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*Review Article*

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# Agronomic and Genetic Biofortification with Fe, Zn of Cereal Crops - A Review

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## ABSTRACT

*Amonge the different nutrients iron and zinc are two important nutrients in the human diet. Their deficiencies in humans lead to a variety of health-related problems. Iron and zinc biofortification of cereals is considered a cost-effective solution to overcome the malnutrition of these minerals. Biofortification aims at either increasing accumulation of these minerals in edible parts, endosperm, or to increase their bioavailability. Agronomic biofortification through fertilizer approaches could complement the existing breeding approach; for instance, foliar application of Zn fertilizer can increase grain Zn above the breeding target set by nutritionists. Iron and zinc fertilization management positively influence their accumulation in cereal grains. Regarding genetic strategies, quantitative genetic studies show the existence of ample variation for iron and zinc accumulation as well as inhibitors or promoters of their bioavailability in cereal grains. Genetically modified cereals developed by modulation of genes involved in iron and zinc homeostasis, or genes influencing bioavailability, have shown promising results. However, iron and zinc concentration were quantified in the whole grains during most of the studies, whereas a significant proportion of them is lost during milling. This makes it difficult to realistically assess the effectiveness of the different strategies. This review focuses on the common challenges and their possible solutions related to agronomic as well as genetic iron and zinc biofortification of cereals.*

***Keywords:*** *Biofortification, bioavailability, Iron, Zinc.*

## INTRODUCTION

Deficiencies iron (Fe) and zinc (Zn) are serious public health issues and important soil constraints to crop production, particularly in the developing world (Cakmak, 2010 & Lyons et al., 2004). Iron deficiency is considered to be the common worldwide nutritional deficiency that affects approximately 20% of the world population. Women and children are especially at risk (Kabata-Pendias & Mukherjee, 2010, Schuler & Bauer, 2012). It is one of the most prevalent and most serious nutrient deficiencies threatening human health in the world, affecting approximately two billion people (Kabata-Pendias, 2010).

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Various physiological diseases, such as anemia and some neurodegenerative diseases are triggered by Fe deficiency (Andreini et al., 2006). Especially those countries are affected by Fe deficiency diseases, where people have low meat intake and the diets are mostly based on staple crops. Human health problems caused by Fe deficiency can be prevented by specific attention to food composition and by choosing a balanced diet with sufficient Fe concentration. Zinc is one of the most important micronutrients in biological systems, and plays critical role in protein synthesis and metabolism. Several of Zn-binding proteins are transcription factors necessary for gene regulation and necessary for more than a half of enzymes and proteins involved in ion transport (Black, 2003). Any decrease in Zn concentration in human body may result in number of cellular disfunctions, including a high susceptibility to infectious diseases, retardation of mental development, and stunted growth of children (Rayman, 2000). Zinc deficiency is considered to be one of major causes of children death in the world. It is responsible for more than 4% of the deaths of children less than 5 years of age (Rayman, 2000). Low dietary intake of Fe and Zn appears to be the major reason for the widespread prevalence of Fe and Zn deficiencies in human populations. The situation is even more adverse in developing countries where more than half of the children and pregnant women are suffering from iron and zinc deficiencies (Seshadri, 1997, 2001; Caulfield et al., 1999). This situation is largely attributed to the high consumption of cereal based foods, rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), and maize (*Zea mays* L.), in these countries (Pfeiffer and Mc Clafferty, 2007). Edible parts (endosperms) of modern cereal cultivars are inherently poor in iron and zinc. Iron and zinc concentration in whole grain of wheat are in the range of 29 to 73 mg/kg and 7 to 85 mg/kg, respectively (Rengel et al., 1999; Cakmak et al., 2004). However, more than 75% of these nutrients are located in the seedy parts other than endosperm which is lost during milling (Slavin et al., 2000; Ozturk et al., 2006). The concentration of iron in the brown rice ranges from 6.3 to 24.4 mg/kg and zinc concentration ranges from 15.3 to 58.4 mg/kg (Gregorio et al., 2000). However, polished rice, the principal form of rice consumed, on an average contains only 2 mg/kg iron and 12 mg/kg zinc (Barry, 2006). In countries with a high incidence of micronutrient deficiencies, cereal-based foods represent the largest proportion of the daily diet (Cakmak, 2008). Cereal crops are inherently very low in grain Zn and Fe concentrations, and growing them on potentially Zn and Fe deficient soils further reduces Fe and Zn concentrations in grain (Cakmak et al., 2010). Among micronutrients, Zn and Fe deficiency is occurring in both crops and humans (White & Zasoski 1999; Hotz & Brown 2004; Welch & Graham, 2004). Zinc and Iron deficiency is currently listed as a major risk factor for human health and cause of death globally. According to a WHO report on the risk factors responsible for development of illnesses and diseases, Zn and Fe deficiency ranks 11th and 12th respectively among the 20 most important factors in the world, 5th and 6th among the 10 most important factors in developing countries (Table 1).

