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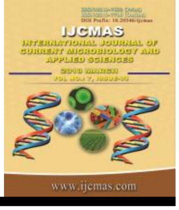

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Dealing with Zinc and Iron Deficiency in Rice: Combine Strategies to Fight Hidden Hunger in Developing Countries

Ritasree Sarma*, H.V. Vijaya Kumara Swamy and H.E. Shashidhar

Department of Plant Biotechnology, University of Agricultural Science,
GKVK, Bengaluru, Karnataka, India
**Corresponding author*

ABSTRACT

Zinc and Iron are essential micronutrient for both plant growth and human health but it is often reported to be deficient in regions where rice is use as staple food. Although significant progresses are made in understanding genetic and molecular mechanism of micronutrient acquisition but these need to be characterize to increase the bioavailability of these micronutrients. Biofortification is suggested to be a sustainable and cost-effective approach in this perspective and for that combination of various agronomic and genetic strategies should be put in place without delay.

Keywords

Iron, Zinc, Biofortification, Malnutrition

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Introduction

Rice is the primary staple food for more than half the world's population and together they directly supply more than 50% of all calories consume by the entire human population (Jia-Yang *et al.*, 2014). Total rice production is increases to 751.9 million tonnes worldwide (FAO, 2017) and among that 90 percent is produce and consume in developing countries. But unfortunately, about 870 million people are suffering from chronic undernourishment globally (Da Silva *et al.*, 2013) and vast majority of them are from developing countries where rice is closely associated with food security and political stability. So, improving the micronutrient status of rice is very important to tackle key nutrition and health related problems of these large numbers of populations, most notably developing countries.

Among the various micronutrients, iron (Fe) and zinc (Zn) are important for both plant growth and human health. In developing countries, iron and zinc deficiencies are reported to be the sixth and fifth highest health risk factor respectively (Freitas *et al.*, 2016; Sharma *et al.*, 2013) causing a high mortality rates. So, overcoming these nutritional deficiencies is need of hour.

Various strategies to improve micronutrient status include food supplementation, food fortification and biofortification (Masuda *et al.*, 2013). Among them biofortification is appears to be the most feasible, sustainable and economical as poor families of developing countries cannot afford other strategies (Nakandalage *et al.*, 2016). For this, selection of effective genetic and crop management approach is of utmost importance.

Importance of zinc

Role in plants

Zinc is one of the key micronutrient involve in regulating various biological and physiological processes in plants. In rice tissues, typical zinc concentration is around 35 to 100 ppm and deficiency symptoms appear when concentration drops below 20 ppm. Zinc deficiency affects photosynthesis due to altered chloroplast pigments (Table 1) (Samreen *et al.*, 2017) and results in short internodes, decrease in leaf size and delayed maturity, sterile spikes, leaves with brown botches and streaks (Abdullah, 2015).

Further it reduces pollen viability leading to fewer grain set and severe yield penalties worldwide (Disante *et al.,* 2010).

Impact in human health

Zinc is one of the important trace elements whose role in human health is undisputable. Cellular zinc homeostasis is important for proper release and action of insulin (Rutter *et al.,* 2016), modulating oxidative stress and various age-related disorder (Prasad, 2013). Insufficient intake of zinc in humans include emotional disorder, weight loss, dysfunctions, atherosclerosis, several malignancies, alopecia, diarrhea (Rutter *et al.*, 2016, Chasapis *et al.*, 2012) decline in immune competence and certain neurological and physiological problem (Roohani *et al.,* 2013).

Importance of iron

Role in plants

Iron is one of the important micronutrient that requires to maintain proper metabolic and physiological processes in plants. It acts as cofactor for many enzymes and proteins of mitochrondria and chloroplast and hence it has major role in life sustaining processes like photosynthesis and respiration. It has role in scavenging of ROS and act as key element to ensure electron flow through the PSII–b6f/Rieske–PSI complex in choloroplast (Zargar *et al.*, 2015). Further insufficient iron uptake leads to iron deficiency symptoms such as interveinal yellowing and chlorosis of emerging leaves, less dry matter production, reduced sugar metabolism enzymes (El-Jendoubi *et al.*, 2014; Das, 2014), seed dormancy (Murgia *et al.*, 2017).

