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# Biofortification of Rice

**Humera Aziz[1*]; Ghulam Murtaza[2]**

[1]Department of Environmental Sciences and Engineering, GC University Faisalabad, 38000, Pakistan

[2]Department of Biological Sciences and Technology, China Medical University, Taichung 40402, Taiwan

[*]**Corresponding Author:** Humera Aziz, Department of Environmental Sciences and Engineering, GC University Faisalabad, 38000, PakistanEmail: humeraaziz.uaf@gmail.com

**Published Date:** January 18, 2020

## Abstract

Rice is an important staple food crop for most of the population around the world. On a global basis, more than 2 billion of population are affected from micronutrients deficiencies and alarming health hazards that result from this type of malnutrition. Folate, Zn and Fe deficiencies are among the most commonly found deficiencies around the world especially in developing countries. Biofortification is the increment in the nutritional contents of food crops by biotechnology or plant breeding. The biofortification of the edible parts of staple foods offers an sustainable and inexpensive measure to combat the prevalent human disorders resulting from micronutrients malnutrition. Rice serve as a suitable target for biofortification as most of the people around world eat rice as a staple diet. The white rice or polished rice consists of insufficient amount of micronutrients which is unable to fulfil every day necessity of micronutrients in food. That's why, the biofortification of rice grains is the solution to this problem. By controlling and facilitating the transportation of micronutrients, including absorption from soil, their translocation and especially their loadings in the grains, it is possible to enhance their concentration in the seeds. This chapter will provide a comprehensive overview of the agents governing micronutrients transport in rice, the transporters and genes involved (uptake and transport) and used for micronutrients biofortification in rice and currently available technologies for micronutrients rice biofortification. Moreover, the progress and future prospects of existing biofortification techniques in rice will also be elucidated in this chapter.

**Keywords:** Micronutrients; Global malnutrition; Rice; Biofortification; biotechnology; Transgenic varieties; Gene

**Abbreviations:** Zn: Zinc; Fe: Iron; Mn: Manganese; GNP: Gross national product; SAM: S-adenosyl methionine; NA: Nicotinamide; DMA: 2-deoxymugineic acid; NAS: Nicotinamide synthase; QTL: Quantitative trait loci; CRISPR: Clustered regularly interspaced short palindromic repeats; TALENS Transcription activator like effector nucleases

## 1. Introduction

Biofortification can be defined as increasing the nutritional value of crops (usually minerals and vitamins) via agronomic practices, conventional breeding of plants or modern biotechnological techniques. It has proved as a best strategy to overcome extensive micronutrients malnutrition especially in developing world. More than half of the world's population relies on rice as a staple food. The aleuone layer of rice seed consists of minor amounts of pro vitamin A and Fe. This bran layer and husk is removed during milling process to get edible endosperm called white rice or polished rice. This is the main cause behind worldwide Fe and vitamin A malnutrition [1,2]. The micronutrients Fe, Zn, and Mn deficiencies are most commonly seen. Fe plays various roles as it acts as a cofactor for many enzymes in the human body. Stoltzfus [3] and Hentze et al [4]. reported that the Fe malnutrition might cause serious disorders in humans like anemia. While, cognitive impairment, immune dysfunction hypogonadism, and growth retardation are often observed in Zn deficient peoples [5]. According to the World Health Organization [6], the predominant micronutrient deficiencies in humans are Zn and Fe deficiencies, which are resulting in over 0.8 million deaths every year by affecting two billion peoples worldwide. Although, the deficiency of Mn is less dominant than Zn and Fe deficiencies among the peoples. However, the pathological issues like chronic birth defects and asthma are most commonly reported in the cases of Mn malnutrition. The combined effects of these micronutrient deficiencies pose a significant threat to human health.

