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REVIEW

# Agronomic Aspects of Zinc Biofortification in Rice (*Oryza sativa* L.)

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**Abstract** Globally, 2.7 billion people suffer from Zn deficiency (ZnD) and 1/3 of the world population living in the poor countries is at the high risk of this deficiency. A staggering number of ZnD deaths occur in South Asia alone. Though the causes of malnutrition are many and complex, one such cause is the dysfunctional food system which is dependent on agriculture. Rice is a staple food for 1/2–2/3 of the world's population and is mainly (90 %) grown in south, southeast and east-Asia. Nearly 50 % of the Indian soil contains inadequate Zn levels and this ZnD in rice–wheat system affects 50 % of rice, particularly, grown under lowland conditions. In order to address the ZnD issue in rice, various agronomic approaches of Zn biofortification can be tested, i.e., selection of cultivars, rate and time of Zn fertilizer application, crop rotation and use of soil microorganisms. Agronomic Zn biofortification is a promising and cost effective method to increase Zn concentration in rice grains. Thus it can save the life of millions of people in Asia, particularly in India. The present article is a modest attempt to analyze the viability of agronomic biofortification in rice grains as a short term and profitable tool to promote Zn concentration that would consequently cure several health hazards commonly visible among humans in the developing countries.

**Keywords** Micronutrient malnutrition · Zinc fertilization · Method of zinc application · Grain Zn content · Crop rotation

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## Introduction

"Green Revolution" met the challenge of feeding the world's poor, by focusing primarily on three staple crops—rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.). These crops provided enough energy and prevented widespread starvation death in many developing countries. However, this agricultural revolution led to rapid rise in micronutrient malnutrition in countries adopting these cropping systems [1]. Many agricultural tools (e.g., diversification, crop selection, fertilizers, cropping systems, soil amendments, livestock production, aquaculture, etc.) could be used to increase the nutrient output of farming systems [2]. Unfortunately, agricultural system followed after the green revolution mostly aimed to increase profitability for farmers and agricultural industries overlooking related aspects of human health. Thus, agricultural systems adopted in developing world failed to provide sufficient micronutrient content in crop produce to meet human needs throughout the year. Therefore, in modern era, sustainable production of nutritious safe foods in sufficient quantity is a challenging task for agricultural scientists.

WHO [3] reports that deficiencies of the mineral micronutrients iron (Fe), zinc (Zn), selenium (Se), and iodine (I) affect more than half of the world population. Zn, Se, Fe, and vitamins A, B, and C have immune modulating functions and thus influence the immunity of a host to infectious diseases and their courses and outcomes [4, 5]. According to WHO, about 2 billion (33 %) of the world population is affected by Zn deficiency (ZnD hereafter) [6, 7], which causes 450,000 deaths of children under 5 years of age every year [8]. In developing countries, ZnD is the fifth major cause of human diseases and mortality [9], with a significant proportion of the Zn related morbidity and mortality occurring in south Asia itself [10].

It is noteworthy that problem of ZnD in human beings predominately occurs in the regions where soil is also deficient in available Zn. Countries like India, Pakistan, China and Turkey are glaring examples of this, where cereals are the major source of calorie intake. Ironically, cereal grains are inherently very low in Zn content. Moreover, growing cereal crops in Zn deficient soil will further decrease Zn content in grains [11].

Against this background, biofortification has emerged as one of the prominent tools to address the micronutrient malnutrition throughout the world. In the present article, an attempt has been made to address a number of issues such as status of rice in nutritional security, status of zinc in Indian soil, role/status of zinc in plant in general and paddy in particular, role of zinc in human health, ways to enhance zinc concentration in rice grain through agronomic biofortification and finally, challenges and future prospectus of agronomic biofortification. The information on the above-mentioned aspects are compiled and reviewed from published literatures in journals of national and international repute, as well as conference proceedings, technical bulletins and books.

## Rice in Relation to Nutritional Security

Globally, rice ranks second to wheat in terms of area harvested (158 Mha) [12] and production (more than 470 M tons of milled rice in 2009) [13]. About 90 % of the rice in the world is grown in south Asia (58 Mha), south-east Asia (43 Mha), and east Asia (31.5 Mha) [14]. It is the main staple food for nearly half [14] to two-third of the world population [15].

