4	6.5	31.2	17.8	15.6
Landraces							
Chittimutyalu	SB	24.9	14.0	9.8	30.5	25.7	24.4
Nahazing	SB	16.8	9.0	5.3	33.6	26.1	23.4
Moirang Phou	SB	17.0	6.9	3.5	37.0	28.5	32.1
Phou Dum	LS	17.2	10.8	5.5	37.2	30.2	23.8
Munga	SB	25.4	15.8	8.0	35.0	28.7	19.6
Deepwater rices							
Jalamanga	SB	25.8	7.0	5.3	28.2	17.5	16.3
Jagabandu	SB	10.1	6.9	5.7	26.6	23.1	21.9
Madhukar	LB	28.6	11.2	7.6	31.2	24.2	22.0
Jalapriya	LB	24.5	8.1	6.6	25.0	21.2	18.4
Dinesh	SB	11.9	7.8	4.7	28.1	25.7	20.0

1. LS = Long slender; SB = Short bold; and LB = Long bold.

this has to be resolved. Even then, the role of XRF is highly commendable due to its simplicity and similarity in the categorization of varieties based on iron content in comparison with the other two machines.

Achievement at DRR through conventional breeding approach

Selections were made in the segregating populations and stabilized lines with high iron and zinc contents with good grain quality and yield were identified. A line derived from the cross between BPT 5204 × Chittimutyalu with short bold grains, semi dwarf with high yield potential (>4.5 t ha^{-1}) and medium duration with high iron (31.2 ppm) and zinc (40 ppm) in brown rice was identified (Fig. 3) possessing good quality characters, viz, good head rice recovery (67.5%), intermediate alkali spreading value (5.01), amylose content (24.05%) and mild aroma. This line was nominated to the AICRIP during *kharif* (rainy season) 2012 and some more fixed lines with high zinc are in the pipeline with different grain types to be nominated to the AICRIP for further testing.

Molecular studies

Several QTLs (quantitative trait loci) have been mapped for micronutrient content in rice grains using various germplasm sources including wild species. Using selective genotyping approach three loci associated with high content of iron and zinc in grain were mapped on chromosome 3, 4 and 8 in Chittimutyalu, a landrace and four loci on chromosome 3, 4, 6 and 12 were mapped in Ranbir Basmati at DRR. Two loci from chromosome 3 and one locus from chromosome 4 were found to be common between the two donors for iron and zinc

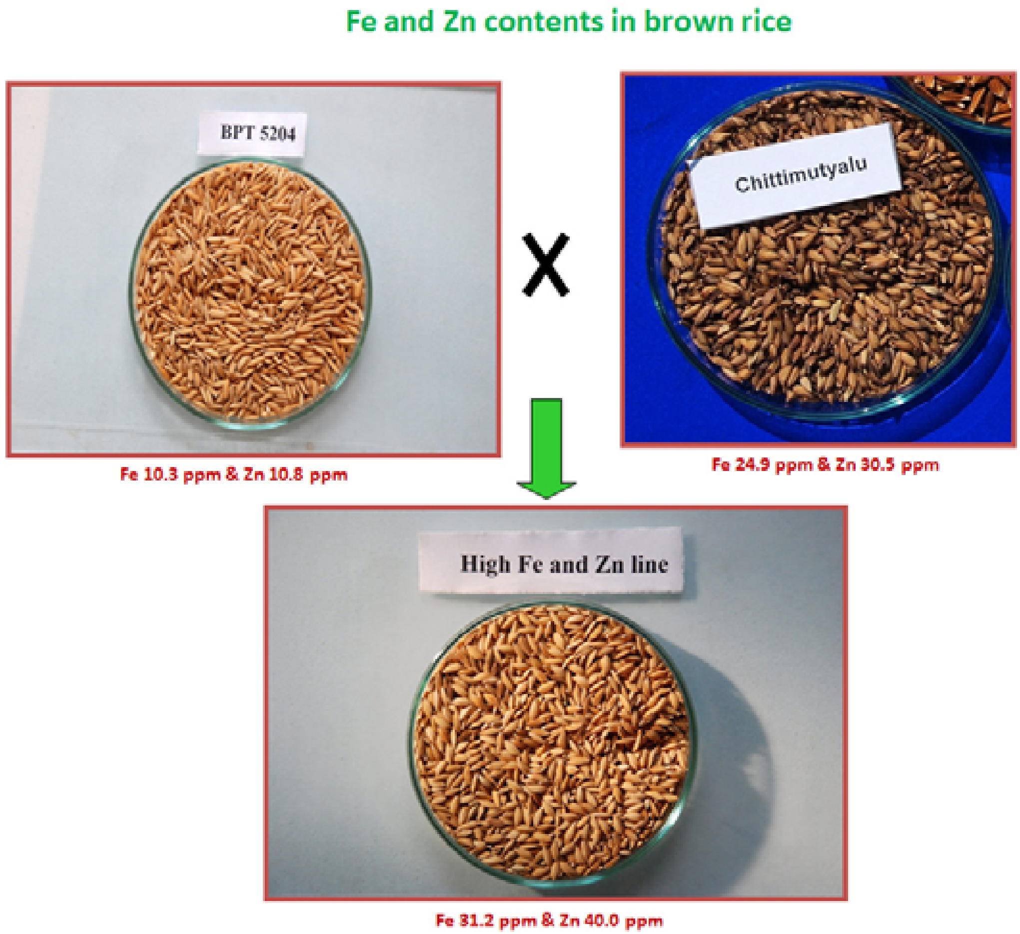

Figure 3. Improved rice selection from the cross BPT 5204 × Chittimutyalu with high iron and zinc contents.