Stein [7] stated that the deficiencies of micronutrients had deteriorated the economic productivity and social welfare by increasing the incidents of diseases worldwide. On a global scale, at present more than 3 billion people are suffering from severe disorders resulting from Fe and Zn malnutrition. The consumption of diets containing less nutrition is the main root cause behind the ever-increasing Fe and Zn deficiencies. Specifically, in developing countries, this kind of malnutrition is the major cause of the decreased GNP by reducing work productivity among individuals. According to a study conducted by Wessells et al. [8], around 18 % of the total world population is affected by Zn malnutrition. Shekari et al. [9] stated that On a global scale, among 20 micronutrients which are categorized as deficient in humans, the number of Zn is the 11$^{th}$ while, in less developed countries total 10 micronutrients are reported as deficient where the Zn is ranked at 5th number. The South Asian population is suffering from severe health problems owing to Zn malnutrition as 95 % of total individuals eat rice as a staple food. Figure 1 overviews the world population (%) suffering from deficiencies of micronutrients.

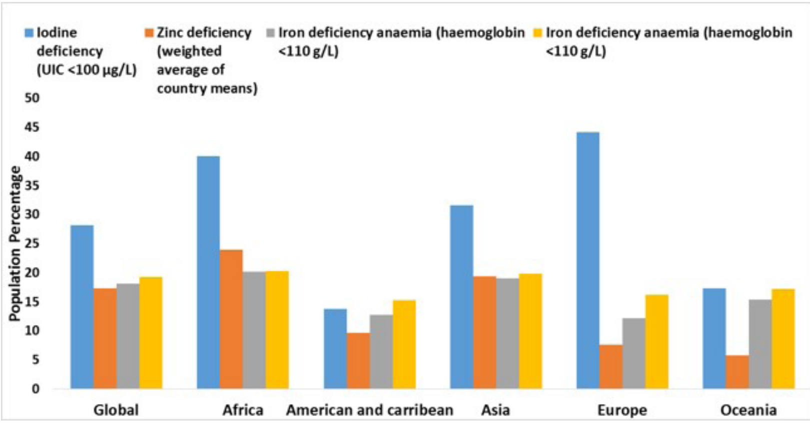


**Figure1:** Population in (%) with deficiencies of selected micronutrients (Source: Black et al [83]).

The Zn and Fe malnutrition arises in the circumstances when people consume a diet with less variation or consume one or two staple foods. Yang et al. [10] reported that in less developed countries, even the population's consumption is based on only rice as a staple food. Moreover, white rice or polished rice is unable to meet regular supplies in foods due to consisting of inadequate quantities of micronutrients [11]. The strategies to alleviate micronutrient's malnutrition include supplementation of micronutrients, nutrient fortification of food on an industrial scale, and grain biofortification. Minhas et al. [12] reported that supplementation of micronutrients and nutrient fortification of food had played well globally to mitigate problems associated with micronutrients malnutrition, but these methods have some shortcomings being expensive and framework requiring for the fortification of foods and the production of micronutrients supplements. Even this malnutrition-related problems may pose even more threat to the people which cannot pay for the non- staple diets rich in micronutrients [13]. Hence, rice biofortification with micronutrients can prove a less costly and defensible strategy to overcome micronutrients malnutrition [12]. In spite of all the above facts that biofortified rice can prove suitable diet, yet very little evidence are available for its usefulness.

## 2. Localization of micronutrients in the seed and rice biofortification

In rice seed, Fe is confined mainly to the scutellum of the embryo and its vascular bundle, endosperm, aleurone layer, and dorsal vascular bundle. As germination proceeds, this Fe is shifted wholly to an embryo, and after 2 days of sowing changes its place to the radicle, leaf primordium, epithelium, and coleoptile [14]. On the other hand, Zn has its maximum portion in the embryo and aleurone laye, and it is disproportionately dispersed to all seed parts [14]. Unlike Mn and Fe, the flow of Zn is very vigorous during the germination process. Mn is found mainly in the embryo and endosperm and as germination starts it started accumulating in the scutellum instead of the coleoptile. Beside these micronutrients, 2-deoxymugineic acid (DMA) and nicotianamine (NA) are also found in the rice seed which during germination are responsible for the chelation and mobilization of micronutrients [14,15,16]. Gregorio et al. [17] reported that different studies highlighted the Fe contents in rice seed with considerable variations. Although Fe is abundantly found in mineral soil but due to lack of solubility, it is not bioavailable, hence in order to increase seed Fe contents supplementation of soil with Fe as a fertilizer is not an effective measure. The fertilization of soil with Zn increase plant growth effectively, but different reports evaluated significantly different rice grain Zn concentration and Zn use efficiency with respect to different rice genotypes [18,19,20,21,22]. The leading factors controlling Fe concentration in the soil are, field conditions like dry and moist soil, carbonates contents and soil pH, while for Zn these are Zn concentration in soil is the Zn status of native soil. [20]. Depending on the Zn status of soil, the Zn concentration may vary from 8 to 47 mg kg−1 within a same variety [21,22] and On the basis of genotype, the Zn concentration in rice grain can be as high as 59 mg kg-1 and as low as 16 mg kg-1 [20].