Worldwide, more than 3.5 billion people depend on rice for more than 20 % of their daily calorie intake [13]. Furthermore, over 2 billion people in Asia alone derive 80 % of their energy needs from rice, which contains 80 % carbohydrates, 7–8 % protein, 3 % fat, and 3 % fiber [16]. Rice protein, though small in amount, is of high nutritional value [17]. It is a rich source of many functional components such as micronutrients, aminobutyric acid (GABA), glutelin, resistant starch, fiber, unsaturated fatty acids, amino acids and free radical scavenger. These components prevent hypertension, nephritic disease, diabetes caused by imbalanced food intake, chemical contamination [18] and protect the body from heart diseases, certain cancers and osteoporosis [19, 20].

Globally, India has the largest paddy cultivating area (44 Mha) and is the second largest producer (89 M tons/annum) of rice but ironically productivity is much below (2.05 t/ha) the world average (2.62 t/ha). In India, rice occupies about 24 % of gross cropped area. It contributes 43 % of total food grain production, 46 % of total cereal production of the country and solely contributes 30 % of the total calories in the Indian diet [21]. Moreover, children under 6 years of age consume 118 g rice/day [22]. Based on population growth trend and per capita availability, future requirement of rice production is projected to be around 215–230 g rice/day, which then would require 109–117 M tons of rice production by 2025 [23].

## Status of Zinc in Indian Soil

Green revolution has provided nutritional security and food sufficiency in the country through increased irrigation facility, introduction of high yielding varieties, and increased use of fertilizers and plant protection measures. However, all these conditions favored cereal based cropping systems, decrease in use of organic manures and crop residues, which in turn have exhausted the inherent pools of micronutrients in the soil. Thus in the regions where there is a widespread deficiency of micro- and minor-nutrients, it therefore becomes critical for achieving sustainable high crop production [24, 25].

Analysis of soil and plant samples from different states of India shows that 48 % of the soil samples and 44 % of the plant samples contain inadequate levels of Zn [26, 27]. The available Zn for plants in Indian soil extracted with diethylene triamine penta acetate (DTPA) constitutes a very small amount (<1 %) of total Zn. DTPA extractable Zn in Indian soil ranges from 0.08 to 20.5 mg/kg soil. The highest rate of ZnD was found in the soil of Madhya Pradesh (62 %) followed closely by Haryana (61 %), Odisha (57 %), Andhra Pradesh (52 %), West Bengal (49 %), Punjab, Uttar Pradesh and Bihar (each 46 %) and the least in the Union Territory of Pondicherry (8 %) [28].

ZnD is the most common micro-nutrient deficiency problem in soil, particularly in rice–wheat cropping systems [29, 30]. Up to 50 % of the rice grown under the lowland (flooded) conditions (paddy rice) may be affected by ZnD [31, 32]. On the other hand, intensive cropping systems remove a large amount of Zn. For example, a harvest of 8 t grain/ha/yr removed 384,744 and 320 g Zn/ha/yr in rice–wheat, maize–wheat and rice–rice cropping systems, respectively [33]. This heavy removal of Zn every year without adequate Zn fertilization has depleted Zn from the native soil [34].

Zn deficiency has been associated with a wide range of soil conditions: high pH (>7.0), low available Zn content, prolonged submergence and low redox potential, high organic matter and bicarbonate content, high Mg:Ca ratio, and high available P [35]. High soil pH appears to be the main factor associated with the widespread ZnD in the calcareous soil of the Indo-Gangetic plains of India [36], whereas perennial wetness is the major cause for ZnD in peat soil and in coastal saline soil [32, 35].

## Form and Availability of Zinc in Paddy Soil

The total amount of zinc in soil is distributed over 5 fractions (or pools) These comprise: (a) The water soluble pool—present in the soil solution; (b) Exchangeable pool—ions bound to soil particles by electrical charges; (c) Organically bound pool—ions adsorbed, chelated or complexed with organic ligands; (d) Pool of zinc sorbed non-exchangeable onto clay minerals and insoluble metallic oxides; (e) Pool of weathering primary minerals [37]. Soluble fractions of Zn can be easily desorbed and easily available to plants but, being potentially leachable, move down through soil profile [38].