**Table 1: Ten leading causes of illness and disease in low income countries (WHO 2002)**

| **Risk factors Ratio** | **(%)** |
|---|---|
| Underweight | 14.9 |
| Unsafe sex | 10.2 |
| Unsafe water | 5.5 |
| Indoor smoke | 3.7 |
| Zinc deficiency | 3.2 |
| Iron deficiency | 3.1 |
| Vitamin A deficiency | 3.0 |
| Blood pressure | 2.5 |
| Tobacco | 2.0 |
| Cholesterol | 1.9 |

Cakmak (2008)

Zinc deficiency in soils and plants is a global micronutrient deficiency problem reported in many countries (Sillanpaa, 1982; Alloway, 2004). Low solubility of Zn in soils rather than low total amount of Zn is the major reason for the widespread occurrence of Zn deficiency problem in crop plants. Global distribution of the regions where Zn deficiency problem has been reported in crop plants (Fig.1). Possibly, there are many other regions or countries where Zn deficiency problem has not been reported or diagnosed. Nearly 50% of the cereal-grown areas in the world have soils with low plant availability of Zn (Graham & Welch, 1996; Cakmak, 2002). Cereal crops represent a major source of minerals and protein in developing world. For example, in most of Central and West Asian countries, wheat provides nearly 50% of the daily calorie intake on average, likely increasing to more than 70% in the rural regions.

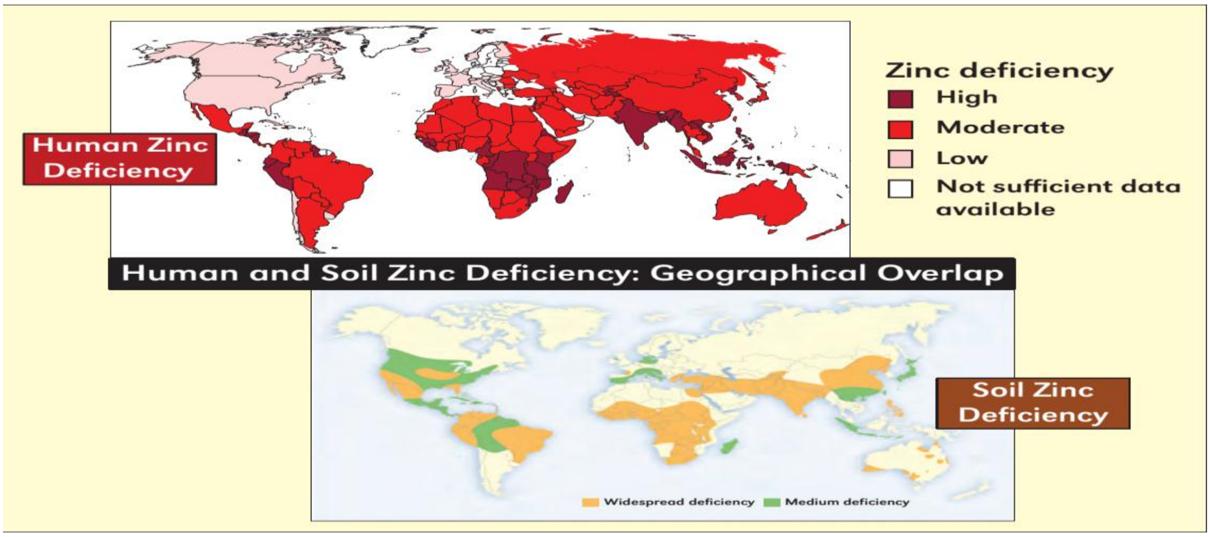

**Fig. 1: Global distribution of Zn deficiency-affected areas (Alloway, 2004)**

**Table 2: Variation for Zn concentration in bread wheat, durum wheat, and their wild relatives, documented in various studies**

| Germplasm | Number of genotypes | Zn concentration (mg/kg) | | Source |
|---|---|---|---|---|
| | | Mean | Range | |
| Bread wheat | 150 | 21.4 | 13.5-34.5 | Zhao et al., 2009 |
| Durum wheat | 10 | 21.4 | 14.0-26.9 | Zhao et al., 2009 |
| Einkorn wheat | 5 | 22.4 | 14.0-26.9 | |
| Emmer wheat | 5 | 22.8 | 20.1-27.8 | |
| *Triticum spelta* | 5 | 22.9 | 16.8-28.0 | Zhao *et al.*, 2009 |
| Winter wheat | 137 | 31.6 | 11.7-64.0 | Karami et al., 2009 |
| Durum wheat (old) | 10 | 36.4 | 33.7-41.4 | Ficco et al., 2009 |
| Durum wheat (modern) | 57 | 33.9 | 28.5-46.3 | Ficco et al., 2009 |
| Durum wheat (advanced) | 17 | 32.7 | 29.1- 40.9 | |
| Wild emmer wheat | 19 | | 39-115 | Gomez-Becerra et al., 2010 |
| Bread wheat (advanced) | 20 | 33.6 | 32.6-34.8 | Joshi et al., 2010 |
| Bread wheat (advanced) | 1300 | 30.5 | 23-52 | Velu et al., 2011a |
| Bread wheat (advanced) | 600 | 30.4 | 16.9-60.8 | Velu et al., 2011b |
| Bread wheat (advanced) | 40 | 32.5 | 29.0-39.5 | Velu et al., 2012 |

