

## Breeding for high grain Fe and Zn levels in cereals

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**ABSTRACT:** Micronutrients are known to play an important role in the metabolism and physiological activities of the human body. Unfortunately, over three billion people in the world are malnourished. The development of crops with enhanced mineral concentration is one of the most sustainable and cost effective approaches for alleviating micronutrient malnutrition. This review focuses on the progress made in the genetic enhancement of element mineral concentration in crops through plant breeding strategies. Biofortification is considered as a suitable strategy of increasing the bioavailable concentrations of an element in edible portions of crop plants through traditional breeding practices or modern biotechnology to overcome the problem of micronutrient deficiencies. Therefore, the combination of conventional breeding with modern genetic engineering approaches and quantitative trait loci (QTL) analysis is important for developing crop cultivars with enhanced micronutrient concentrations to improve human health.

**KEYWORDS:** Bioavailability, biofortification, iron, micronutrient deficiency, zinc.

### 1 INTRODUCTION

Micronutrient malnutrition has been designated as the most serious challenge to humanity (Copenhagen Consensus, 2008) as two-thirds of the world's population is at risk of deficiency in one or more essential mineral elements (White and Broadley, 2009; Stein, 2010). The concern is more crucial for developing countries, especially in children, given that the statistics of malnutrition in these countries are high. More than half of the total populations in developing countries are reported to be affected by micronutrient deficiency and therefore are more susceptible to infections and impairment of physical and psycho-intellectual development (WHO, 2005). The mineral elements most commonly lacking in human diets are iron (Fe) and zinc (Zn) (White and Broadley, 2009; Stein, 2010), whereas vitamin A and other essential minerals such as Calcium (Ca), Copper (Cu), Magnesium (Mg), and Iodine (I) can be deficient in some population's diets as well (Genc *et al.*, 2005). These deficiencies are caused by habitual diets that lack diversity (over dependence on a single staple food); situations of food insecurity, where populations do not have enough to eat (WHO, 2002); and low intake of vegetables, fruits, and animal and fish products that are rich sources of minerals. The widespread deficiencies of Fe and Zn in developing countries are mostly due to monotonous consumption of cereal-based foods with low concentration and reduced bioavailability of Fe and Zn (Graham *et al.*, 2001).

Micronutrient malnutrition greatly increases mortality and morbidity rates, diminishes cognitive abilities of children and lowers their educational attainment, reduces labor productivity, stagnates national development efforts, contributes to continued high population growth rates and reduces the livelihood and quality of life for all those affected (Welch and Graham, 1999). In an attempt to reverse this scenario, research has been carried out to improve nutrient concentrations in edible crops, which is generally known as biofortification (Mayer *et al.*, 2008; Bouis *et al.*, 2011). Biofortification of staple crops can be a sustainable and cost-effective approach to combat malnutrition (Meenakshi *et al.*, 2010), especially for rural populations in remote, low-rainfall areas, with limited access to diverse diets, commercially fortified foods or supplements

(Saltzman *et al.*, 2013). Genetic variation of grain micronutrient densities in adapted genetic materials is the basic requirement for biofortification breeding programs, and thus needs to be assessed beforehand.

The understanding of the genetic basis of accumulation of micronutrients in the grains and mapping of the quantitative trait loci (QTL) will provide the basis for devising the plant breeding strategies and for improving grain micronutrient content through marker-assisted selection. Developing micronutrient-enriched staple plant foods, either through traditional plant breeding methods or via molecular biological techniques, is a powerful intervention tool that targets the most vulnerable people (Bouis, 2000). This review focuses on the progress made in genetic enhancement of micronutrients in staple food crops, with emphasis on Fe and Zn in cereals, in solving the problem of micronutrient deficiency.

## 2 MICRONUTRIENT BIOAVAILABILITY

The total amount of a micronutrient in a plant food does not represent the actual micronutrient content of the food that is utilizable by the consumer. The bioavailable amount must be determined independently using methodologies especially developed for such purposes. In human nutrition terms, bioavailability is commonly defined as the amount of a nutrient in a meal that is absorbable and utilizable for metabolic processes in the body (Welch and Graham, 2004). Indeed, only a small portion of accumulated minerals in edible parts is bioavailable leading to certain segments of the vegetarian population being at risk for Fe and Zn, and other trace elements deficiency. Thus, determining the bioavailability of Fe and Zn in the genetically enhanced new lines is an important aspect of crop biofortification programs. The levels of bioavailable Fe and Zn in staple food crop seeds and grains are as low as 5% and 25%, respectively (Bouis and Welch, 2010). Breeders should therefore consider the bioavailability of micronutrients and the micronutrient concentration while breeding.