The uptake, translocation, metabolism, and plant use of Zn is inhibited by high P availability, particularly due to the high rate of application of P fertilizer [39]. Concentration of Zn in soil solution generally increases temporarily after flooding, but it can also decrease with time [40, 41]. However, in the acid soil, Zn availability decreases after flooding owing to an increase in pH and the precipitation of $Zn(OH)_2$. On the other hand, if an alkali soil is submerged, the pH of the soil decreases resulting in increase in Zn solubility [42]. Decreased availability of Zn in submerged soil could be attributed to the formation of insoluble compounds such as franklinite ($ZnFe_2O_4$), ZnS (under intense reducing condition), $ZnCO_3$ (owing to high partial pressure of $CO_2$ arising from the decomposition of organic matter at later period of soil submergence), $Zn(OH)_2$ (at a relatively higher pH), and adsorption of soluble $Zn^{2+}$ by oxide minerals, e.g. sesquioxides, carbonates, soil organic matter and clay minerals [43].

## Zinc in Rice Plant

The essentiality of Zn in plants was first shown in maize [44], and subsequently in barley and dwarf sunflower [45]. In rice field, ZnD was first noticed in sixties in the *tarai* 'foot hills of the Himalayan' soil [46]. Plant response to ZnD occurs in terms of decrease in membrane integrity, susceptibility to heat stress, decreased synthesis of carbohydrates, cytochromes, nucleotide, auxin and chlorophyll. Further, Zn-containing enzymes are also inhibited, which include alcohol dehydrogenase, carbonic anhydrase, Cu–Zn-superoxide dismutase, alkaline phosphatase, phospholipase, carboxypeptidase, and RNA polymerase [47]. It binds with more than 500 different proteins [48].

### Deficiency

During the green revolution more emphasis was given to cereal production with massive use of nitrogen (N) and phosphorus (P) fertilizers which caused reduction in plant Zn uptake from soil reducing its mobility within the plants [2, 49]. Its deficiency results in the reduced ability of the rice plant to support root respiration during flooded conditions [50]. Typical Zn concentration in rice tissue level is between 25 and 100 ppm and deficiency symptoms appear when this level falls below 20 ppm [51]. In rice, ZnD causes multiple symptoms which usually appear 2–3 weeks after transplantation of rice seedlings, i.e. leaves develop brown blotches and streaks that may fuse to cover older leaves entirely, plants remain stunted and in severe cases may die, while those that recover will show substantial delay in maturity and reduction in yield [35, 52, 53].

### Critical Concentration

In most crops, the typical leaf Zn concentration required for adequate growth approximates 15–20 mg Zn/kg DW [47]. Critical Zn concentration in rice plant is noted and summarized in Table 1.

## Role of Zinc in Human Health

The cases of human ZnD among poorly growing adolescent boys were firstly noticed in Egypt [54]. ZnD is ranked as the 5th leading risk factor for diseases (e.g. diarrhoea and pneumonia in children) in the developing countries [3]. It is required for the activity of >100 enzymes [55] and binds to nearly 2,800 human proteins (corresponds to 10 % of human proteome) [56]. Almost 40 % of the Zn-binding proteins are transcription factors needed for gene regulation and the 60 % enzymes and proteins involve in ion transport [56]. Zinc is also a critical micronutrient required for structural and functional integrity of biological membranes and for detoxification of highly aggressive free radicals [57].

Any alteration in Zn homeostasis or any decrease in Zn concentration of human body will, therefore, result in a number of cellular disturbances and impairments such as: immune dysfunctions and high susceptibility to infectious diseases, retardation of mental development, and stunted growth of children [8]. These adverse health consequences of ZnD vary with age, for example, low weight gains, diarrhoea, anorexia and neurobehavioural disturbances are common during infancy, whereas skin changes, blepharoconjunctivitis and impairments in linear growth are more frequent among toddlers and schoolchildren [58]. Manifestations among the elderly include hypoguesia (reduced ability to taste sweet, sour, bitter, salty and umami), chronic nonhealing leg ulcers and recurrent infections are also the causes of ZnD [59].

The dietary allowance of Zn for infants is 3–5 mg/day, while for children of 1–10 years it is 10 mg/day. For adults, the dietary allowance is 15 mg/day for men, 12 mg/day for women and 16–19 mg/day for lactating women [60]; however, these intake limits meet seldom. According to an estimate, over 25 % of the total population in India is at the risk of inadequate Zn intake [54]. Actually, there is no store for Zn in human body. Therefore, these bioavailable Zn from food or supplements must supply Zn on a regular basis [61]. The current burden of ZnD in India amounts to 2.8 million disability-adjusted life years (DALYs) lost, 2.7 million from mortality and 1,40,000 from morbidity, 70 % of which occur among infants [62].