Biofortified staple foods may not deliver equally high levels of minerals and vitamins per day, compared to supplements or fortified food products, but they can increase micronutrient intake for the resource-poor people who consume them daily, and therefore complement existing approaches (Bouis et al., 2011). Thus, biofortification of cereal crops with Zn and Fe is a high-priority global issue. Harvest Plus (www.harvestplus.org) is the major international consortium to develop new plant genotypes with high concentrations of micronutrients by applying classical and modern breeding tools (*i.e.* genetic biofortification). Although plant breeding is the most sustainable solution to the problem, developing new micronutrient-rich plant genotypes is a protracted process and its effectiveness can be limited by the low amount of readily available pools of micronutrients in soil solution (Cakmak, 2008). Application of Zn and Fe containing fertilizers (*i.e.* agronomic biofortification) is a short-term solution and represents a complementary approach to breeding. Exploiting the genetic variability and biotechnological approach to the development of plants with high Fe and Zn content may be an effective method to improve the human nutrition, but, unfortunately, it is not very cost effective and requires significant amount of time. Agronomic approaches such as application of Fe and Zn to plant growth media, called "agronomic biofortification" (Bilski, et al., 1995), seems to be a very cost-effective, fast and practical approach to improve Fe and Zn concentration in cereal crops. Increasing the Zn and Fe concentration of food crop plants, resulting in better crop production and improved human health is an important global challenge. The provision of grains with higher micronutrient levels is a challenging task for breeders, but one that would complement the use of supplemental fertilizers, particularly on soils inherently low in these nutrients. This review synthesizes the progress made in biofortification approaches, including genetic and agronomic biofortification strategies by traditional cross breeding and fertilizer management strategies, respectively.

## RESULTS AND DISCUSSION

### 1. Agronomic biofortification

### 1.1Minerals Fertilization

### 1.1.1 Iron fertilization

Soil iron fertilization is believed to have little or no effect on iron concentration of grains (Narwal et al., 2010). Thus much of the work has been focused to identify the effects of foliar application on iron accumulation in grains. Foliar application is reported to increase iron concentration by 20% to 70% in the grains of bread wheat (Shukla & Warsi, 2000; Habib, 2009; Zeidan et al., 2010; Zhang et al., 2010). Recently, effects of foliar application of different forms of iron fertilizer at different plant developmental stages were studied in rice and it was shown that application of the DTPA-Fe form at the anthesis stage resulted 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 (Shukla & Warsi, 2000; Fang et al., 2008; Habib, 2009; Shi et al., 2010; Zeida et al., 2010).

### 1.1.2 Zinc fertilization

It is generally found that zinc deficiency in human beings is associated with zinc-deficient soils (Cakmak et al., 1999, Alloway, 2009). This led to numerous studies to identify the effect of soil or foliar zinc fertilization on grain zinc concentration under varied agro-ecological conditions. Soil application of zinc resulted in 20% to 90% and 60% to 250% increase in grain zinc concentration in bread wheat (*Triticum aestivum* L.) and durum wheat (*Triticum durum* L.), respectively (Shukla & Warsi, 2000; Yakan et al., 2001; Shivay et al., 2008; Cakmak, 2010; Zhao et al., 2011; Stomph et al., 2011). Foliar application of zinc resulted in even a higher increase in grain zinc concentration than soil application in both bread and durum wheat ( Habib, 2009; Zeidan et al., 2010; Zhang et al., 2010; Khoshgoftarmanesh et al., 2013). Studies of natural variation revealed the existence of notable differences for zinc accumulation in wheat grains between different wheat cultivars in response to soil and foliar application of zinc (Khoshgoftarmanesh et al., 2013). Moreover, the timing of foliar application of zinc was also found to be crucial in determining the wheat grain zinc contents. Foliar application of zinc around flowering time was shown to produce the highest increase in zinc contents in the endosperm of wheat grains (Cakmak et al., 2010). Applying Zn during the grain development stage contributes to increased grain Zn concentration (Zhang et al., 2010) as foliarly-applied Zn can be absorbed by the leaf epidermis and then transported to other plant parts via the xylem and phloem (Haslett et al., 2001). These findings suggested that both the zinc uptake and the remobilization are important factors that determine the zinc concentration in wheat grains, and natural variation for response to zinc fertilization is present in this species. Phloem is the only vascular tissue to reach the developing wheat grains, therefore zinc has to leave the xylem at some stage and become actively loaded into the phloem to reach the grains (Patrick & Offler, 2001). In rice, the impact of soil or foliar application of zinc on increasing grain zinc concentration is not as strong as in wheat; generally, an increase in the range of 10% to 60% is found in grain zinc concentration in response to zinc fertilization (Shivay et al., 2007; Fang et al., 2008; Phattarakul et al., 2009). Similarly, very low increase in grain zinc concentration (up to 40%) was observed after soil application of zinc fertilizer in maize (Kanwal et al., 2010). Foliar application of zinc, besides influencing grain zinc concentration, could also increase iron concentration in wheat, rice, and maize grains (Fang et al., 2008; Habib, 2009; Aref, 2010; Zeidan et al., 2010), and reduce cadmium toxicity and accumulation in cereals grown on cadmium-contaminated sites (Jiao et al., 2004; Koleli et al., 2004). These findings strongly support the utilization of zinc fertilizer in cereal cultivation to produce zinc and iron-enriched cereals, but the success may depend upon the cultivar being used and agro-ecological conditions.