Impact in human health

Iron is the most abundant transition metal involve in various biological processes. Almost two-thirds of the body iron is found in the hemoglobin present in circulating erythrocytes, 25% is contained in a readily mobilizable iron store and the remaining 15% is bound to myoglobin in muscle tissue and in a variety of enzymes involved in the oxidative metabolism and many other cell functions (IOM, 2001).

Abnormal iron homoeostasis can induce cellular damage through hydroxyl radical production which can cause the oxidation and modification of lipids, proteins, carbohydrates, DNA and leads to various neuro generative diseases like Alzheimer's disease and Parkinson's disease (Ward *et al.*, 2014). Further iron deficiency anaemia is a major problem affecting around 2 billion people in both developed and developing countries (WHO, 2016).

Table.1 Chlorophyll contents (mg kg^{-1}) on dry weight basis in mungbean varieties at different concentrations of Zn in solution culture

Zn treatment	V1	V2	V3	V4	Mean±St.dv
Control	35.7f	73.45de	93.12 cd	105.93c	78.55b 30.63
1µM	36.81f	145.30b	210.82a	221.01a	153.5a 84.71
2 µM	64.54e	146.07b	210.57a	226.08a	161.9a 73.52
Mean±St.dv	45.69c	123.6b	171.5a	184.4a	
	16.34	41.71	67.88	67.95	

V1 = Ramazan, V2 = Swat mungI, V3 = NM92, V4 = KMI.St. d = standard deviation. The mean followed by similar letter (s) are not significantly different at *P* = 0.05.

Table 2. Effect of different forms of foliar Zn fertilization on the percentages of solubility, retention, transported and uptake efficiency of Zn among three rice cultivars

Treatments	Cultivars[a]					
	Hai7	Bing91185	Biyuzaonuo	Hai7	Bing91185	Biyuzaonuo
	Solubility (%)			Retention (%)		
Control	29.17 c	30.58 c	25.70 b	14.53 c	14.91b	14.39 c
Zn-EDTA	30.75 c	31.21 bc	26.06 b	14.82 bc	14.96 b	15.04 bc
Zn-Citrate	30.90 bc	31.13 bc	26.20 b	14.93 bc	15.78 b	15.11 bc
ZnSO4	32.68 a	32.24 a	28.54 a	16.68 ab	18.29a	16.80 ab
Zn-AA	31.64 ab	32.62 a	27.73 a	17.54 a	18.80a	17.53 a
Zn effect by f-test[b]	*	*	***	*	***	*
	Transport (%)			Uptake efficiency (%)		
Control	9.35 b	16.09 b	8.99 c	6.95 c	9.48 b	6.01 b
Zn-EDTA	9.61 b	16.23 b	9.57 c	7.52 bc	9.73 b	6.39 b
Zn-Citrate	11.08 ab	16.15 b	9.96 bc	8.02 b	9.94 b	6.57 b
ZnSO4	13.27 a	18.43 ab	13.53 a	9.79 a	11.84 a	8.66 a
Zn-AA	13.05 a	19.11 a	12.42 ab	9.68 a	12.39 a	8.30 a
Zn effect by f-test [b]	**	*	*	***	***	***

aDifferent letters after number in the same column designated significant difference by LSDP,0.05.b Significant effects: NS = not significant at P.0.05*at P,0.05; **at P,0.01;***at P,0.001.

Table.3 Main effects of cultivation system, genotype, and Fe application on shoot dry weight, shoot Fe concentration, and shoot Fe content of rice at tillering stage

Main effects and factors within main effects	Shoot dry weight (kg ha−1)	Shoot Fe concentration (mg kg−1)	Shoot Fe content (g ha−1)
Cultivation system			
Aerobic	935 a	294 b	27 b
Flooded	825 b	393 a	32 a
Genotype			
Qiuguang	659 c	378 a	25 b
K150	788 bc	295 b	23 b
Han72	759 bc	361 ab	27 b
89B-271-17(hun)	898 b	332 ab	30 ab
Han277	1059 a	348 ab	36 a
Han297	1119 a	348 ab	36 a
Average	880	344	30
Fe application			
0 (kg ha−1)	819 b	328 a	25 b
30 (kg ha−1)	941 a	358 a	34 a

For each main effect, values in a column followed by the same letter are not significantly different (*P* >0.05).