## 3. Techniques for Biofortification of Rice

**Agronomic (Non-genetic) practices**

Various agronomic practices are there in use to for obtaining biofortification of crop grains and may prove more efficient for enhancingthe nutritional quality of crops as well as yield. The examples of such non-genetic approaches are management practice, fertilizer application, and adding O.M in the soil for improving the availability of nutrient elements [23]. The specific nutrient element may be applied as fertilizer either in soil or to the leaves via foliar application to increase its concentration in that plant and its edible portion. From these two methods, the foliar application of a specific element is more effective in this regard compared to soil application [24]. Moreover, the fertilization of specific element for biofortification may prove more fruitful by adopting integrated soil fertility management approach, which includes a combination of both organic and inorganic fertilizers. It has been proven that agronomic practices like foliar fertilization have enormous potential for increasing micronutrient contents in the rice and its edible part [25,26,27,28].

**Conventional breeding**

The most promising solution to combat micronutrients deficiencies is to develop genotypes after breeding having more vitamins and minerals. Earlier, the rice breeding programs were emerged with the purpose to produce high yield rice cultivars while the development of rice cultivars having high nutritional contents was ignored [29]. Later on, the qualitative enhancement gained importance along with the quantitative increment of rice grains. The breeding of rice cultivars having high micronutrient contents is sped up by germplasm screening of wild species, traditional varieties, and landraces to induce variations in alleles. For increasing Zn and Fe concentration of rice grains, the breeding programs are started with germplasm screening. Later many modern and more developed germplasms evolved. The preservation of these germplasms was done using gene banks containing a pool of genetic variation required for breeding [30]. Kumar et al. [31] reported Zn and Fe concentration in rice grains ranging from 10 – 39 and 10 – 44 mg kg-, Respectively using this approach. On the other hand, Anuradha et al. [31] screened out 126 brown rice cultivars and gave a range for Zn from 27 to 67 mg kg-1 and for Fe 6 to 72 mg kg-1. While, for Fe concentration, 52 rice genotypes were screened out by Jahan et al. [32] who reported a range for Fe from 1.3 to 100.5 mg kg-1. An inbred line of rice 185M was tested for bioavailability in comparison with its parent Swarna and reported three times and two times more bioavailability for Zn and Fe respectively with this line in the presence of ascorbic acid at National Institute of Nutrition, India in an in a vitro cell system. Beside it, the high Zn inbred lines IET 23814 and IET 24775 were also screened out in India which gave promising results. Although, the conventional breeding approach gained considerable success regarding Zn and Fe biofortification in rice, many of these evolved genotypes were failed in attaining consumers and farmers acceptance due to the reasons that in most cases in inbred lines Fe increment was in aleurone layer which is removed during the milling process. The Zn and Fe rich breeds often showed less yield. Beside it, lack of awareness among consumers regarding buying biofortified rice and lack of special prices for farmers in the market for biofortified rice [33].

