

## Review Article

# Enhancement of Iron and Zinc in Rice Grain through Biofortification Approach

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## ABSTRACT

Rice (*Oryza sativa* L.) being most important staple food among cereal grains for large part of world's human population, for the major source of carbohydrate and even protein. Rice does not supply minerals adequately when consumed in polished form particularly for iron (Fe) and zinc (Zn). Micronutrient malnutrition affects over three billion people worldwide, So Bio-fortification is an effective way of combating micronutrient deficiencies. The selection of best candidate genotype and availability genetic variability accessions will fulfill the conventional breeding approaches, but Marker Assisted Selection (MAS) combining with the modern molecular breeding will have the greater levels of micronutrient enrichment program.

### Keywords

Rice,  
Micronutrients, Fe,  
Zn and MAS

## Introduction

Rice (*Oryza sativa* L.), is one of the agronomically and nutritionally important cereal crop and most important staple food among cereal grains for a large part of the world's human population mainly in the developing countries, being the major source of carbohydrate and even protein. It provides around 21 per cent of dietary energy and 15 per cent of protein to global population (IRRI, <http://irri.org/about-rice/rice-facts/rice-basics>).

It is a major source of food for more than 2.7 billion people on a daily basis and is planted on about one-tenth of the earth's arable land. The area under its cultivation is 163 million hectares producing 718 million tons and a productivity of 4.4 tons/ha in the world (FAOSTAT, 2013). India is the

second largest rice producing country with a cultivated area of 45 million hectares, production of over 148 million tons and mean productivity of 3.2 tons/ha.

Unfortunately, rice does not supply minerals adequately when consumed in polished form, and thus it is a poor source of essential micronutrients such as iron and zinc. Malnutrition is a large and growing problem in the developing world. Micronutrient malnutrition, and particularly iron and zinc deficiency affect over three billion people worldwide, mostly in developing countries and Rice is the most amenable crop for molecular genetic studies due to its small genome size, enriched genetic map, availability of entire genome sequence and relative ease of transformation.

Improvement in agronomic traits of rice is bound to affect a sizeable population, since it is a primary source of sustenance. Rice is used as staple food, in countries where the per capita consumption is very high ranging from 62 to 190 kg/year. Thus, even a small increase in the nutritive value of rice can highly contribute to human nutrition (Graham *et al.*, 1999).

Among all micronutrients which are essential for sustaining human health, iron, zinc, iodine and vitamin A have been reported to be most at risk of malnutrition (Welch and Graham, 2004). The main reason for human mineral malnutrition is the relatively low content of cation minerals in plant based foods in combination with the abundance of anti-nutrient compounds that severely reduce their bioavailability. Deficiencies in bioavailable iron (Fe), zinc (Zn) and other essential cation minerals in human food, causing mineral malnutrition, affect a large proportion of the world population.

Iron and Zinc deficiencies are among the most prevalent micronutrient deficiencies in humans, affecting two billion people and causing more than 0.8 million deaths annually (WHO, 2003). Zinc deficiency causes most important health risk factors in developing countries and worldwide. Amongst children, zinc deficiency is commonly associated with diarrhea, pneumonia, stunting and child mortality. Recent epidemiological studies reported that whole-grain intake (such as brown rice) is linked to disease prevention against cancer, cardiovascular disease, diabetes and obesity (Slavin, 2003). These deficiencies account for decreased work productivity, reduced mental capacity, stunting, blindness, increased child mortality, and elevated morbidity and mortality (Baishya *et al.*, 2015).

Earlier studies were mostly confined to the production of high yielding varieties, but currently the focus has shifted to enrichment of micronutrients in staple food crops like rice and wheat which helps in ameliorating the problems of micronutrient deficiency in the form of hidden hunger in the human population.

Scientists have coined the term “biofortified” for genotypes that deliver increased levels of essential nutrients or vitamins. Biofortification, when applied to staple crops, such as rice, is a sustainable approach, provided that access to the technology in the form of seeds is unrestricted (Anuradha *et al.*, 2012). Biofortification of food crops with iron and Zinc remains a priority area of research. Iron (Fe) and Zinc (Zn) are basic nutrient elements for plants, which assist metabolism and development in plant parts.