## Agronomic Biofortification of Zinc in Rice

Adoption of micronutrient deficiencies controlling strategies like supplementation and fortification has benefited only certain sections of the society. Thus new approaches become imperative for sustainable micronutrient deficiency alleviation in burgeoning urban and rural populations. In recent years, an alternative solution, called biofortification of staple crops, is being sought to mitigate the problem of micronutrient malnutrition [63–68]. Biofortification is the process of increasing the natural content of bioavailable nutrients in crop plants [69–72]. Zn biofortification of rice, could save between 1.6–2.3 million DALYs [62] and 0.4–1.5 million DALYs [73] per year in India and China, respectively. This corresponds to a reduction of the burden of ZnD in India and China by 18–56 % [22, 62] and 15–60 % [73], respectively. Due to higher natural zinc content in existing wheat varieties, the impact of the wheat biofortification is lower in comparison to the rice biofortification.

It is generally believed that biofortification is only possible by generating nutritionally improved crop varieties through conventional plant breeding and modern biotechnology [74] and grain-Zn composition will thereby remain unaffected by the Zn-fertility status of the soil [75]. Contrary to the earlier myth, it is now confirmed that Zn concentration in rice grains can be enhanced with Zn fertilization [76]. Biofortification can be achieved by two distinct ways: by enriching bioavailable micronutrients in edible portion of plants through (1) breeding or genetic engineering i.e. genetic biofortification [70, 77, 78], and (2) through agricultural interventions (judicious fertilizer use) i.e. agronomic biofortification [65, 79, 80].

Genetic biofortification of food crops faces several problems viz., (1) high funds and resource base requirement; (2) long term process for achieving research goals; (3) rejection by the farmers, as the bio-fortified cultivars may not be superior to conventional cultivars in terms of yield; (4) negative response from the consumers, as newly developed cultivars may differ in quality; (5) export issues, a nation adopting genetically modified (GM) crop cultivars may not be able to export the produce to the countries restricting consumption of GM foods; (6) intellectual property rights, and (7) ethical problems with GM cultivars [81].

Thus, agronomic biofortification is a win–win approach for developing countries [78], which relies on exploitation of micronutrient dense cultivar [82], applying zinc fertilizers to seeds, soil and/or foliar, at rates greater than those required for maximum yield, in order to increase the uptake of Zn into the plants and its translocation into seeds [83–85]. This could be a more sustainable and cost effective strategy to improve Zn concentrations in rice grains [79, 86]. Application of soil microorganism [87] and selection of suitable crop rotation [88] has also been found very promising to increase zinc concentration in rice grain (Fig. 1).

Keeping these facts in view recently, a global zinc fertilizer project called HarvestZinc Project (www.harvestzinc.org) has been initiated under the HarvestPlus Program. The project aims at evaluating the potential of Zn-containing fertilizers for increasing Zn concentration of cereal grains (e.g., wheat, rice and maize) and improving crop production in different target countries (e.g., India, China, Pakistan, Thailand, Turkey, Mozambique, Zimbabwe and Brazil). Basically, zinc fertilization is adopted to keep sufficient amount of available Zn in soil solution as well as to maintain adequate Zn transportation to seeds during reproductive growth stage.

Agronomic biofortification of Zn in rice is a relatively new field which includes strategies like application of mineral fertilizers and/or improvement of the solubilization and mobilization of mineral elements in the soil. When crops are grown on soil where mineral elements are immediately unavailable, targeted application of soluble inorganic fertilizers to roots or to leaves is therefore practised. Other such strategies include selection of appropriate micronutrient dense cultivars and crop rotation, which increases Zn concentration in edible portion.

### Providing the Appropriate Form and Amount of Zn-Fertilizer for Increasing the Zinc Density in Grain

There are six major sources of Zn used for ameliorating ZnD, namely : (a) Zinc sulphate heptahydrate ($ZnSO_4.7H_2O$) (21–22 % Zn); Zn sulphate monohydrate ($ZnSO_4.H_2O$) (33 % Zn) of which 98 % is water-soluble; (b) sparingly soluble Zn oxide (ZnO) (67–80 % Zn); (c) Zn