Table4 Zn concentrations in shoot and root of rice under different water regimes and Zn source treatments

Genotype	Zn treatment	Shoot Zn concentration (mg/kg)		Root Zn concentration (mg/kg)	
		CF	AWD	CF	AWD
Nipponbar	Control	50.2b	54.6b	86.9c	90.7c
	ZnSO4	60.7a	63.6a	130.6a	143.9a
	Zn-EDTA	59.6a	61.3a	119.5b	117.7b
	Mean	56.9B	59.8B	112.3A	117.5B
Jiaxing27	Control	62.0b	65.3b	96.2c	102.1b
	ZnSO4	68.7a	72.6a	144.5a	153.5a
	Zn-EDTA	66.2ab	71.5a	130.0b	149.9a
	Mean	65.6A	69.8A	123.6A	135.1A

Within a column, means followed by different letters are significantly different at *P*<0.05 according to Duncan's multiple range test. Lower-case and upper-case letters indicate comparisons among three Zn treatments and between two genotypes, respectively

Table 5 Iron and Zn concentrations in individual plant tissues of transgenic progeny classified as high-yield(CHY) and low-yield(CLY)in the OE-*OsNAS*/IR64 and OE-*OsNAS*/Esp progenies

Progeny type	Concentration (mg g^{1} DW)											
	Root		Stem/sheath		Non-flag leaf		Flag leaf		Panicle		Grain	
	Fe	Zn	Fe	Zn	Fe	Zn	Fe	Zn	Fe	Zn	Fe	Zn
OE-OsNAS/IR64												
NS (*n* D 3)	6267	18.7	273	7.8	237	10.2	205	9.8b	89b	16.9b	14.4b	15.3b
+C HY (*n* D 6)	7350	18.5	258	12.0	251	10.6	2131	1.3b	107ab	11.1b	18.0b	23.2b
+LY(*n* D 4)	9150	22.5	283	26.2	253	15.6	193	16.6a	122a	34.6a	28.8a	55.9a
P-value $_{\text{(progenytyp)}}$	n.s.	n.s.	n.s	.n.s.	n.s	.n.s.	n.s.	**	*	**	**	***
OE-OsNAS/Esp												
NS (*n* D 3)	8000	25.0	207	9.0	295	13.2	295	15.2	155	10.0	16.1b	20.2b
+ HY (n D 10)	8120	43.4	234	19.0	337	13.7	296	17.9	157	15.2	28.6a	38.3a
+ LY(*n* D 3)	6333	59.0	243	16.0	423	10.53	201	4.2	173	16.13	6.1a	63.0a
P-value $_{\text{(progenytyp)}}$	n.s.	n.s.	n.s	.n.s.	n.s	.n.s.	n.s.	n.s.	n.s.	n.s	***	***

n.s.,not significant.Within each column,values with different letters represent significant differences between progeny type at the 5%leve lbyHochberg'sGT2test.The values givenaremeans.*P < 0.05, **P < 0.01, ***P < 0.001. NS,nullsegregants;DW,dryweigh

Agronomic strategy for improving iron and zinc uptake

Application of fertilizers

Nitrogen (N) is an essential macronutrient (Sarwar *et al.*, 2010) which helps to improve translocation of other micronutrients like iron and zinc in various plants. Better N nutrition promotes protein synthesis, which is a major sink for Fe and Zn and enhances the expression Zn and Fe transporter proteins, such as ZIP family transporters (Cakmak *et al.*, 2010), YSL protein synthesis and nitrogenous compounds formation, such as NA and DMA, both of which participate in Zn and Fe transport in rice (Slamet-Loedin *et al.*, 2015). So, application of N fertilizer could improve Fe and Zn in rice grains but effect varies depending on genotypic different and rate or method of application. Split application of nitrogen fertilizer in proper time corresponding to plant requirement found to be effective and help to increase Fe content of rice grain and enhance rice grain nutritional value (Fei *et al.,* 2008). N fertilizer rate combined with Zn application method show a clear increase in both grain yield and Zn content as the N fertilizer level increased from 200 to 300 kg/ha. Fe and Zn content in different parts of rice plant may be affected by nitrogen fertilizer thus increasing the nitrogen fertilizer up to 160kg/ha has reported to improve Fe and Zn concentrations in brown rice by 28.96%, and 16.0% for IR64 and by 22.16% and 20.21% for IR68144 compared with control (Hao *et al.,* 2007).