### **Bio-fortification**

Micronutrient enrichment in food crops through the process of biofortification has the potential to combat deficiencies in humans. Biofortification is the process of increasing the bioavailable concentrations of an element in edible portions of crop plants through traditional breeding practices or modern biotechnology. Biofortification is an effective and cheaper alternative to traditional ways of combating micronutrient deficiencies, i.e., healthy food (which is often expensive), micronutrient supplementation and food fortification. Biofortification under Harvest Plus program are underway for six staple foods: rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), maize, (*Zea mays* L.), cassava (*Manihot esculenta* Crantz), orange-fleshed

The best candidate genotypes selected for biofortification program should fulfill the

criteria of high productivity; show considerable health impact for micronutrient enrichment levels; the trait for micronutrient must be relatively stable across various environmental conditions and climatic zones and consumer acceptance for taste and cooking quality (Goto *et al.*, 1999., Gregorio *et al.*, 1999., Welch, 2001 and Welch and Graham, 2004).

Successful crop improvement program depends on the availability of sufficient genetic variability that arises from genetic diversity (Rana and Bhat, 2004). There is a wide genetic variability in rice among and between cultivars / varieties which is useful for trait improvement in breeding programmes.

There is a lot of scope for increasing the concentration of these elements in rice grains through marker assisted breeding (MAS) as there is availability of large genetic variability in micronutrient concentration in rice grains. However, for efficient breeding, knowledge about the genetics of the observed variation and an insight into the genotype by environment (G x E) interaction are essential.

Modern conventional breeding techniques, including molecular marker-assisted selection, may be very useful in accelerating the development of more nutritious rice varieties. Combining high yields and high grain nutritional value thus appears to be possible without any genetic manipulation.

### **Improvement of iron and zinc concentration in plants**

Staple crops that are micronutrient-enriched, either through traditional breeding or molecular biological techniques, are powerful tools that can help the people who are most vulnerable to micronutrient

malnutrition (Welch, 2002). Increasing the amounts of micronutrient metals stored in seeds and grains of staple food crops increases the yield potential of these crops when they are sown in the micronutrient-poor soils so prevalent in the developing world (Welch, 2002).

Earlier investigation has indicated that micronutrient enrichment traits are available within the genomes of major crops; as a result, improvements in micronutrient concentration can be made without adversely affecting yield. Furthermore, enrichment traits appear to be stable across soil types and climatic environments (Welch and Graham, 2004). Further research is needed to determine if increasing levels of micronutrients in staple foods can significantly improve the nutritional status of people suffering deficiency (Welch and Graham, 2004).

Selecting elite germplasm having enormous genetic potential for high iron and zinc in breeding programs can bring necessary variability of these micronutrients in staple food crops like rice. Total variability of a trait is divided into genotypic variability and phenotypic variability. The estimate of variability suggests the variation in heritable portion of a trait that could be transferred from parent to offspring in response to selection (Hallauer and Carena, 2009).

High yield along with more nutrients is important for present day healthy diet. Since 1992, researchers at IRRI have been evaluating the genetic variability of iron concentration in rice grain (Gregorio *et al.*, 2000).

### **Iron deficiency in plants**

Iron deficiency in plants is a major problem worldwide because of low iron availability

in the aerobic environment and at biological pH, especially in the calcareous soils that cover about one-third of the surface of the earth (Yang and Volker, 1999; Rengel, 2005).

There were two major strategies by which plants can overcome iron deficiency. Strategy I for plants, dicotyledons and non-graminaceous monocotyledons, iron efficiency is a function of a number of induced responses by plant roots; primarily, an increased rate of reduction reactions ( $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$ ) at the root surface, an increased rate of rhizosphere acidification, increased release of phenolic compounds, e.g., caffeic and chlorogenic acid, and the accumulation of citric acid in plant roots (Hell and Stephan, 2003). Three types of root membrane-bound Fe(III) reductases have been suggested for strategy I plants. There is a standard reductase, which occurs in the plasma membranes of all higher plant species but does not reduce chelated iron compounds, and inducible and constitutive reductases, which can reduce Fe (III) in chelates from various origins. Apparently, inducible reductase takes effect upon the increased activity of constitutive reductase under iron stress conditions (Rengel, 2002).