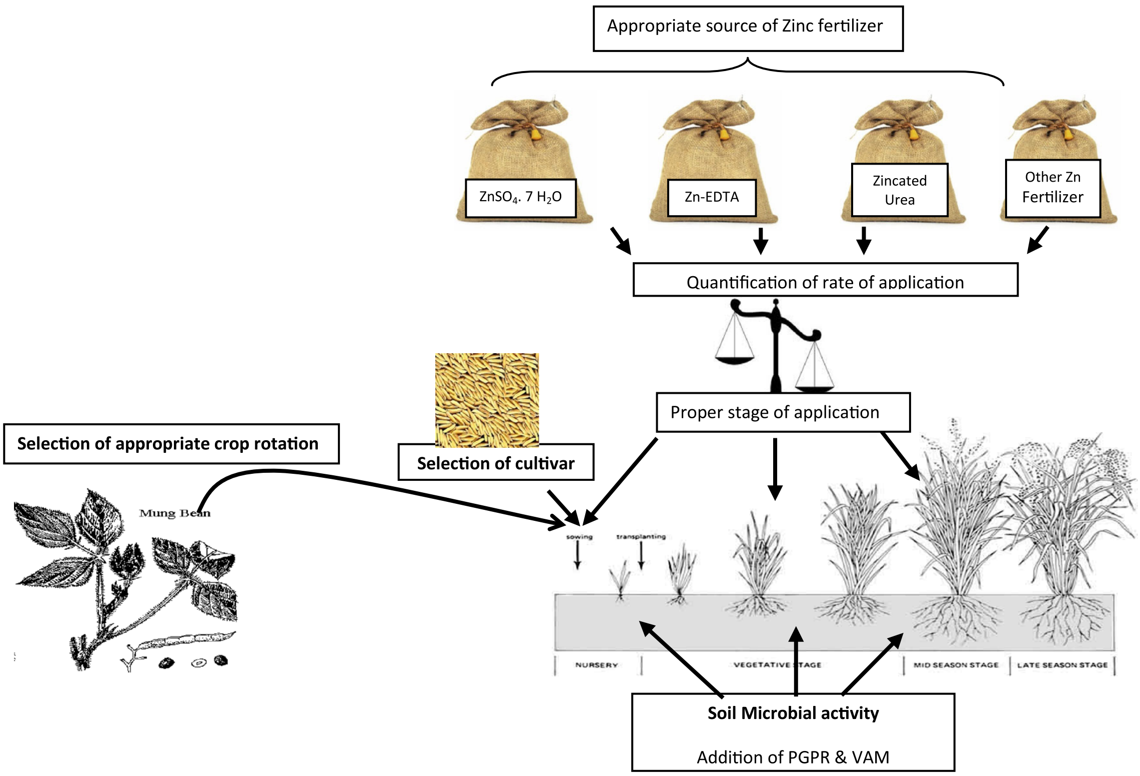

**Fig. 1** Major approach for agronomic zinc biofortification

carbonate ($ZnCO_3$) (56 % Zn); (d) Zn phosphate ($Zn_3(PO_4)_2$ (50 % Zn); (e) Zn frits (4–16 % Zn); (f) Zn chelates (12–14 % Zn) and Teprosyn-Zn slurry (55 % Zn) [26]. Generally, Zn sources should be 40–50 % water-soluble to be an efficient Zn fertilizer [89, 90].

Evaluation of different Zn sources [Zn-enriched farmyard manure (Zn-FYM), Zn-tetra-ammonia complex sorbed on FYM and Zn-ethylenediaminetetraacetate (Zn-EDTA)] on lowland rice production under green house study showed that Zn concentration in rice grain was highest for Zn-FYM and lowest for the $ZnSO_4$. It was also observed that lowest Zn concentration in rice straw following Zn-EDTA application could have been due to the mobilization of most of the absorbed Zn to the grains from vegetative tissues; thus Zn-EDTA recorded highest mobilization index of 0.93 as compared to ~0.50 for all the other Zn sources [91]. Similarly, Naik and Das [42] also found better response of Zn-EDTA over $ZnSO_4.7H_2O$, because application of chelated-Zn facilitates greater absorption and maintains Zn in soil at a steady rate as compared to $ZnSO_4$.

As Zn-EDTA is being costlier, so in general, soil Zn application at the rate 5–17 kg Zn/ha in the form of $ZnSO_4$ is recommended for crops [92, 93]. In India, zinc sulphate is the most common source of Zn, due to its high water solubility, easy availability and relatively low price as compared to other sources [27]. In India, 5 kg Zn/ha in the form of $ZnSO_4$ is recommended for correcting ZnD in rice [28, 94]. Furthermore, green house study showed that application of 5.0 mg Zn/kg soil significantly increases Zn concentration in rice grain [88]. However, $ZnSO_4$ is relatively cheaper product but still it is quite costly for small farm holders. So farmers skip its application resulting in reduced crop yields. Another factor that discourages the farmers from applying Zn in India is spurious $ZnSO_4.7H_2O$ sold by unscrupulous traders [95]. Currently, an attempt is being made by the Indian fertilizer industry to produce Zn-coated urea (also referred to as zincated urea) that would permit the rice growers to have an easy access to Zn [96]. $ZnSO_4.H_2O$ is generally used for coating urea because it contains 33 % Zn and also lesser quantities are needed for coating. It was found that application of 2.0 % $ZnSO_4$–coated urea showed highest grain Zn concentrations in basmati rice [96]. It is worth mentioning that Zn fertilization not only increases Zn concentration in seeds but also reduces phytate content and phytate:zinc ratio of the seeds, making it more bioavailable to consumers [11].