Bioavailability of iron and zinc is known to be influenced by various dietary components, which include both inhibitors and enhancers of absorption. Among inhibitors, phytic acid (PA), tannins, dietary fiber and calcium are the most potent, while organic acids are known to promote iron absorption (Sandberg, 2002; Hambidge *et al.*, 2010). Phytate, a complex of phytic acid and mineral elements, decreases the bioavailable concentration of nutrient elements, and thus leads to health problems, such as Fe and Zn deficiency, for populations whose diets are based mainly on cereals and legumes (Liu *et al.*, 2006). These compounds are normal plant metabolites and only small changes in their concentration may have significant effects on the bioavailability of micronutrients.

Several studies have demonstrated the negative effect of phytate on Zn and Fe absorption, causing nutritional deficiencies both in animals and humans (Lönnnerdal, 2002). A study on pearl millet showed that Fe was chelated by phytates and insoluble fibers, whereas Zn was almost exclusively chelated by phytates. Similarly, in the case of higher fiber and tannin contents, the chelating effect of these compounds was higher than that of phytates (Lestienne *et al.*, 2005a). In the Western Highlands of Guatemala, 412 infants were randomized to receive low-phytate or control maize. Within each maize group, infants were further randomized to receive a zinc supplement or placebo. Length, weight, and head circumference were measured at 6, 9, and 12 mo of age. No significant differences between the 2 maize groups or between the Zn supplement and placebo groups were observed indicating no effect on linear growth in Guatemalan infants with dietary phytate reduction, zinc supplement, or their combination (Mazariegos *et al.*, 2010). Studies in animals have shown the positive effect of diets containing low phytate maize to improve the use of minerals (Veum *et al.*, 2001; Li *et al.*, 2000). Thus, the inhibitory effect of phytate should be taken into account when assessing Fe and Zn deficiencies.

Recent technological advancements have improved accuracy and precision of methods used in the study of bioavailability and absorption of trace elements. Currently two models are used to evaluate mineral bioavailability in foods and diets, presenting great variability of results, and they include: *in vitro* and *in vivo* models (Hemalatha *et al.*, 2007; Vitali *et al.*, 2007). *In vivo* investigations generally include work with rats or clinical studies with humans. *In vitro* methods involve determining the soluble and/or dialyzable fraction of the mineral and are important as screening techniques (Fairweather-Tait *et al.*, 1995). Due to the phytic acid influence on mineral absorption, researchers have also used the molar ratio of phytic acid/mineral as a simpler and less costly method to estimate the Fe and Zn bioavailability in food (Lestienne *et al.*, 2005a; Lazzari, 2006; Abebe *et al.*, 2007). *In vitro* and *in vivo* studies on the availability of Fe in a nutritional formulation indicated low Fe availability and absorption in humans (Bueno *et al.*, 2013).

## 3 BIOFORTIFICATION: A TOOL FOR IMPROVED HUMAN HEALTH

Breeding staple cereal crops dense in minerals is a low-cost, sustainable strategy to ameliorate micronutrient malnutrition for people living in developing countries which cannot afford pulses, fruits, vegetables, fish and animal products enriched with micronutrients in the diet (Martinez *et al.*, 2007; Cakmak, 2008). A Combination of strategies involving food fortification, pharmaceutical supplementation and dietary diversification has been suggested to fight micronutrient

malnutrition (Stein *et al.*, 2005). However, neither of these strategies has been universally successful in developing countries, largely due to lack of safe delivery systems, stable government policies, appropriate infrastructures and continued adequate investment (Lyons *et al.*, 2003; Misra *et al.*, 2004). Thus, a complimentary solution to micronutrient malnutrition has been proposed and termed as 'biofortification' (Bouis, 2003). Biofortification, a new approach to combat micronutrient deficiencies, is defined as the process of increasing the concentration and/or bioavailability of essential elements in the edible part of the plant by traditional plant breeding or genetic engineering (White and Broadley, 2005). By definition, the focus of plant breeders and biofortification initiatives is on breeding crops with a high density and increased bioavailability of nutrients. HarvestPlus ([www.harvestplus.org](http://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. Most Zn fertilization studies have focused on increasing grain yield, though grain Zn concentration is also starting to be addressed (Cakmak, 2009). The various methods of Zn application may differentially influence yield and grain Zn concentration. The most effective method for increasing grain Zn is the soil + foliar application method, which may result in an about 3-fold increase in grain Zn concentration (Cakmak *et al.*, 2010). Applying Zn during the grain development stage contributes to increased grain Zn concentration (Zhang *et al.*, 2010).