Strategy II plants, which consist of the *graminaceae*, respond to iron deficiency by the increased release of phytosiderophores (Rengel, 2002). Strategy II plants also possess membrane-bound standard reductases that are capable of reducing electron donor molecules such as ferricyanide, but they do not possess the inducible and constitutive reductases of Strategy I plants (Yang and Volker, 1999).

### **Zinc deficiency in plants**

It has been estimated that zinc deficiency is the most widespread micronutrient

deficiency affecting production and quality of cereals, such as wheat, rice, and other crops. Genotypes of plants vary widely in their tolerance of zinc deficient soils. Tolerance to zinc deficiency is termed “zinc efficiency,” and defined as the ability of a genotype to grow and yield well in soils too deficient in available zinc for a standard cultivar (Yang and Volker, 1999).

Zinc enters the plant mainly via root absorption of  $\text{Zn}^{2+}$  from the soil solution. Because of the low zinc concentration in the soil solution, supply of zinc by mass flow is limited and diffusion is the major process by which zinc reaches the roots.

Therefore, root morphology and vitality characteristics are crucial in how efficiently the plant explores for zinc in the soil. Less work has been done on understanding the mechanisms of zinc uptake than iron uptake in higher plants; however, zinc uptake appears to be a function of transport across the plasma membrane, which is largely metabolism-dependent, and genetically controlled (Yang and Volker, 1999).

For example, zinc-efficient wheat genotypes release more phytosiderophores than do inefficient genotypes (Rengel, 2002). The speculated mechanisms of zinc uptake in the plant include thermodynamic transport of zinc, driven by an electrochemical potential gradient across the membrane; transport through an  $\text{H}^+$ -ATP-ase ion pump; the involvement of zinc-chelate transport system; and ion channels (Yang and Volker, 1999).

A number of attributes are characteristic of zinc-efficient genotypes, such as more and finer small roots ( $\leq 0.2$  mm), the release of zinc chelating phytosiderophores, and the efficient use and compartmentalization of zinc within cells (Rengel, 2002).

## **Molecular breeding strategy for improving grain iron and zinc concentrations**

Exploitation of large genetic variation for improving the grain iron and zinc concentrations existing in cereal germplasm is an important approach to minimize the extent of iron and zinc deficiencies in the developing world. Plant breeding programs involving biofortification of staple food crops such as rice and wheat require screening of germplasm for varieties and elite lines having iron and zinc dense grains to be used as donor parents. Using this method, plant breeders search seed or germplasm banks for existing varieties of crops which are naturally high in nutrients. Then they cross-breed these high-nutrient varieties with high-yielding varieties of crops, to provide seed with high yields and increased nutritional value. A study conducted by Garcia-Oliveira *et al.*, (2009) in 85 introgression lines grown during 2005 and 2006 revealed highest quantities of zinc with a combined mean value of 27.1 ppm, whereas iron was found in the lowest quantities with a mean performance of 9.6 ppm. It is suggested that the rice based diet should contain 14.5 ppm iron (Johnson *et al.*, 2011) and 24 ppm zinc.

### **Iron and Zinc Concentrations in Brown and Polished Rice Grain**

Several studies have reported the evaluation of germplasm and on the advanced breeding lines for grain iron and zinc content (Brar *et al.*, 2011 and Gregorio *et al.*, 2000). 11,400 rice samples of brown (unpolished) and milled rice were evaluated for iron and zinc during 2006-2008 by Martínez *et al.*, (2010). They found that brown rice had 10–11 ppm iron and 20–25 ppm zinc while milled rice had 2–3 ppm iron and 16–17 ppm zinc. It has been estimated that more than 70 % of

micronutrients are lost during polishing (Sellappan *et al.*, 2009). Thus rice grain iron content will also vary with degree of milling / polishing.