**Rice biofortification by increasing the number of metal chelators**

Rice plants secrete small molecules called phytosiderophores belonging to family mugineic acid. These help to uptake and transport micronutrients from roots towards shoots and then to the grains. These phytosiderophores have the mechanism for the solubilization of Mn, Cu, Zn, and Fe [34]. The synthesis pathway of these mugineic acid family phytosiderophores has been illustrated in the Figure 2. Nicoitinamide can also bind metal ions through chelation. Nozoye et al. [35] stated that these mugineic acid family phytosiderophores are secreted into the rhizosphere via mugineic acid 1 transporter. It is an antiporter belonging to a superfamily, which is a major facilitator [35]. Inoue et al. [36] reported that the transporter, which is the member of a family yellow stripe like (YSL) is responsible for facilitating uptake of the metal-phytosiderophore complex by plants. According to Haydon and Cobbett, [37] in the vacuole, the transporter of NA 1 is found, which is known as one of the best Zn facilitators. It has been proven that in rice plants if DMA and NA concentration is increased, the grain metal contents were also increased. These results were later confirmed by Wirth et al. [38], (Lee et al. [39], and Johnson et al. [11], who reported the significant increment in the rice grain concentration of Zn and Fe through overexpressing the Pvferritin and AtNAS1, OsNAS2 and OsNAS3, and OsNAS1-3 transporters respectively.

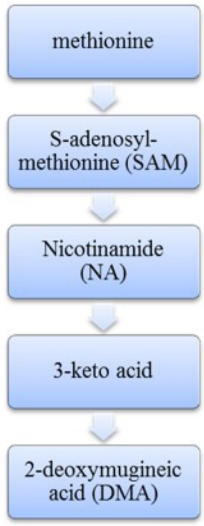


**Figure 2:** Synthesis pathway of mugineic acid family phytosiderophores (Source: Suzuki et al., 2012)

## Rice biofortification by utilizing metal transporters

The uptake of metal ions in rice plants is positively correlated with the metal transporters belonging to various families [40]. The transporters often reported involving in Fe regulation are, OsNRAMP5, OsNRAMP1, OsIRT2, OsIRT1 [41,42, 43,44] respectively. In case of DMA and NA chelated metals, the Fe(III) – DMA is transported by OsYSL15, OsYSL16, and OsYSL18 while, Fe – NA or Mn – NA are transported by OsYSL2 [40]. The PEZ1 and PEZ2 are phenolics efflux transporters involved in apoplastic phenolics secretion in xylem and roots and help to facilitate the apoplasmic Fe transport after solubilizing it [45,46]. According to the findings of Kobayashi and Nishizawa, [47], the loadings of xylem is governed by TOM1, and the transportation from xylem to phloem is regulated by OsYSL2, OsYSL16, and OsYSL18 transporters. Moreover, the translocation of micronutrients towards seeds as well as their uptake from the soil is controlled by OsNRAMP5, OsYSL15, OsIRT, and OsIRT2. A significant increment in the Mn and Fe contents in rice grains was obtained by overexpressing phloem localized transporter OsYSL2 [48]. Lee et al. [49] and Lee and An, [50] reported the increased concentration of Zn and Fe in rice lines by the overexpression of OsYSL15 and OsIRT1 transporters, respectively. Hence, in rice grains, the flow of Fe can be considerably increased by controlling the temporal and special genes expression, specifically in the case of metal transporters. Metal biofortification in rice is positively affected by supplementary transporters.

## Transgenic Practices for rice Biofortification

The biotechnological practices such as plant alteration play a significant role for increased metal contents in rice grains. The plant alteration is carried out by utilizing genes encoding phytase, nicotianamine synthase, ferritin (a Fe storage protein), metal transporters and metal chelators. The types of selected genes and the promoters utilized for the transformation of rice plants for increasing micronutrient concentration in rice grains have been reviewed in Table 1. The more promising results can be achieved by expressing multi genes, for example, the combination of NAS genes and ferritin genes (six fold increment) compared to single gene expression (two-fold increment). An experiment was conducted by using promoter OsGluB1 found in the endosperm of rice to produce transformed rice plant which express SoyferH1 gene (a soybean ferritin gene) [51]. The resulting transformed rice exhibited 2-fold increment in Fe concentration in endosperm and 3-fold increment in brown grains. These findings made the way that a further increment in rice endosperm Fe concentration can be achieved by facilitating absorption of Fe from the soil and then its transport within the plant. In order to increase Fe uptake, the rice endosperm was supplied with phytase (thermo tolerant) obtained from a fungus, and metallothionein-like proteins rich in cysteine were overexpressed. The results showed 130-fold increment in phytase contents in rice grains with high Fe and cysteine peptide contents. In another study, by utilizing nicotinamine aminotransferase (NAAT) gene (from barley), the transformed rice plants were developed, which showed high Fe uptake from soil [52].