### Providing Zn Fertilizer at Appropriate Time for Increasing Zinc Density in Grain

Split application (½ at basal and ½ at grand tillering stage) of $ZnSO_4.7H_2O$ (10 and 20 kg Zn/ha) recorded higher Zn content in grain and straw over single basal application, whereas, application of Zn-EDTA (1.0 kg Zn/ha) at similar stage has no effect on Zn content in rice grain [42], but split application of Zn i.e. 0.5 kg Zn/ha at grand tillering stage (14 DAT) and 0.5 kg Zn/ha at panicle initiation stage (49 DAT) significantly increased Zn content in grain and straw [97].

### Adopting Appropriate Method of Zn-Fertilizer Application for Increasing the Zinc Density in Grain

Now it is experimentally proven that soil and/or foliar applications of Zn fertilizers greatly contribute to grain Zn concentrations [11, 98]. It is observed that sometime basal Zn fertilizer application has small effect/no effect in increasing grain Zn concentration which might be due to immobilization of Zn in older shoot tissue. Under such situation, foliar application of Zn in lowland rice significantly increases Zn concentration, but in Zn-rich upland soil foliar application has no significant effect on grain Zn concentration [99]. The timing of foliar applications is critically important, and it is observed that application after the growth stage of 'milk' [100] is more effective for loading zinc into grain. Cultivar difference also responds variably with foliar Zn application, viz., grain Zn concentrations in IR74 shows a very small response to foliar sprays. This may be due to the fact that genotype either possesses a higher resistance for Zn translocation to grains or is less capable to absorb foliar Zn [99]. Application of Zn as seed/soil plus foliar is most appropriate for agronomic biofortification resulting in 3.5-fold increases in Zn concentrations in grain [11]. It is worth mentioning that in the second phase of HarvestPlus Zinc fertilizer project (2011–2014) special attention is paid to motivate farmers to include Zn in their soil and foliar fertilization programs [101].

### Appropriate Choice of Crop Rotation for Increasing Zinc Density in Grain

Crop rotations also influence grain Zn concentration in rice. Field experiment showed that significantly higher Zn concentration in grain was recorded when aromatic hybrid rice was grown after incorporation of cowpea and mungbean residues, which were significantly better than summer fallow [88].

### Exploiting Micronutrient Dense Cultivar Selection

Rice cultivars have different abilities in accumulating micronutrient in grain when grown under similar conditions. Screening of nearly 1,000 rice genotypes at International Rice Research Institute (IRRI) reveals that grain Zn concentrations ranged from 15.9–58.4 mg/kg [64]. Experimental findings indicate that genotypic differences in rice became significant in the Zn deficient soil and further increased with increasing native soil-Zn status in lowland soil, but were less pronounced in the upland soil where native soil Zn status was high and grain Zn concentrations were also high for all genotypes. This indicates that right selection of cultivars is essential for increasing grain Zn concentration in rice (lowland) grown in highly Zn deficient soil. At highly Zn deficient location, IR74 had significantly lower grain Zn concentrations as compared to RI L597, Jalmagna and IR68144, whereas RIL507 was intermediate [99]. Tolerant cultivars may have lower Zn requirements or translocate relatively more Zn from roots to shoots [102].

### Use of Arbuscular Mycorrhizae and PGPR for Increasing Zinc Density in Grain

Mycorrhizae have been shown to increase plant absorption of nutrients, whose uptake is limited by diffusion through the soil matrix to the roots [103]. Increasing plant nutrition is particularly important in soil of heterogeneous [104, 105] and low nutrient status [105, 106]. A good review on role of arbuscular mycorrhizas in improving plant zinc nutrition under low soil zinc concentrations was presented by Cavagnaro [107].