The distribution of minerals in rice kernels is not uniform. About 50% of the mineral content is located in the bran layer and 10% in the embryo; both will be removed when producing white rice. White rice only contains 28% of the total ash of brown rice (Hunt *et al.*, 2002), ash content being an index for mineral content.

In a study conducted by Lucca *et al.*, (2003), the concentrations of minerals in whole rice grains, hulls, brown rice, bran and polished rice were quantified by inductively coupled plasma mass spectroscopy. The *in vivo* mineral distribution patterns in rice grains and shifts in those distribution patterns during progressive stages of germination were analyzed by synchrotron X-ray microfluorescence. The results showed that half of the total zinc, two thirds of the total Fe, and most of the total K, Ca and Mn were removed by the milling process if the hull and bran were thoroughly polished.

At present, optimization of rice processing could be an effective and practical way to increase the levels of minerals in rice products. Optimization of milling and polishing procedures could help reduce the inevitable loss of minerals, while parboiling and fortification could help increase mineral contents in final rice products (Mannar and Gallego 2002; Mohapatra and Bal 2004; Singh Gujral *et al.*, 2002).

### **QTL base to develop micronutrient rich rice**

Many agriculturally important traits such as yield, quality and some forms of disease resistance are controlled by many genes and

are known as quantitative traits (also 'polygenic,' 'multifactorial' or 'complex' traits).

From the past two decades, the major effort in breeding has changed from traditional phenotypic-pedigree based selection systems to molecular genetics with emphasis on QTL identification and Marker Assisted Selection (MAS). MAS is an excellent tool for selecting beneficial genetic traits that are difficult to measure, that exhibit low heritability and/or are expressed late in development (Davies *et al.*, 2006., Wilde *et al.*, 2007 and Ender *et al.*, 2008).

The regions within genomes that contain genes associated with a particular quantitative trait are known as quantitative trait loci (QTLs) (Collard *et al.*, 2005).

A major breakthrough in the characterization of quantitative traits that created opportunities to select for QTLs was initiated by the development of DNA (or molecular) markers in the 1980s (Collard *et al.*, 2005). One of the main uses of DNA markers in agricultural research has been in the construction of linkage maps for diverse crop species. Linkage maps have been utilized for identifying chromosomal regions that contain genes controlling simple traits (controlled by a single gene) and quantitative traits using QTL analysis (Mohan *et al.*, 1997).

QTL analysis is based on the principle of detecting an association between phenotype and the genotype of markers. Markers are used to partition the mapping population into different genotypic groups based on the presence or absence of a particular marker locus and to determine whether significant differences exist between groups with respect to the trait being measured (Tanksley, 1993; Young, 1996).

A significant difference between phenotypic means of the groups, depending on the marker system and type of population, indicates that the marker locus being used to partition the mapping population is linked to a QTL controlling the trait (Collard *et al.*, 2005).

### **QTL to gene approach**

Efficiency of marker-aided selection in breeding programs depends on the strength of linkage between molecular markers and the target trait. QTL to gene approach is method to identify a novel gene from the QTL sequence of specific traits. Traditionally, anonymous molecular markers are used to establish linkage with a phenotype. However, even for tightly linked markers, the effectiveness of marker aided selection is greatly diminished by the occasional uncoupling of the marker from the trait during many cycles of meiosis in a breeding program. With the availability of large genome databases, it is now possible to predict putative function of a gene based on sequence information, thus enabling the identification of candidate genes involved in a particular biochemical pathway. These candidate genes, or DNA sequences with predicted function, are used as molecular markers to associate with phenotypes expressed in segregating populations or genetic stocks (Thorup and Kearsey, 2000).

### **Transporters**

A variety of transporters provide an efficient tool for transporting ions from the soil into the roots, then distributing them throughout the plant. Genes involved in this transport have now been identified from plants (Grotz and Guerinot 2006).