**Table 3: The effect of iron or zinc fertilization on accumulation of these minerals in cereal grains**

| Species | Variety | Nutrient method | Nutrient added | Percent increase in grain zinc (mg/kg) | Percent increase in grain iron (mg/kg) | References |
|---|---|---|---|---|---|---|
| *Triticum aestivum* L | K 7410 | Soil | 25 kg/ha Zn | 30 | NIb | Shukla and Warsi 2000 |
| *Triticum aestivum* L. | Baldo | Soil | 45 kg/ha Zn | 20 | NDa | Yakan et al., 2001 |
| *Triticum aestivum* L | NDa | Soil | 5.2 kg/ha Zn | 30 | NDa | Shivay et al., 2008 |
| *Triticum durum* Desf. | Balcali | Soil | 21 kg/ha Zn | 250 | NDa | Cakmak 2010 |
| *Triticum aestivum* L. | Zhengmai 9023 | Soil | 45 kg/ha Zn | 30 | NDa | Zhao et al., 2011 |
| *Triticum aestivum* L. | SAMNYT-16 | Soil | 5 mg Zn kg−1 | 10 | NDa | Stomph et al., 2011 |
| *Triticum aestivum* L |  | Foliar | 150 g/ha Zn<br>150 g/ha Fe | 420<br>90 | 50<br>70 | Habib 2009 |
| *Triticum aestivum* L. | Sakha-93 | Foliar | 0.5% Zn<br>1% Fe | 210<br>20 | 20<br>60 | Zeidan et al., 2010 |
| *Triticum aestivum* L. | Jing 411 | Foliar<br>Foliar | 1.8 kg/ha Fe<br>1.8 kg/ha Fe and Zn | Nib<br>40 | 30<br>10 | Zhang et al., 2010 |
| *Oryza sativa* | NDa | Soil | 5.2 kg/ha Zn | 50 | NDa | Shivay et al., |

| | | | | | | |
|---|---|---|---|---|---|---|
| L. | | | | | | 2007 |
| *Oryza sativa* L | Wuyunjing 7 | Foliar<br>Foliar | 0.9 kg/ha Zn<br>1.8 kg/ha Fe | 20<br>10 | 30<br>NIb | Fang et al., 2008 |
| *Oryza sativa* L. | TDK 7 | Soil<br>Foliar | 50 kg/ha Zn<br>0.5% Zn | 10<br>60 | NIb<br>NDa | Phattarakul et al., 2009 |
| *Zea mays* L. | FHY-421 | Soil | 54 kg/ha Zn | 40 | NDa | Kanwal et al., 2010 |
| *Zea mays* L. | NDa | Soil | 24 kg/ha Zn | NDa | 50 | Aref 2010 |

NDa- for no data was available.

NIb -for no increase in grain iron or zinc concentration in response to iron or zinc fertilization.

## 2. Genetic biofortSiciation

Genetic biofortification is a strategy that uses plant breeding techniques to produce staple food crops with higher micronutrient levels, reducing levels of anti-nutrients and increasing the levels of substances that promote nutrient absorption (Bouis, 2003). It offers a sustainable solution to malnutrition problems by exploring natural genetic variation to develop mineral-dense crop varieties (Pfeiffer & Mc Clafferty, 2007). Plant breeders screen existing accessions in global germplasm banks to determine whether sufficient genetic variation exists to breed for a particular trait. They then selectively breed nutritious cultivars of major staples, rich in Zn and Fe concentrations and with substances that promote the bioavailability of Zn and Fe.

### 2.1 Constraints to the breeding approach

#### 2.1.1 Soil pH

A successful breeding program for biofortifying cereals and other food crops with Zn is dependent on the size of plant available Zn pools in soil. In order to increase Zn accumulation in grain required for a measurable biological impact, sufficient amount of plant-available Zn must be maintained in soil. However, in many cereal-growing areas, soils have a variety of chemical and physical problems that significantly reduce solubility and impair root absorption of Zn. Among the soil chemical factors, soil pH plays the most important role in Zn solubility in soil solution. In a pH range between 5.5 and 7.0, Zn concentration in soil solution is decreased by 30-fold to 45-fold for each unit increase in soil pH, thus increasing a risk for development of Zn deficiency in plants (Marschner, 1993). As shown in Fig.2 increasing soil pH stimulates adsorption of Zn to soil constituents (e.g., metal oxides, clay minerals) and reduces desorption of the adsorbed Zn (Fig. 2). (Lindsay, 1991) reported that at pH 5.0 the concentration of $Zn^{2+}$ in soil solution is sufficiently high, about $10^{-4}$ M (6.5 mg $kg^{-1}$). When soil pH increased from 5 to 8, concentration of soil solution $Zn^{2+}$ is reduced 1,000 times and becomes nearly $10^{-10}$ M (approx. 0.007 mg $kg^{-1}$). Consequently, an increase in soil pH is associated with very sharp decreases in concentrations of Zn in plant tissues (Sarkar & Wyn Jones, 1982; Marschner, 1995).