Recently published studies report clear increases in Fe and Zn absorption when biofortified pearl millet grain of Indian origin is consumed by young women or children (Cercamondi *et al.*, 2013; Kodkany *et al.*, 2013). Another study showed strong positive correlation ( $r = 0.73$ ) between Zn and Fe, showing that simultaneous selection for high Zn and Fe densities could be very efficient (Burger *et al.*, 2014). Several studies reported high correlation between Zn and Fe in pearl millet (Velu *et al.*, 2007; Govindaraj *et al.*, 2009; Bashir *et al.*, 2014), and wheat (Gomez-Becerra *et al.*, 2010a; Velu *et al.*, 2012). In wheat iron and zinc correlate positively (Zhang *et al.*, 2010) and highest concentrations (up to 85 mg/kg) were detected in landraces as well as in wild and primitive relatives (Ortiz-Monasterio *et al.*, 2007; Peleg *et al.*, 2008a). In India, application of Zn-coated urea fertilizer significantly improved both grain yield and grain Zn concentrations (Shivay *et al.*, 2008).

Conventional plant breeding and genetic engineering both involve changing genotype of targeted crops with the aim of developing plants carrying genes that support the accumulation of bioavailable minerals. The way of reaching this goal differs between the two approaches (Gomez-Galera *et al.*, 2010a). The main nutrients targeted for biofortification are betacarotene, iron and zinc. Most work is currently done on traditional plant breeding techniques, exploiting the variability of mineral concentrations found in different germplasms (Qaim *et al.*, 2007). Not all crops have the genetic potential to meet desired micronutrient levels with traditional plant breeding, and therefore genetic engineering has to be applied to achieve sufficient improvements (Borg *et al.*, 2009). It is suggested that genetic modification is an excellent approach to obtain high micronutrient concentrations (Bouis, 2007) and that genetically modified organisms (GMO) have the potential for increased agricultural productivity.

Another genetic engineering approach for increasing the bioavailability of Fe in diets is the reduction of dietary phytate. This sugar-like molecule binds a high proportion of dietary Fe, so that the human body is unable to absorb the Fe. Lucca *et al.* (2001) introduced a fungal gene for the enzyme phytase. This breaks down phytate, thus improving the bioavailability of Fe in rice diets. Wei *et al.* (2012) reported that foliar Zn fertilization reduced the phytic acid content and increase the accumulation of bioavailable Zn in the polished rice.

#### 4 EXPLOITING EXISTING GENETIC VARIATION: PREREQUISITE FOR BIOFORTIFICATION

Exploitation of genetic variation is the basis for crop improvement through plant breeding (Ortiz-Monasterio *et al.*, 2007). Genotypic variation for micronutrient accumulation in grain has been reported in staple crops such as pearl millet, rice, maize and wheat. Mean and range for grain iron and zinc concentrations documented in various studies are presented in Table 1.

##### PEARL MILLET

Pearl millet has naturally relatively high concentrations of both iron and zinc and there is demonstrated potential to increase these levels further by plant breeding. Several reports indicate the existence of large variability for grain Fe and Zn concentrations in pearl millet. For example, a recent study by Pucher *et al.* (2014) of 72 pearl millet accessions from West and Central Africa (WCA) assessed in Niger showed moderate ranges in mineral density (24.2 to 48.7 mg kg<sup>-1</sup> for Fe and 19.8 to 43.4 mg kg<sup>-1</sup> for Zn). A study focusing on the grain mineral density of 225 Sudanese pearl millet accessions evaluated in Sudan also found wide variation for Fe and Zn ranging from 19.7 to 86.4 mg kg<sup>-1</sup> for Fe and 13.5 to 82.4 mg kg<sup>-1</sup> for Zn (Bashir

et al. 2014). Rai *et al.* (2013) revealed two-fold variation for Fe density (31 to 61 mg kg<sup>-1</sup>) and Zn density (32 to 54 mg kg<sup>-1</sup>) among 122 commercial and pipeline hybrids of pearl millet developed in India. Other studies on grain Zn and Fe densities in pearl millet materials reported significant variability for Fe and Zn (Velu *et al.*, 2007; Velu *et al.*, 2008a; Gupta *et al.*, 2009; Govindaraj *et al.*, 2013).