Zinc-regulated transporter, iron-regulated transporter-like proteins (ZIPs) generally

contribute to metal-ion homeostasis by moving cations into the cytoplasm (Colangelo and Gueriot 2006). This group is found at every phylogenetic level, including archaea, bacteria, fungi, plants and mammals (Grotz and Gueriot 2006). *Arabidopsis* contains 16 members in that family (Maser *et al.*, 2001). There, ZIP1, ZIP2, ZIP3 and ZIP4 proteins functionally complement a yeast strain defective in zinc uptake (Grotz and Gueriot 2006).

### **The ZIP family**

The ZIP family takes its name from the first members to be identified 'ZRT, IRT-like Protein'. IRT1 (iron-regulated transporter) is an *Arabidopsis* cation transporter that is expressed in the roots of iron deficient plants and ZRT1 and ZRT2 (zinc-regulated transporter) are, respectively, the high- and low-affinity zinc transporters of yeast. At this time, over 25 ZIP family members have been identified.

These genes fall into roughly two subfamilies based on amino acid similarities. Subfamily I includes 15 genes in plants (11 from *Arabidopsis*, two from tomato, one from pea and one from rice), two yeast genes (ZRT1 and ZRT2), and a gene from the protozoan *Trypanosoma brucei*. Subfamily II includes 8 genes in the nematode *Caenorhabditis elegans*, one gene in *Drosophila* and two genes in humans (Gueriot, 2000).

Most ZIP proteins are predicted to have eight potential trans-membrane domains and a similar membrane topology in which the amino- and carboxyterminal ends of the protein are located on the outside surface of the plasma membrane. ZIP proteins range from 309 to 476 amino acids in length; this difference is largely due to the length

between trans-membrane domains III and IV, designated the 'variable region' (Gueriot, 2000).

### **The NRAMP family**

The NRAMP (Natural Resistance-Associated Macrophage Protein) protein family, with members also in *Drosophila melanogaster*, *Saccharomyces cerevisiae* and rice (OsNRAMP1), was defined by a highly conserved hydrophobic core with 10 transmembrane domains, several invariant charged residues and other features typical of transporters or channels.

Two groups presented evidence for the involvement of plant NRAMP proteins in iron transport. Curie *et al.*, (2000) showed that both rice and *Arabidopsis* NRAMP1 were able to complement the *fet3fet4* yeast mutant, defective in low- and high-affinity iron transport, with the *Arabidopsis* protein being more efficient than the rice one. They also showed that *AtNramp1* transcript accumulated in response to iron deficiency in roots. Furthermore, overexpression of AtNRAMP1 in *Arabidopsis* increased its resistance to toxic iron concentrations. Thomine *et al.* showed that *AtNramp3* and *AtNramp4* were more efficient than *AtNramp1* in complementing the iron uptake yeast mutant *fet3fet4*. Iron starvation resulted in up-regulation of expression of the *Arabidopsis Nramp* genes. Disruption of *AtNramp3* led to increased cadmium resistance and overexpression of the same gene led to cadmium hypersensitivity. It was suggested that the AtNRAMP3 protein may be involved in iron uptake in roots, with its contribution relative to IRT1 being dependent on pH (Thomine *et al.*, 2000).

As Rice being staple food for more than half the world population across the globe.

Polishing of rice grain, however, is an essential process which is carried out by all rice industries and commercial farmers to remove the aleurone layer that would otherwise make the rice seed rancid during long storage, which lead to loss of micronutrients (Fe and Zn) in the grain and leading to many disorders. So opting the approach of Biofortification for enhancing the iron and zinc concentration combined with molecular methods and mining of various transporters will define the aspects in micronutrient values in rice grain.

### **Future Line of Work**

Combining the traits of high iron, zinc and high grain yield with potential of plant breeding techniques could be aided by candidate gene markers.

Many more genes associated with iron and zinc content in rice have to be explored and the bioinformatics resources can be utilized to a greater extent to improve efficiency.

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