An estimation of soil Zn and application of Zn fertilizer to Zn deficit soil is important for Zn biofortification (Mallikarjuna Swamy *et al.,* 2016). But the response to Zn fertilizer has been shown to differ across rice genotypes, methods of application and soil conditions (White *et al.,* 2011). Foliar application of Zn fertilizers has shown better results than soil application for increasing grain Zn concentration, but the magnitude of this increase is not consistent across genotypes (Table 2) (Mabesa *et al.,* 2013). Application of Fe fertilizer is direct and effective method for enhancing Fe content in rice grain (Li *et al.,* 2016). Among the various iron forms chelated iron sulphates results in higher root iron concentrations while a higher leaf iron concentration is observed when iron citrate is used. Effects of foliar application of different forms of iron fertilizer at different plant developmental stages are studied in rice and it is shown that application of the synthetic chelating agents like DTPA-Fe form at the anthesis stage results in about 20% increase in iron content of polished rice grains (He *et al.,* 2013). In addition to grain iron concentration, iron fertilization positively influences the grain zinc concentration in rice and wheat (Zeidan *et al.,* 2010, Zaigham *et al.,* 2014)

Water management

Rice is a semi aquatic crop grown under lowland condition but as the fresh water crisis increasing day by day, rice is now grown under various irrigation management options like always aerobic, always anaerobic and many variations along the aerobic-anaerobic spectrum (Bouman *et al.,* 2007). In aerobic conditions, rice is grown as a dry field-crop in irrigated not in flooded, fertile soils (Gao *et al.,* 2006). But shifting from anaerobic to aerobic condition has benefits and risking of micronutrient status of grains in different soil types which need to be understand. In aerobic conditions nitrogen is uptake as nitrate which may cause an imbalance in the cation/anion ratio, resulting in exudation of OH^- into the rhizosphere with a subsequent rise in soil pH and redox potential. A higher redox potential can accumulate much more oxidized Fe^{3+} which is not readily available for plant uptake (Zuo *et al.,* 2011).

While in flooding condition, Fe- oxides are dissolved when the Fe^{3+} is reduced to Fe^{2+} which weakens the oxide stability and increases its water-solubility (Kirk, 2004).

This releases much more Fe into the soil solution which is nearly sufficient for plant uptake. In both aerobic and flooded condition, application of ferrous sulphate significantly increases shoot Fe concentration and shoot Fe content at tillering stage but at physiological maturity, grain iron is found significantly lower in aerobic than in flooded plots (Table 3) (Xiaoyun *et al.,* 2012).

Under anaerobic conditions, Zn forms as insoluble zinc sulphide (Bostick *et al.,* 2001) and insoluble carbonate mixtures (Kirk, 2004) which plant cannot uptake. While increase oxidation under aerobic condition decrease Zn precipitation as ZnS (Carbonell-Barrachina *et al.,* 2000) and further increase availability of iron oxidizing/reducing bacteria, AM fungi associated with root-induced rhizosphere processes such as exudation of Zn chelators and have positive effect on nutrient availability (Gao *et al.,* 2017).

Alternative wetting and drying (AWD) is one of the promising water saving technology which is widely adapted in many rice producing countries (Lampayan *et al.,* 2015). It combines both the beneficial effects of aerobic and anaerobic cultivation system which potentially decrease water inputs by 5%–35% when compared with Continuous flooding (CF) with the yield of rice grain either being maintained (Chapagain *et al.,* 2010).

Although for iron, it does not seem to be promising for increasing iron content in grain (Nortona *et al.,* 2017) but shows effective for increment of grain zinc content alone or when combine with various zinc fertilizer treatments (Table 4) (Wang *et al.,* 2014).