#### 2.1.2. Soil moisture

Transport of Zn to root surface in soils occurs predominantly *via* diffusion (Wilkinson et al., 1968), and this process is highly sensitive to soil pH and moisture. Soil moisture is a key physical factor providing suitable medium for an adequate Zn diffusion to plant roots. This role of soil moisture is very critical in soils with low Zn availability (Rattan & Deb, 1981; Marschner, 1993). Zinc nutrition of plants is, therefore, adversely affected under water stressed conditions, particularly in regions where top soils are usually dry during the later stages of crop growth. Thus, development of Zn deficiency stress and decreases in grain yield due to Zn deficiency were found to be more severe under rainfed compared to irrigated conditions, as shown under field conditions (Bagci et al., 2007).

#### 2.1.3. Soil organic matter

Soil organic matter plays a critical role in solubility and transport of Zn to plant roots (Obrador et al., 2003). In a study with 18 Colorado soils, there was a strong inverse relationship between the contents of soil organic matter and soluble Zn concentrations in the rhizosphere (Catlett et al., 2002). Turkish soils are characterized by low levels of organic matter and high soil pH (Cakmak et al., 1996). In a survey of 1,511 soil samples from Turkey conducted by (Eyupoglu et al., 1994), it has been found that diethylenetrinitrilopentaacetic acid (DTPA)-extractable Zn concentrations are inversely related to soil pH and soil organic matter. In general, soils containing less than 0.5 mg DTPA-extractable Zn are considered potentially Zn deficient that may respond well to Zn fertilizers (Lindsay & Norvell, 1978).

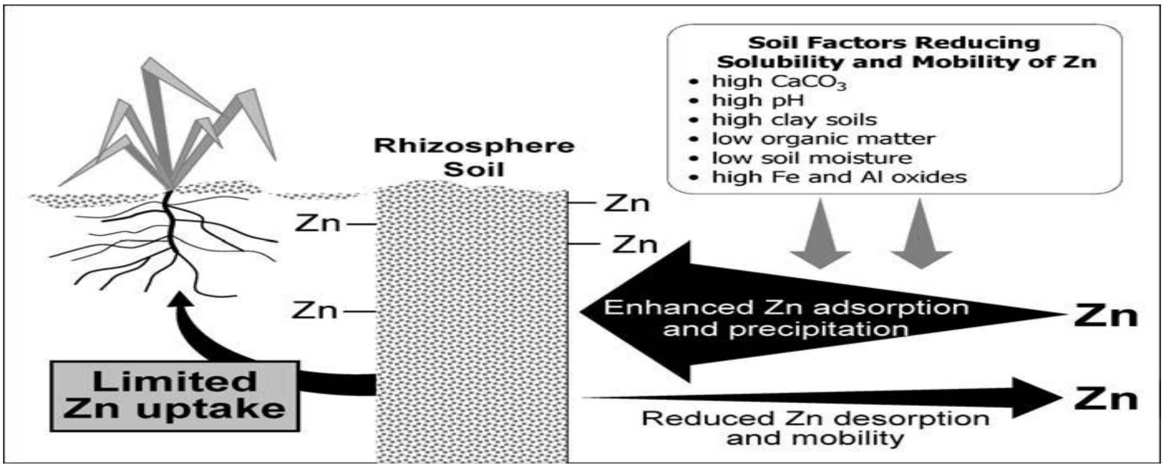

**Fig. 2: Major soil chemical and physical factors affecting availability of Zn to roots**

## 2.2. Breeding approach

### 2.2.1. Precision phenotyping

Several soil and environmental variables such as pH, temperature, radiation, precipitation, organic matter, and soil texture have the potential to influence concentration and solubility of micronutrients to plant roots (Cakmak, 2008; Joshi et al., 2010). Precision phenotyping is a vital tool in breeding wheat germplasm with stable and high Zn concentration. Early and advanced generation materials should be evaluated in locations where soil Zn is homogeneous and not limiting. This can be achieved in at least two ways: (1) by applying a high rate of Zn fertilizer to homogenize the area and ensure that Zn is not limiting; and (2) by identifying areas that are naturally homogeneous, with non limiting levels of soil Zn. The latter may be done by planting a systematic check cultivar in a given area and developing maps using geo-statistics that show variability for Zn grain concentration (Ortiz-Monasterio et al., 2010). It is important to maintain an adequate amount of available Zn and Fe in the soil during the crop growth period. Soil application of Zn-containing fertilizers may improve grain Zn concentrations in wheat depending on the severity of Zn deficiency in soil (Cakmak, 2008; Zou et al., 2012).