#### **RICE**

Large genetic variation exists for grain iron and zinc in rice germplasm accessions and this variation can be exploited in breeding programs to enhance Fe and Zn content in the grains (Graham *et al.*, 1999; Welch and Graham, 2004). A recent study by Jahan *et al.* (2013) of 52 rice genotypes screened for Fe concentration revealed a wide range of variation with Fe concentration ranging from 1.32 mg/kg to 100.45 mg/kg. Anuradha *et al.* (2012) reported Fe concentration ranged from 6.2 mg/kg to 71.6 mg/kg among 126 accessions of brown rice genotypes and the local accessions had the highest Fe. Neelamraju *et al.* (2012) reported Fe concentration in brown rice ranged from 6 mg/kg in Athira to 72 mg/kg in *O. nivara* and Zn concentration from 27 mg/kg in Jyothi to 67 mg/kg in *O. rufipogon*. Kumar *et al.* (2012) reported large variation for Fe and Zn contents in rice grains ranging between 9.6-44.0 mg/kg and 9.9-39.4 mg/kg, respectively. Significant genetic variation was reported for Fe and Zn in indica and aromatic rice varieties (Brar *et al.*, 2011). Another study showed wide variation for micronutrient levels recorded among the 46 tested rice genotypes, which ranged from 4.8 to 22.7 mg/kg for grain Fe and 13.95 to 41.73 mg/kg for grain Zn content (Banerjee *et al.*, 2010). A study by Jiang *et al.* (2007) among 274 rice genotypes revealed mineral content in grains ranging from 0.98 to 26.78 mg/kg for Fe and 13.3 to 43.7 mg/kg for Zn.

#### **MAIZE**

Maize is a major component of the daily diet of many of the neediest people of the world, and was selected as a target crop for the HarvestPlus biofortification program (Nestel *et al.*, 2006). The development of an efficient breeding program to increase minerals concentration in maize depends on the presence of genetic variability in this species (Menkir, 2008). A study evaluating the kernel Fe and Zn of 67 diverse maize genotypes grown during 2006–08 indicated significant variation for both the micronutrients. Prasanna *et al.* (2011) evaluated kernel iron (Fe) and zinc (Zn) concentrations in a set of 30 diverse maize genotypes during rainy (*kharif*) season of 2006, 2007 and 2008. The ranges of kernel Fe and Zn concentrations were 11.28–60.11 mg/kg and 15.14–52.95 mg/kg, respectively, across the three years. Queiroz *et al.* (2011) reported significant variability in the contents of zinc (17.5 to 42 mg.kg<sup>-1</sup>) and iron (12.2 to 36.7 mg.kg<sup>-1</sup>) in 22 tropical maize inbred lines with different genetic backgrounds. Significant differences in the Fe and Zn concentrations in maize have been reported in many genotypes in trials conducted in Mexico and Zimbabwe by Bänziger and Long (2000) and in Nigeria by Menkir (2008).

#### **WHEAT**

A series of studies has shown that there is a two- to three-fold difference in the Fe and Zn concentrations within wheat germplasm and/or wheat cultivars. A recent study among 81 cultivars of bread wheat showed the concentration of grain Fe varied by 1.64 fold, ranging from 41.4 to 67.7 mg kg<sup>-1</sup>, grain Zn by 2.03 fold, from 36.4 to 73.8 mg kg<sup>-1</sup> (Badakhshan *et al.*, 2013). Research by Velu *et al.* (2011) among a diverse range of wheat core-collection accessions of diverse origin revealed the existence of large variability for Zn and Fe concentrations. Another study evaluating a set of high yielding lines under field conditions showed Zn values ranged between 15 and 35 mg/kg but reached as high as 43 mg/kg in some genotypes, while the Fe concentration ranged from 20 to 60 mg/kg (Oury *et al.*, 2006). Morgounov *et al.* (2007), Zhao *et al.* (2009) and Chatzav *et al.* (2010) reported significant genetic variability for Fe and Zn among wheat genotypes.