Breeding and transgenics approach

Plant breeding (e.g., genetic biofortification) approach is thought to be the cost effective and eco- friendly approach for improving micronutrient status of rice in developing countries. For developing variety with high micronutrient, germ plasm screening is done initially to find out the genetic variation among the existing genetic resource (Slamet-Loedin *et al.,* 2015, Howarth *et al.,* 2017). There is abundant genetic variation for the grain Zn and Fe concentration in both brown and polished grains in the rice germplasm. Different wild relatives, landraces, aus and aromatic accessions, deep water rice and coloured rice are the best sources of high grain Zn. Wild species of rice such as *O. nivara, O. rufipogon, O. latifolia, O. officinalis,* and *O. granulata* also contain high amounts of Zn, around 2–3 fold higher than in the cultivated rice with Zn concentration varying from 37 mg/kg to 55 mg/kg in non-polished grains (Impa *et al.,* 2013; Anuradha *et al.,* 2012; Banerjee *et al.,* 2010).

The world's first Zn enriched rice variety is released in 2013 by the Bangladesh Rice Research Institute (BRRIdhan62), which is claimed to contain 20–23mgZnkg^{-1} for brown rice (Harvest plus, 2015) while another variety by Directorate of Rice Research (DRR-Dhan 45) is released in India with over all mean zinc content of 22.6ppm in polished rice, develop through conventional breeding without compromising yield using the material from Harvest Plus (Balasubramanian, 2016). While in case of iron, rice germplasm has a very narrow genetic variability for endosperm iron content. Iron content changes depending on varieties, IR64 (12.58-12.88mg/Kg), Jasmine 85 (12.84-18.50 mg/Kg) and OMCS2000 (11.77-14.78 mg/Kg) and about 2/3 of iron is lost through milling (Tran *et al.,* 2004). Other advance strategy like mutation breeding also gaining importance in this regard. A number of IR64 mutants produced by the treatment with Sodium azide, a mutagen, is reported to have high Zn. Three IR64 mutant lines viz., M-IR-180, M-IR-49 and M-IR-175 has more than 26 mg kg −1 Zn in polished rice as against 16 mg kg −1 in IR64 has been reported (Jeng *et al.,* 2012) A combinatorial approach using both hybridization and induced mutation is also found to be effective to develop new cultivar expressing several improved traits like improve aroma and high iron content (Cua, 2016).

Although various approaches are trying from last15 years to reach the 30% EAR (Estimated Average Requirement) nutritional targets for iron and zinc concentrations in polished rice grains (Bouis *et al.,* 2011) but still it remains a major challenge. This 30% EAR was calculated as 13 μ g g−1 Fe and 28 μ g g−1 Zn in polished grains taking into account of 90% micronutrient retention after processing and 10% bioavailability for Fe and 25% bioavailability for Zn (Trijatmiko *et al.,* 2016). In this aspect transgenic approach can be a better option.

Several studies exhibit the associated increase in Fe and Zn content in rice grain by over expression or activation of various transporters genes. Over expression of three rice NAS homologous proteins, (OsNAS1, OsNAS2, and OsNAS3) resulted in 2-fold increase in Fe and Zn concentration in polished rice (Sasaki *et al.,* 2014) while over expression of *OsHMA3* enhance the uptake of Zn by up regulating the ZIP family genes in the roots (Johnson *et al.,* 2011).

Combined improvement of iron, zinc and β-carotene content in rice endosperm are improve by expressing Arabidopsis *Nicotianamine Synthase 1* (*Atnas1*), Bean *Ferritin* (*PvFERRITIN*), bacterial *Carotene Desaturase* (*CRTI*) and maize *PHYTOENE Synthase* (*ZmPSY*) in a single genetic locus (Singh *et al.,* 2017).

High yielding rice line with Zn and Fe biofortified in polished grains can also be develop by overexpressing OsNAS2 in various genotypes (Table 5) (Singh *et al.,* 2017) Further field evaluation of transgenic events is also reported to be successful without a yield penalty or altered grain quality where NASFer-274 containing rice (*OsNAS2*) and soybean ferritin (*SferH-1*) genes is use in a single locus insertion (Cua, 2016).

Iron and zinc deficiency are the most common type of micronutrient malnutrition where population of all groups in all the region of world is get affected. So, for effective and sustainable solution of this problem a complete understanding of iron and zinc uptake, translocation and further allocation to reproductive organs is needed. Agronomic interventions for increment of micronutrient status are effective but it is erratic, depends on cultivar and environment.

Genetic intervention is a cost effective and sustainable strategy but for that further exploitation of wide genetic variety of rice germplasm is necessary. Consequently, new combined agronomic and genetic strategy should be developed to address this problem of malnutrition for people whose staple diet is rice.

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