### 2.2.2. High throughput screening methodology

Selecting genotypes with higher micronutrient concentrations requires fast, accurate, and inexpensive methods of identifying nutrient dense genotypes. Traditionally, elemental analysis was conducted using inductively coupled plasma-optical emission spectrometry (ICP-OES) (Zarcinas et al., 1987), but this method requires expensive equipment, highly trained analysts, contamination free reagents, and extensive sample preparation. Consequently, many biofortification programs, especially those in more remote locations, have not been able to conduct their own analyses and have had to send samples to better-equipped laboratories elsewhere. Alternative, colorimetric approaches have been developed for Zn and Fe analysis in different cereal crops (Choi et al., 2007; Velu et al., 2006, 2008). Though simpler to perform than ICP-based methods, these approaches are only semi-quantitative and laborious when applied in large scale screens. Furthermore, energy-dispersive X-ray fluorescence spectrometry (EDXRF) was standardized by Paltridge et al., (2012) for measuring Zn and Fe concentrations in whole grain wheat. The high throughput, low cost XRF screening technique allows screening large number of breeding lines to discard low Zn/Fe lines and the selected high Zn/Fe lines could be tested with ICP for confirmation. The promising genotypes with significantly high Zn and Fe levels along with other essential agronomic features will be considered for large scale multi location testing and variety release.

### 2.2.3. Genotype x environment (G x E) interaction

Breeding for high Zn concentration is complicated by environmental conditions, particularly soil composition (Trethowan, 2007). Therefore, despite advances in breeding for uptake efficiency or mobilization to the grain, grain Zn concentration is limited by Zn availability in the soil (Ortiz- Monasterio et al., 2011). Significant genotype location interactions have been observed for Zn and Fe in wild and improved wheat cultivars (Gomez-Beccara et al., 2010a). The best high Zn lines developed at CIMMYT, Mexico, and evaluated in a multi location trial in India's Eastern Gangetic Plains (EGP), revealed that wheat grain Zn concentrations were highly unstable (Joshi et al., 2010) as the performance of the elite lines varied across locations and years. Another reason for greater G x E interaction for Zn concentration may be its quantitative inheritance, as reported in maize and rice (Gregorio et al., 2000; Long et al., 2004). Other reports

also suggest quantitative control in wheat (Trethowan, 2007). A recent study tested biofortified wheat lines at multiple locations in South Asia and revealed high heritability and high genetic correlation between locations for grain Zn, suggesting that G x E may not be a serious issue in breeding high Zn wheat genotypes (Velu et al., 2012).

### 2.2.4. Breeding target and target population

General estimates of breeding targets for Fe and Zn were derived based on the expected bioavailability percentage, daily intake of cereals per capita, type of food preparation, and estimated average requirements (EAR). The preliminary breeding target for primary target countries of Pakistan and Northern India is to increase Fe and Zn levels by 25 and 10 mg/kg, respectively, above the baseline, which is the mean of popular varieties currently grown in the region. Within the wider gene pool, there is sufficient genetic variability for Zn concentration to develop cereals varieties with increased Zn levels in the grain. There is also promising genetic variability for Fe, but due to its lower bioavailability, the target levels for Fe need to be high, and achieving them may not be easy.

### 2.2.5. Increasing Iron and Zinc Bioavailability

The bioavailability of iron and zinc can be increased by reducing the concentration of inhibitors which hinder the human absorption of dietary iron and zinc or increasing the concentration of enhancers which favor iron and zinc absorption. Cereal foods are the most important part of both human and animal diets. They provide proteins, carbohydrates, fiber, vitamins, minerals, antioxidants, and phytochemicals. Yet, they contain anti nutritional factors (ANF) such as phytate, tannin, and certain insoluble fibers which interfere with the absorption of iron and zinc (Hambidge et al., 2010; Petry et al., 2012). The ANFs contain negatively charged groups which results in the creation of mostly insoluble complexes with numerous divalent and trivalent cations, thus making them unable to be absorbed during intestinal digestion. In cereals, phytate being the most predominant form of ANFs (Lestienne et al., 2005) have been the subject of numerous studies. Phytic acid (PA, also known as myo-inositol hexaphosphate) is the primary storage form of phosphorus (P) in seeds, typically accounting for 60% to 90% of the total seed phosphorus and contributing as much as 1.5% to the seed dry weight (Bohn et al., 2008). The negatively charged phosphates in PA strongly bind to metallic cations (such as K, Mg, Mn, Fe, Ca, and Zn) to form a mixed salt called phytin or phytate (Bohn et al., 2008). Metal cation-phytate complexes can be formed in 2 ways: simple phytate-mineral complexes or fibre-phytate-mineral complexes. The stability and solubility of the metal cation-phytate complexes depend on the individual cation, the phytate-to-cation molar ratio, pH value, and presence of other compounds in the solution (Greiner et al., 2006). Phytate is predominantly found in the protein bodies of embryo and aleurone layers (Steadman et al., 2001), where there is a high deposition of minerals as well. The chelation of iron and zinc with PA has a strong negative effect on absorption of these minerals in humans and other monogastric animals that largely lack the phytase enzyme, which is required to degrade phytate. Different processing methods like mechanical, thermal, and bioprocessing techniques have been employed to degrade phytate