Rice plants readily form mycorrhizal associations under upland [108] and submerged conditions [109]. Under waterlogged transplanted paddy, inoculation with *Glomus etunicatum* L., vesicular–arbuscular mycorrhizal fungi (VAMF) increased the content of Zn in rice grain, and the highest content of Zn was observed in the NPK+$ZnSO_4$ treatment. The interaction of source and inoculation showed that the content of Zn in the NPK+$ZnSO_4$+VAMF treatment was significantly higher than in other treatments [87]. Similarly, inoculation of mix strain of plant growth

**Table 1** Critical concentrations of zinc in different plant tissues of rice

| Tissue | Critical concentration (mg Zn/kg dry matter) | Reported from country |
|---|---|---|
| Whole seedling[a] | 15.00 | India |
| Plant top, pre-flowering[a] | 17.40 | India |
| 3rd leaf from top[a] | 16.0–23.5 | India |
| Whole plant[b] | 15.0–22.0 | India |
| Whole shoot (Deficient)[c] | <10.0 | Philippines |
| Whole shoot (Deficiency very likely)[c] | 10.0–15.0 | Philippines |
| Whole shoot (Deficiency likely)[c] | 15.0–20.0 | Philippines |
| Whole shoot (Sufficient)[c] | >20.0 | Philippines |

[a] Srivastava and Gupta [122]

[b] Takkar [51]

[c] Dobermann and Fairhurst [123]

promoting rhizobacteria (PGPR) significantly increased the concentration of Zn in the rice grain over the control without Zn and chelated Zn-EDTA treatment. However, it did not increase the Zn concentration in the rice straw, thereby recorded highest Zn mobilization efficiency index to rice grain over rice straw [110].

## Challenges for Agronomic Biofortification of Staple Foods

There are several challenges for biofortification of crops such as:-

### Setting appropriate target levels for the zinc content of biofortified staple foods

A great difficulty exists in setting appropriate target levels for the Zn content of biofortified staple foods as it differs with population, processing practices, and inclusion of other foods which can also result in large differences in the Zn content and bioavailability in the staple food [111].

### Retention of zinc in biofortified staple food

Micronutrients are mostly concentrated in germ and/or aleurone layer of grain, with lesser amount in endosperm. During milling process, these fractions are lost thus micronutrient content is reduced substantially. Zinc retention in milled food can range from 20 to 60 % of the whole grain content, depending on the grain and the extraction rate [1]. An un-hulled rice (paddy) contains 27–42 mg Zn/kg grain [95], and the polished rice contains only 13–15 mg Zn/kg grain [112]. A diet of 300–400 g cereals/day will supply only 4–6 mg Zn/day in case of rice and 11–18 mg Zn/day in case of wheat [113]. For population that consumes the whole grain (that includes aleurone and germ), it is relatively easy to achieve the target micronutrient increment through biofortified grain. However, large population in developing countries consume refined grains, thus pose challenge for the success of biofortification programme [111].

### Bioavailability of zinc from agronomic biofortified staple food

Absorption of available Zn from biofortified staple food depends on interaction of several common plant food components in human gastrointestinal tract during digestion to alter their bioavailability [111, 114]. For the success of biofortification programme, there is an urgent need to promote synergistic food combinations [115].The proportion of zinc that is absorbed from typical diets appears to range from about 18 to 34 % [111].

Only a part of Zn present in cereals is bioavailable due to the phytate. Around 80 % of the phosphorus stored in cereal seeds is present as phytate [114]. In rice, the phytate content varies from 0.14 to 0.60 % [113]. Phytate:Zn molar ratio in rice ranges from 3.07 to 11.27 [116, 117] and its ratio above 15 in food is associated with reduced Zn bioavailability [113, 118]. It is expected that lower phytate content of staple foods would lead to improved zinc bioavailability and hence would increase dietary zinc adequacy [111].

However, there is evidence that dietary phytate has anticarcinogenic and antioxidant effects, and that it may enhance the absorption of dietary Cu [114]. Furthermore, it was found that low-phytate content lowers seedling survival and growth, especially on the low-P soils [119], and reduces yield of barley mutants [120]. Thus, it is essential to maintain proper balance while reducing phytate content to increase grain Zn concentration, because this modification may extend beyond bioavailability to broader issues of public health and agronomic feasibility [111].

### Determining biological impact of biofortified staple crops

To accept viability and cost-effectiveness of biofortification, its biological impact on health and development of nutrient-deficient population needs to be demonstrated. Till date, no efficacy studies with zinc-biofortified staple foods have been conducted [111].