**Table 1.** Variation for Fe and Zn content in major crops documented in various studies

Crop	No. of entries	Mean	Range	Source
<b>Fe concentration (mg/kg)</b>				
Pearl millet	225	42.9	19.7 - 86.4	Bashir <i>et al.</i> (2014)
	72	38	24.2 - 48.7	Pucher <i>et al.</i> (2014)
	120	45.5	30.1 - 75.7	Velu <i>et al.</i> (2007)
	68	55	42 - 79.9	Velu <i>et al.</i> (2008a)
	30 S1's	46.7	29.9 - 77.2	Gupta <i>et al.</i> (2009)
Wheat	82	50.6	41.4 - 67.7	Badakahshan <i>et al.</i> (2013)
	600	39.7	26.3 - 68.8	Velu <i>et al.</i> (2011)
	150	38.2	28.8 - 50.8	Zhao <i>et al.</i> (2009)
	66	38	25 - 56	Morgounov <i>et al.</i> (2007)
	30	30.8	17.5 - 48.7	Khodadadi <i>et al.</i> (2010)
Maize	30	29.8	11.3 - 60	Prasanna <i>et al.</i> (2011)
	22	24.2	12.2 - 36.7	Queiroz <i>et al.</i> (2011)
Rice	274	5.4	0.98 - 26.8	Jiang <i>et al.</i> (2007)
	46	-	4.8 - 22.7	Banerjee <i>et al.</i> (2010)
	20	-	9.6 - 44.0	Kumar (2012)
<b>Zn concentration (mg/kg)</b>				
Pearl millet	225	40.3	13.5 - 82.4	Bashir <i>et al.</i> (2013)
	72	34.9	19.8 - 43.4	Pucher <i>et al.</i> (2014)
	120	43.9	24.5 - 64.8	Velu <i>et al.</i> (2007)
	68	38	27.2 - 50.2	Velu <i>et al.</i> (2008a)
	30 S1s	44.6	30.7 - 63	Gupta <i>et al.</i> (2009)
Wheat	82	49.6	36.4 - 73.8	Badakahshan <i>et al.</i> (2013)
	600	30.4	16.9 - 60.8	Velu <i>et al.</i> (2011)
	150	21.4	13.5 - 34.5	Zhao <i>et al.</i> (2009)
	66	28	20 - 39	Morgounov <i>et al.</i> (2007)
	30	35.7	22.4 - 52.7	Khodadadi <i>et al.</i> (2010)
Maize	30	29.6	15.1 - 53	Prasanna <i>et al.</i> (2011)
	22	27.2	17.5 - 42	Queiroz <i>et al.</i> (2011)
Rice	274	26	13.3 - 43.7	Jiang <i>et al.</i> (2007)
	46	-	14 - 41.7	Banerjee <i>et al.</i> (2010)
	20	-	9.9 - 39.4	Kumar (2012)

## 5 MICRONUTRIENT CONCENTRATION AND GRAIN YIELD

Iron (Fe), zinc (Zn) and copper (Cu) are essential micronutrients for plants and humans (Asad and Rafique, 2000; Hao *et al.*, 2007). A deficiency of one of these nutrients can greatly reduce plant yield and even cause plant death. A recent study on micronutrient density in pearl millet showed no significant correlation between grain yield and Zn and Fe densities (Bürger *et al.*, 2014). Govindaraj *et al.* (2009) studied correlations between agro-morphological traits and densities of four minerals (P, Ca, Zn and Fe) in pearl millet, where no association with grain yields was observed for all four. However, other studies on pearl millet have reported significant negative to no correlations between Zn (Fe) density and grain yield (Velu *et al.*, 2008a; Gupta *et al.*, 2009; Rai *et al.*, 2012). Negative correlations between the concentrations of Fe and Zn in grain and grain yield were reported in many studies in wheat, although the strength of these relationships was influenced greatly by the environment (White and Broadley, 2009). There were obviously significant negative correlations between yield and Zn concentration with the correlation coefficients ranging from -0.67 to -0.41, while there was no significant correlation for Fe (Oury *et al.*, 2006; Morgounov *et al.*, 2007; Ficco *et al.*, 2009; Zhao *et al.*, 2009). Grain yield and grain Fe were found negatively associated in maize ( $r = -0.26$ ) and sorghum ( $r = -0.32$  to  $-0.36$ ) (Reddy *et al.*, 2005; Chakraborti *et al.*, 2009). Grain yield and grain Zn were negatively associated in sorghum ( $r = -0.46$  to  $-0.54$ ) (Reddy *et al.*, 2005). However, Ananda *et al.* (2012) reported negative correlation between grain yield and mineral contents in rice.

## 6 HERITABILITY ESTIMATES OF GRAIN IRON AND ZINC CONCENTRATIONS

The inheritance of nutritional traits appears to be mostly quantitative, influenced by the environment, but more specific to source genotypes (Cichy *et al.*, 2009; Blair *et al.*, 2009). To determine whether iron and zinc concentration in a particular crop can be improved by traditional breeding methods, it must be known to what extent these traits are heritable. Heritability is a measure of genetic differences among individuals in a population, not simply of whether or not a trait is inherited (Gomez-Becerra *et al.*, 2010b). There are two types of heritability, broad-sense heritability ( $h^2_{bs}$ ) and narrow-sense heritability ( $h^2_{ns}$ ), with former less sensitive to environments than the latter (Klug and Cummings, 2005).