metabolism. In the segregating population derived from Samba Mahsuri/Chittimutyalu, recombinant sd1 gene from Samba Mahsuri and aroma gene from Chittimutyalu were identified with maximum background genome of Chittimutyalu and high concentrations of iron and zinc in grains. Attempts were also made to identify the regions associated with iron and zinc contents in the grains. Molecular breeding involving the utilization of DNA markers for selection of plants based on the iron and zinc contents is suggested to be the best strategy.

Conclusion

- Plant breeding and biotechnology tools are good for fighting micronutrient malnutrition.
- The final permanent solution to micronutrient malnutrition is breeding staple foods that are dense in minerals and vitamins to provide a low-cost, sustainable strategy for reducing levels of micronutrient malnutrition.
- Molecular marker technology expedites the development of rice varieties with improved iron and zinc contents through identified genomic regions.
- Iron and zinc contents in brown and milled rice of national and international germplasm need to be estimated for identification of donors for future deployment in the nutritional breeding program and also to get mapping information on association of iron and zinc contents in grains.
- Rice lines in the genetic background of elite rice varieties possessing optimum concentration of zinc in the endosperm will be developed and released for cultivation.

References

Anonymous. 2013. 4^{th} advance estimates, Indian agristat. New Delhi, India: Ministry of Agriculture.

Bouis HE. 2002. Plant breeding: A new tool for fighting micronutrient malnutrition. Journal of Nutrition 132:491–494.

Graham RD, Senadhira D, Beebe S, Iglesias C and **Monasterio I.** 1999. Breeding for micronutrient density in edible portions of staple food crops: conventional approaches. Field Crops Research 60:57–80.

Graham RD and **Welch RM.** 1996. Breeding for staple food crops with high micronutrient density. Working papers on agricultural strategies for micronutrients no. 3. Washington, DC, USA: International Food Policy Research Institute.

Gregorio GB. 2002. Progress in breeding for trace minerals in staple crops. Journal of Nutrition 132:500–502.

Gregorio GB, Senadhira D, Htut H and **Graham RD.** 2000. Breeding for trace mineral density in rice. Food and Nutrition Bulletin 21:382–386.

Kandoi A, Bhatia BD, Oandey S, Sen PC and **Satya K.** 1991. Cellular immunity status in anaemia in pregnancy. Indian Journal of Medical Research 94:11–15.

Maret W and **Sandstead HH.** 2006. Zinc requirements and the risks and benefits of zinc supplementation. Journal of Trace Elements in Medicine and Biology 20(1):3–18.

McClain, Craig J, Edward J, Kasarskis Jr and **Allen JJ.** 1985. Functional consequences of zinc deficiency. Progress in Food and Nutrition Science 9:185–226.

Monasterio I, Palacios-Rojas N, Meng E, Pixley K, Trethowan R and **Pena RJ.** 2007. Enhancing the mineral and vitamin content of wheat and maize through plant breeding. Journal of Cereal Science 46:293–307.

Nestel P, Bouis HE, Meenakshi JV and **Pfeiffer WH.** 2006. Biofortification of staple food crops. Journal of Nutrition 136:1064–1067.

O'Brien KO, Zavaleta N, Caulfield LE, Dong-Xiao Yang and **Abrams SA.** 1999. Influence of prenatal iron and zinc supplements on supplemental iron absorption, red blood cell iron incorporation, and iron status in pregnant Peruvian women. American Journal of Clinical Nutrition 69:509–515.

Sandstead HH. 1985. Requirements of zinc in human subjects. Journal of the American College of Nutrition 4:73–82.

Scholl TO, Hediger ML, Fischer RL and **Shearer JW.** 1992. Anemia vs. iron deficiency: increased risk of preterm delivery in a prospective study. American Journal of Clinical Nutriton 55:985–988.

Welch RM and **Graham RD.** 2004. Breeding for micronutrients in staple food crops from a human nutrition perspective. Journal of Experimental Botany 55:353–364.

Wingerd J, Christianson R, Lovitt WV and **Schoen EJ.** 1976. Placental ratio in white and black women: relation to smoking and anemia. American Journal of Obstetrics & Gynecology 124:671–675.

Wu JG, Shia C and **Zhanga X.** 2003. Estimating the amino acid composition in milled rice by near-infrared reflectance spectroscopy. Field Crops Research 75(1):1–7.

Zimmermann MB and **Hurrell RF.** 2002. Improving iron, zinc and vitamin A nutrition through plant biotechnology. Current Opinion in Biotechnology 13(2):142–145.