Fundamentally, complete step-wise evaluation of efficacy is very complex which involves in vitro and animal testing in the laboratory as well as assessment of nutritional, health, agricultural, societal, environmental and economic impacts on the community [121]. According to Johns and Eyzaguirre [115], considering the complexity of the problem and the limited understanding of the issues, evaluation methods of the potential impacts of biofortified foods are uncertain and potentially problematic. These will likely be time consuming and expensive.

### Creating awareness among the farmers regarding biofortification

Local farmers may be willing to adopt biofortification programme but they have limited knowledge about the different issues related to biofortification. Extensive extension and training programmes are therefore needed to aware the farmers about this programme. Without adequate information, farmers are likely to make inappropriate decisions that are potentially harmful to their health and harvest.

Increased cost of fertilization

In India, it is the small and marginal farmers who dominate the agriculture, and farm without any government support. Therefore they are burdened with the additional cost of fertilization and this will ultimately affect their economic security.

In spite of these challenges, biofortification programme is found to be highly viable and short term solution for mitigation of ZnD in human beings. For further success and maximum results of this programme, following issues need to be addressed.

## Future Prospects of Agronomic Zinc Biofortification

1. Data generated on soil zinc status is more than a decade old. Thus there is an urgent need to prepare a recent map of zinc status of rice soil using GPS/GIS. This will facilitate prediction of periodic change in zinc status and forecast of ZnD in soil.
2. Development of Zn containing customized products, complex fertilizer formulation, fortified organic manures and Zn containing compounds which would be compatible with herbicides and fungicides in tank mixes for foliar application.
3. Development of mathematical models to predict and measure rate of depletion of Zn reserves in soil; to study crop response to applied Zn fertilizers, and to formulate suggestions to farmers about the choice of Zn fertilizer, their rate and proper time of application to enhance Zn concentration in grains.
4. To evaluate interaction of Zn with other nutrients, physical and environmental factors, and plant biotic and abiotic stresses.
5. To explore the potential mechanisms of Zn availability by blue-green algae, VAM and PGPR.
6. Screening of Zn responsive and tolerant varieties under different rice-production system.
7. Evaluation of suitable crop rotation and specific stage of Zn application on cultivar, based on crop duration and ecological requirement under different rice-production systems.
8. Conducting research on performance and efficiency of various Zn fertilizers (especially chelated compounds) under different rice-production systems, particularly the water saving ones.
9. Carrying out research to understand the physiological mechanism of Zn uptake and translocation by root, sequestration in leaves, and its partitioning in grains.
10. To urge the Government of India to undertake more initiatives to promote the use of zinc in agriculture. In fact, in National Food Security Mission (NFSM), there is a provision to provide Rs. 500/ha to the farmers for the use of micronutrients; to create awareness among consumer to avoid misconception; to upgrade the skills of the extension agencies to highlight the increasing need of zinc in crop and human nutrition; and finally to demonstrate research results on agronomic biofortification to farmers and extension workers.
11. Also, to coordinate collaborative micronutrient research with agronomists, soil scientists, physiologists, breeders, biotechnologists, pathologists, and microbiologists, besides the animal and human nutrition scientists.

## Conclusion

Thus the importance of Zn in soil and human health and how agronomic biofortification can enhance the ZnD through proper experimentation in agro-research can be easily understood. The realization of the necessity of facing challenges in biofortification and how certain steps should be undertaken to eradicate such hurdles is significant. Besides the discussion on biofortification, its process, problems, prospects and findings one can now conclude that—Zn is an important micronutrient for the metabolic functioning of both humans and plants. ZnD is listed as a major risk factor for human health and also a cause of millions of death globally, with a significant proportion in south Asia. ZnD in humans predominately occurs where soil is deficient in available Zn and where cereals are cultivated as major source of calorie intake. To mitigate ZnD in humans, supplementation and fortification can provide solution to limited population. Thus biofortification of staple food is an alternative solution to mitigate micronutrient malnutrition.

Rice is a major staple food of south Asia including India and it is mostly grown in Zn deficient soil. Enhancement of Zn concentration in rice grain through genetic biofortification is a long term strategy associated with some degree of uncertainty. Thus agronomic biofortification offers a reliable, short term and cost effective approach for increasing Zn concentration in rice grain, which can save millions of poor population from Zn malnutrition, especially, in rice growing regions. A number of promising strategies for agronomic biofortification of rice have been documented. Further, there is a need for policy and research interventions to make agronomic biofortification adoptable at the farmers' level.

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