**Table 1:** Some selected studies utilizing genes and their promoters for Zn and Fe biofortification of rice (Source: Bashir et al. [81], Kok et al. [82])

| Promoter of gene | Gene | Rice cultivar | Zn/Fe Fold Increase | Reference |
|---|---|---|---|---|
| Glu-B1 | SoyferH-1 | Japonica cv. Kitaake | … /3.0 | Goto et al. [51] |
| Glb-1 | PvFerritin+ rgMT | Japonica cv. Taipei 309 | … /2.03 | Lucca et al. [70] |
| Glu-B1 | SoyFer | Indica cv. IR68144 | 1.4/3.7 | Vasconcelos et al. [71] |
| Glu-B1; Glb-1 | SoyFer | Japonica cv. Kitaake | 1.1/3.0 | Qu et al. [72] |
| Genomic fragments | HvNAS1, HVNAS1+HvNAAT, IDS3 | Japonica cv. Tsukinohikari | 1.0/1.0, 1.1/1.1, 1.3/1.4 | Masuda et al. [73] |
| CaMV 35S, Glb-1, Glb-1 | AtNAS1+, Pvferritin+, Afphytase | Japonica cv. Taipei 309 | 1.6/6.3 | Wirth et al. (74) |
| Actin | HvNAS1 | Japonica cv. Tsukinohikari | 2.3/3.4 | Masuda et al. [16] |
| Activation tagging | OsNAS3 | Japonica cv. Dongjin | …. /2.2 | Lee et al. [49] |
| Ubi | OsIRT1 | Japonica cv. Dongjin | 1.1/1.1 | Lee and An [50] |
| OsSUT1 | OsYSL2 | Japonica cv. Tsukinohikari | … /4.4 | Ishimaru et al. [48] |
| GluB1 | OsNAS1 | Japonica cv. Xiushui 110 | 1.3/1.0 | Zheng et al. [75] |
| CaMV 35S | TOM1 | Japonica cv. Tsukinohikari | 1.6/1.2 | Nozoye et al. [35] |
| CaMV 35S | OsNAS1, OsNAS2, OsNAS3 | Japonica cv. Nipponbare | 1.4/2.2, 2.2/4.2, 1.4/2.2 | Johnson et al. [11] |
| Activation tagging | OsNAS2 | Japonica cv. Dongjin | 2.7/3 | Lee et al. (39,76) |
| OsSUT1, Glb-1, Glb-1. Glu-B1, Act | OsYSL2+, SoyFerH2+, HvNAS1 | Japonica cv. Tsukinohikari | 1.6 /6 | Masuda et al. [77] |
| OsGluA2 | OsFer2 | Indica cv. Pusa-Sugandh II | 1.4/2.1 | Paul et al. [78] |
| OsVIT1 or OsVIT2 T-DNA | OsVITs | Japonica cv. Zhonghua11 | … /1.4 | Bashir et al. [79] |
| AtIRT1, Pvferritin | MsENOD12B, OsGlb1 | Japonica cv. Taipei 309 | …. /4.3 | Boonyaves et al. [80] |

## Quantitative Trait Loci (QTL)

Quantitative Trait Loci (QTL) are genetic loci regulated by many genes and control quantitative characteristics. The transformed varieties can be can be produced utilizing marker-assisted selection, by the transformation of QTLs. This can be achieved by the Identifying and characterizing genomic regions responsible for increased concentration of rice Zn and Fe [53]. For the purpose of mapping, the consistent and strong methods are compulsory for analyzing Fe and Zn contents in rice grains. This QTL mapping technique has been applied for the identification of various genes involved in Zn and Fe uptake from the soil, their translocation within the rice plants and storage in the grains [54]. Various researchers have presented their work through interspecific and intraspecific crosses utilizing QTL mapping for increasing Zn and Fe contents in rice grain [55, 56,57,31,58]. The markers RM7488 and RM3322 are involved in Zn and Fe concentration in rice. In rice grains, the QTLs governing Zn and Fe contents are found on chromosome 12 and chromosome 7 [59]. Later findings suggested that by targeting the same regions on the chromosome, the cereal grains Zn and Fe contents can be aggravated [60]. For the identification of genes responsible for Zn and iron biofortification in rice, QTLs meta-analysis was carried out which discovered 22 meta-QTLs and genes associated GRMZM2G178190 and GRMZM2G366919 and marked as NRAMP i-e natural resistance-associated macrophage protein genes [61]. The techniques of functional validation and expression analysis can be implied for further characterization of gene sequence inside the QTL regions through knocking out or overexpression of gene responsible. The genes within the QTLs can be identified if the genome sequence is available. The rice seed Zn and Fe concentration are positively correlated with the expression pattern of genes within Zn and Fe QTLs [62]. Furthermore, the information coded on these genes can be utilized for the production of functional markers for breeding programs like marker-assisted selection (MAS), fine mapping of QTLs/saturation in order to enhance nutritional characteristics of rice grains.