Recently, Govindaraj *et al.* (2011, 2013) and Bashir *et al.* (2014) reported high estimates of  $h^2_{bs}$  in pearl millet and they suggested predominance of additive gene effects in the inheritance of the nutritional traits. Both high  $h^2_{bs}$  for grain Fe (65 to 71.2%) and Zn (65 to 80%) (Gupta *et al.*, 2009) and  $h^2_{ns}$  for grain Fe (80%) and Zn (77%) (Velu, 2006) have been reported in pearl millet, which indicates that substantial portion of total variation for Fe/Zn is due to genetic effects. In wheat, estimates of  $h^2_{bs}$  ranged from 90.62% for Fe in 2010 to 90.90% for Zn in 2011 (Badakhshan *et al.*, 2013). Rawat *et al.* (2009) reported high  $h^2_{bs}$  for grain Fe (0.98) and Zn (0.96) in wheat genotypes. [95]Khodadadi *et al.* (2014) reported  $h^2_{bs}$  of grain Fe and Zn in wheat was 0.74 and 0.61 in 2009, 0.85 and 0.92 in 2010 respectively. Chakraborti *et al.* (2010) reported high  $h^2_{bs}$  for grain Fe (78% and 73%) and grain Zn (71% and 76%) in maize. Both moderate  $h^2_{bs}$  (54%) and high  $h^2_{ns}$  (78 to 82%) were reported for grain Zn in common bean (Cichy *et al.*, 2005; da Rosa *et al.*, 2010). Thus it appears that heritability estimates for grain Fe and Zn densities are high enough to permit effective selection for these traits in several staple food crops.

## 7 MARKER ASSISTED SELECTION AND QUANTITATIVE TRAIT LOCI ANALYSIS

The rapid development of DNA marker technology provides great opportunities to enhance nutritive values of traditionally cultivated crops and grains. Molecular markers augment conventional plant breeding for efficient and precise identification or selection of a trait of interest linked to them. During the past few decades; molecular markers have been immensely used in plant breeding and related genetic studies. They are used in assessment of genetic variability and characterization of germplasm; estimation of genetic distances between populations, inbreds and breeding materials; genetic mapping; detection of monogenic and quantitative trait loci (QTLs); marker assisted selection; increase the speed and quality of backcrossing to introgress desirable traits from distantly related varieties to elite germplasm; identification of sequences of useful candidate genes, etc. (Kesawat *et al.*, 2009; Kumar *et al.*, 2009; Miah *et al.*, 2013). The identification of genes and quantitative trait loci (QTLs) and DNA markers that are linked to them is accomplished via QTL mapping experiments. Since then, there have been thousands of QTL studies carried out in different species of plant kingdom. Molecular markers have been used to identify the genetic regions involved in grain Fe and Zn content in several species of crop plants. QTL information was therefore collected from previous QTL studies which reported major cereal grain Fe and Zn content and presented in Table 2.

In a study conducted in wheat, nine additive and four epistatic QTLs were identified for Fe and Zn, among which six and four, respectively, were effective at the two environments (Xu *et al.*, 2012). Zhi-en *et al.* (2014) reported QTLs for Fe and Zn concentration in two wheat recombinant inbred line populations. Four QTLs each associated with Fe and Zn concentrations were identified in the SC population explaining 5.4-9.5 and 5.5-8.6% of the phenotypic variance, respectively. Four QTLs for Fe concentration were detected in the CC population on chromosomes 4A, 4D, 5A, and 5B explaining 9.2-19.1% of the phenotypic variance while three QTLs affecting Zn concentration were identified on chromosomes 3D, 4D and 5B individual accounting for 14.5, 15.9 and 13.8% of the phenotypic variance, respectively. Peleg *et al.* (2009) found 11 QTL on chromosome 2A, 5A, 6B, 7A and 7B for Fe and 6 QTL on chromosome 2A, 2B, 3A, 4B, 5A, 6A, 6B, 7A and 7B for Zn. Shi *et al.* (2008) identified 4 QTL for grain zinc concentration (mg/kg) on wheat chromosomes 4 and 5 contributing 11.9% and 10.9% to the variance whereas for grain zinc content ( $\mu\text{g}/\text{seed}$ ) 7 major QTL were found on chromosomes 2 and 7 in a double haploid wheat population. QTL mapping researches have also been conducted on micronutrient content in maize (Lung'aho *et al.*, 2011, Qin *et al.* 2012, Šimic *et al.*, 2012; Jin *et al.*, 2013). A total of 5 significant QTLs controlling grain Zn and Fe content were detected in maize  $F_{2:3}$  mapping population (Jin *et al.*, 2013). For Zn content, 4 QTLs were identified on chromosomes 2, 5 and 10, whereas for Fe content, only one QTL was found on chromosome 5. Several mineral QTL co-localized with each other for two sets of  $F_{2:3}$  populations such as the QTL for zinc kernel (ZnK), zinc concentration (ZnC), iron kernel (FeK) and iron concentration (FeC) on chromosome 2, QTL for Znk, FeK and FeC on chromosome 9 and QTL for ZnK and ZnC on chromosome 7, were identified by Qin *et al.* (2012). Lung'aho *et al.* (2011) reported three modest QTL for grain iron concentration (FeGC) and ten QTL for grain iron bioavailability (FeGB) from an Intermated B73xMo17 (IBM) recombinant inbred (RI) population of maize.