(Frontela et al., 2011). The extent of dephytinization was shown to vary depending upon the rigor and intensity of processing operations. Usually, phytate is quite stable to cooking for prolonged periods (Schlemmer et al., 2009). Likewise, extrusion cooking seems to have little or negative impact on the iron bioavailability (Hurrell et al., 2002). The decreased bioavailability of minerals was attributed to the loss of phytase activity during extrusion cooking. Owing to water solubility, phytate level may decrease during soaking (Afify et al., 2011). Further reduction can be achieved by soaking at the optimal temperature (55°C) and pH (5.5) for phytases (Schlemmer et al., 2009). Among cereals, rye is said to contain high phytase activity (5000 to 7000 U/kg) followed by wheat (1200 to 3000 U/kg) and barley (1000 to 2300 U/kg) (Greiner and Konietzny, 2006). Relatively lower phytase activity has been observed in oats (100 to 500 U/kg), rice (150 to 350 U/kg), and corn (70 to 150 U/kg) (Steiner et al., 2007). Endogenous phytases are activated during proofing of dough in bread-making (Schlemmer et al., 2009). The germination/malting of cereals are another such technique, believed to be quite helpful in increasing the bioavailability of iron and zinc in cereals (Krishnan et al., 2012). Thus, it is anticipated that reduction in dietary phytate content is likely to result in an improvement in iron and zinc absorption. Accordingly, for the past few years, it is being advocated that iron and zinc malnutrition could be combated by reducing seed PA. Different breeding and genetic engineering approaches that can help to developed cereal varieties with low-phytate.

### 2.2.6. Breeding Approaches to Reduce Phytate

Exploring natural variation may help us identify novel genetic mechanisms to reduce seed phytate in cereals. The quantitative genetics approach is relatively more developed in a model plant, *Arabidopsis thaliana*, where various QTL(Quantitative Trait Locus) mapping studies have been performed. A recombinant inbred lines (RILs) population developed by crossing Ler and Cvi was characterized for variation in seed phytate concentration and thereby used for QTL mapping (Bentsink et al., 2003). The L*er* × Cvi RILs displayed more than 2-fold variation for phytate concentration. More than 50% of the RILs displayed positive or negative transgression suggesting that new allelic combinations generated by mixing genomes of the 2 individuals offer great potential to reduce seed phytate concentration. Further, the authors mapped QTLs controlling seed phytate and found that one of the QTLs located on top of the chromosome 03 accounted for more than 60% of the total variance. Recently, 3 additional *A. thaliana* RIL populations were used for mapping QTLs of seed phytate concentration (Ghandilyan et al., 2009). Positive as well as negative transgression for seed phytate concentration was consistently observed in all the populations, and QTLs for seed phytate concentration in these populations were also mapped to the top of chromosome 3 that explained more than 30%

of the total variance. Thus, top of chromosome 3 in Arabidopsis seems very crucial to determine the variation in seed phytate concentration, and high percentage of variance explained by the QTLs in studies conducted under variable environmental conditions suggests that the underlying gene could have major and stable influences on the trait. A similar study was undertaken in rice to map the QTLs of seed phytate concentration, and iron and zinc concentration in rice seeds using doubled haploid population (DH) of IR64 × Azucena (Stangoulis et al., 2007). Interestingly, only positive transgression was observed for seed phytate concentration in this DH population in contrast to Arabidopsis thaliana RIL populations. In DH population of rice accessions, two QTLs, one on chromosome 05 and another on chromosome 12, respectively, explained 24% and 15% of the total variance. Thus, it is anticipated that breeding using mutation breeding may seriously hamper seed germination and plant performance, which will ultimately affect yields.