**Utilization of non-coding microRNAs**

In order to enhance Zn and Fe concentration in rice grains, specific non-coding tiny RNAs can also play a role and are called microRNAs. Beside it, these also have a role in various metabolic and biological phenomena like plant stress responses, cell wall biosynthesis, and plant development [63]. According to Fischer et al. [64], these microRNAs are involved in the absorption and translocation of various mineral elements inside the plants. These microRNAs also have a role in the regulation of gene expression after transcription through controlling translational repression or degradation of mRNA [65]. These are tiny non-coding RNA molecules having subsets of 22 endogenous nucleotides [64]. Agarwal et al. [66] Explored the role of microRNAs in controlling Zn and Fe concentration in rice plants by reporting the microRNAs expression analysis with respect to nutrient homeostasis and biognenesis. The identification of four microRNAs have been made in response to Fe deficiency are miR156, miR171, miR169, and miR172 [66].

**Gene editing**

This technique involves engineering methods based on nucleases to create substitutions, mutations, and precise incisions. The engineering approaches include CRISPR (clustered regularly interspaced short palindromic repeats)/CRISPR-associated systems (Cas), and TALENS (transcription activator-like effector nucleases). Shan et al. [67] developed 2-acetyl-1-pyrroline containing fragrant rice by targeting OsBADH2 gene using the TALENs engineering technique. However, recently, the CRISPR/Cas techniques have been gained more importance due to high efficiency and ease of engineering compared to TALENS [33]. Recent studies have used CRISPR/Cas technique to develop herbicide and disease (blast lesion) resistant rice [68,69]. These studies revealed that CRISPR could be an effective tool for the replacement of genes which will be helpful for the nutrient enrichment of rice grains. The editing of genes involve in the absorption, translocation and loadings of Zn and Fe in rice and their promoter sequences has been made possible using this technique. OsYSL15 OsDMAS1, OsNAS2, OsIRT2, OsYSL2 and OsNAS2 genes have been identified involving in Zn and Fe homeostasis [31,62]. Due to high specificity and precision the gene editing techniques have more advantages than other methods with respect to Zn and Fe enhancement of rice grains The precise gene expression of responsible genes and the creation of desirable alleles by using genome editing techniques is extremely useful for the micronutrients biofortification of rice [1].

**Conclusion and future prospects**

Rice biofortification with micronutrients is an effective technique to combat micronutrients deficiencies among the population which relies on rice as a staple food. It has gained enormous attraction for researchers, industries, and especially in developing countries suffering from micronutrients malnutrition among individuals. The biofortified cultivars of rice are being developed by supplying nutrient elements by fertilizer, conventional rice breeding programs and advanced biotechnological strategies. The genetic techniques like genes and QTLs mapping for enriched Zn and Fe have made possible the discovery of target genes which can be utilized for the production of biofortified rice grains by means of conventional or transgenic practices. For the micronutrients enrichment of rice grain future, Research should be focused on the combination of agronomic and advanced genomic practices for increasing uptake, transport, and storage of micronutrients in rice grains. The advanced transgenic tools like QTLs, MicroRNAs, and genome editing practices like CRISPR and TALENS have led to considerable improvement in the biofortification of rice with Zn and Fe and the characterization, mapping and engineering of specific genes involved in Zn and Fe homeostasis.

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