For iron and zinc concentration in rice grains, 12 QTLs were detected for iron and zinc co-located on chromosomes 7 and 12 (Anuradha *et al.*, 2012). Seven QTLs for Fe and six QTLs for Zn were identified each explaining >30% phenotypic variance in rice accessions (Neelamraju *et al.*, 2012). Garcia-Oliveira *et al.* (2009) reported two QTL for Fe on chromosomes 2 and 9 and 3 QTL for Zn on chromosomes 5, 8 and 12. Three QTL for Fe on chromosome 2, 8 and 12, while two QTL for Zn on chromosomes 1 and 12 and a common QTL for Fe and Zn accounted for 13% to 18% variation were identified by Stangoulis *et al.* (2007). Marker-assisted selection is useful in improving the efficiency of selection early in the breeding cycle helping to improve characters with low heritability.

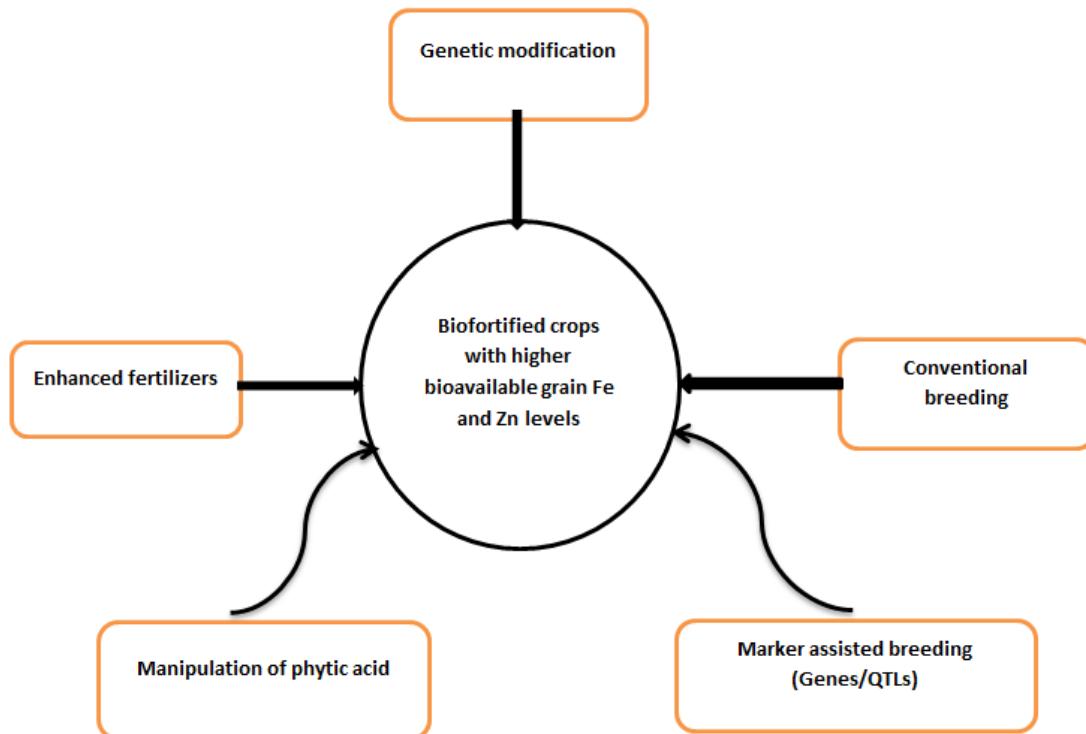
**Table 2. Summary of recent QTL studies considered for Zn and Fe content in major cereals**