### 2.2.7. Genetic Engineering Approaches to Reduce Phytate

Phytases, a special class of phosphatases, catalyze the sequential hydrolysis of PA to produce less phosphorylated myo-inositol derivatives and inorganic phosphate. Seed phytate concentration decreases by expressing heterologous phytases in cereals either under the control constitutive promoters or tissue-specific promoters. A thermo-stable phytase gene of *Aspergillus fumigates* (*phyA*) was expressed in rice endosperm under the control of glutelin promoter which resulted in a 130-fold increase in phytase activity in transgenic seeds (Lucca et al., 2001). This much phytase activity was considered to be sufficient to completely degrade phytic acid in a simulated digestion experiment. However, the results were not confirmed after boiling the rice for 20 min in water where only 8% of the total phytase activity was retained. (Brinch-Pedersen et al., 2000) transformed *Aspergillus niger* phytase encoding gene (*phyA*) in wheat using 2 expression cassettes. In the 1st, an *α*-amylase signal peptide sequence was inserted between the promoter and the phytase coding region (Ubi-SP-Phy), and in the 2nd no *α*-amylase signal peptide was inserted (Ubi-Phy). The Ubi-SP-Phy transgenic seed exhibited up to a 400% increase of phytase activity, while up to 56% increase was found in Ubi-Phy plants. In maize, over expression of *Aspergillus niger* phytase gene *(phyA2*) in seeds using a construct driven by the maize embryo specific globulin-1 promoter resulted in about 5000% increase in phytase activity and 30% decrease in seed phytate concentration (Chen et al., 2008). During most of these studies characterization of the transgenic for agronomic characteristics was not performed, and in the absence of such data it is very difficult to evaluate the effectiveness of this approach in increasing iron and zinc bioavailability without affecting plant performance. On the other hand, a very novel and interesting approach has been used in maize and soybean to silence the genes involved in the biosynthesis of PA (Shi et al., 2007). It was found that maize *lpa1* mutants are defective in a MRP ATP-binding cassette (ABC) transporter that is more highly expressed in embryos, but also in immature endosperm, germinating seeds, and vegetative tissues. The expression of this transporter was silenced in an embryo-specific manner. The concentration of PA in seeds of maize transgenic was found to be reduced by up to 87% depending upon the transgenic line, and the transgenic plants were not affected in grain yield or seed germination in contrast to the *lpa* mutants. Similarly, silencing of MRP transporter in sorghum decreased the PA concentration in seeds by 80% to 86%, and a consequent increase in iron and zinc absorption was observed when analyzed in Caco-2 cell lines (Kruger et al., 2013). Rice transgenic developed by silencing *RINO1*, a gene involved in the biosynthesis of PA, in embryo and aleurone layer-specific manner exhibited up to 67% decrease in PA and were unaffected in grain yield, seed germination, and plant performance (Kuwano et al., 2009). These remarkable findings indicate the possibility to produce GMO cereal with low PA and without affecting agronomic performance by silencing the expression of transporters involved in the biosynthesis of PA.

**Table 4: Biofortified varieties of Rice, Wheat and Perl millet recommended for different part of India with increasing Fe and Zn content**

| Crop | Variety | Zn (ppm) | Fe (ppm) | Increase Zc (ppm) | Increase Fe (ppm) | Adaptation |
|---|---|---|---|---|---|---|
| Rice | CR Dhan310 | NDa | NDa | NDa | NDa | Odisa, M.P, U.P. |
| Rice | DRR Dhan 45 | 22.6 | NDa | 12-16 | NDa | Karnataka,T.N, A.P,Telangana. |
| Wheat | WB 02 | 42.0 | 32.2 | 40.0 | 28-32 | Punjab, Haryana, Delhi, Western UP, Himachal Pradesh, J and K |
| Wheat | HPBW 01 | 40.6 | 40.0 | 32.0 | 28-32 | Punjab, Haryana, Delhi, Western UP, Himachal Pradesh, J and K |
| Pearl Millet | HHB 299 | 41.0 | 73.0 | 30-35 | 45-50 | Punjab, Haryana, Delhi, Rajasthan, Gujarat, Maharashtra, T.N. |
| Pearl Millet | AHB 1200 | NDa | 73.0 | NDa | 45-50 | Punjab, Haryana, Delhi, Rajasthan, Gujarat, Maharashtra, T.N |

NDa- for no data was available.

(Yadav et al., 2017)

**Table 5: Biofortified varieties of Maize recommended for different part of India**

| Crop | Variety | Pro vitamin A (ppm) | Lysine (%) | Tryptophan (%) | Adaptation |
|---|---|---|---|---|---|
| Maize | Vivek QPM9 | 8.15 | 2.67 | 0.74 | Himachal Pradesh, Uttarakhand, North East Stats, Maharashtra, Karnataka, T.N, A.P, Telangana |
| Maize | PUSA HM4 | - | 3.62 | 0.91 | Uttarakhand, Punjab, Haryana, Delhi,U.P. |
| Maize | PUSA HM4 | - | 4.18 | 1.06 | Maharashtra, Karnataka,T.N, A.P, Telangana |

(Yadav et al., 2017)

## CONCLUSION

It is clear that genetic and agronomic biofortification offer sustainable solutions to the escalating micronutrient-related malnutrition problems. Genetic and agronomic biofortification approaches are actually not separate solutions; they are complementary and synergistic. Bioavailability of iron and zinc from cereal grains can be increased by genetic interventions by modulating accumulation of either anti-nutrient agents or prebiotics. Enhanced accumulation of iron and zinc in cereal grains can be achieved by fertilization or genetically manipulating iron and/or zinc homeostasis-related genes. However, it is not fully known whether it is feasible to increase the accumulation of these minerals up to desired levels in the edible parts of cereals by these strategies under field conditions. Moreover, it is not clearly identified how agronomic or genetic biofortification interventions affect the accumulation of toxic heavy metals such as cadmium and arsenic in edible portions of cereal grains. Future research should involve analyzing the accumulation of iron and zinc, as well as other heavy metals, in the edible parts of cereal grains rather than whole grains. It is also advised that the impact of genetic modifications on the agronomic performance of crops, including grain yield, drought tolerance, insect resistance, disease resistance, and so on should also be assessed. In addition, the focus should be on studies involving field crop trials and human beings as experimental subjects to analyze the effectiveness of agronomic or genetic biofortification.

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