QTL study	Population type	Parents	No. of envir	Software & method	Chromo	some	No. of QTL	Range of Fe	R <sup>2a</sup> Zn
					Fe	Zn			
<b>Wheat</b>									
Zhi-en <i>et al.</i> (2013)	RIL	SHW-L1/Chuanmai32	2	IciMapping 2.0;	2, 5, 7	2,3,4,5	4	5.4-9.5	5.5-8.6
		Chuanmai42/Chuannong	2	CIM	4, 5	3,4, 5	7	9.2-19.1	13.8 - 15.9
Peleg <i>et al.</i> (2009)	RIL	Langdon/G18-16	3	MultiQTL Package; IM	2,5,6,7	2,3,4,5,6,7	17	2.0 - 18.0	1.0 - 23.0
<b>Maize</b>									
Jin <i>et al.</i> (2013)	F2:3	Inbreds 178/P53	1	WinQTLCart 2.5; CIM	5	2,5,10	5	16.89	5.85 - 17.5
Šimic <i>et al.</i> (2012)	F4	B84/Os6-2	3	PLABQTL; CIM	2, 6, 8	4	4	6.8-7.5	7.8
Qin <i>et al.</i> (2012)	F2:3(Mus) F2:3(Mos)	Mu6/SDM Mo17/SDM	2 2	QTL IciMapping v3.0; FRA	2, 9	1, 2, 6, 7, 9, 10	14	10-21.1	6.3-21.3
Lung'aho <i>et al.</i> (2012)	RIL	B73/Mo17	6	QTL Cartographer v2.5; CIM	2, 5, 9	-	3	9.3-12	
<b>Rice</b>									
Anuradha <i>et al.</i> (2012)	RIL	Madhukar/Swarna	1	QTL Cartographer v2.5; CIM & MIM	1,3,5,7,12	1,3,5,7,12	14	69 - 71	29 - 35
Garcia - oliveira <i>et al.</i> (2009)	ILs	Teqing/Oryzaruifipogon	1	Map Manager QTx; QTxb17	2	5,8,12	4	5 & 7	11.0 - 19.0
Zhang <i>et al.</i> (2011)	DH	japonica JX17/indicaZYQ8	1	QTL MAPPER 1.6; IM	-	4, 6	2	-	10.83 - 12.38
Stangoulis <i>et al.</i> (2007)	DH	IR64/AZUCENA	1	QTL Cartographer v2.5; CIM	2,8,12	1,12	5	14.0 - 18.0	13.0 - 15.0

<sup>a</sup> Coefficient of determination: percentage of phenotypic variance explained by the QTL; CIM: Composite Interval Mapping; FRA: Forward Regression Analysis; MIM: Multiple Interval Mapping

## 8 FUTURE PROSPECTS FOR BREEDING HIGH-YIELDING CULTIVARS WITH IMPROVED FE AND ZN LEVELS AND/OR BIOAVAILABILITY

Most of plant breeding research in this area is now being concentrated on increasing the micronutrient content in the edible parts of plants species. Various biotechnological approaches like gene transfer and use of DNA markers have emerged as powerful tools to complement conventional methods of breeding by generating genetic variability necessary for grain micronutrients and reducing the time taken to produce cultivars with improved iron and zinc. Efficient molecular marker techniques now allow the genes to be tagged, and thus markers can be used to facilitate crossing the genes into new breeding lines with the target micronutrient. This may present a challenge to improve Fe and Zn grain concentrations because lower application of nitrogen fertilizer correlates to lower Fe and Zn grain concentrations (Cakmak *et al.*, 2010). Breeding/molecular techniques should be used to lower the level of the anti-nutrients like phytic acid (Sandberg, 2002).



**Figure 1. Future prospects for breeding high-yielding cultivars with improved levels of bioavailable Fe and Zn.**

## 9 CONCLUSION

There is very compelling global human health and nutritional evidence to convince plant breeders that micronutrient density traits should be primary objectives in their work targeted to the developing world. Therefore, biofortification is of great importance in enriching seeds with mineral micronutrient levels. Conventional plant breeding and marker-assisted selection offer good opportunities to increase the bioavailable micronutrient contents of edible parts of major staple food crops. Based on a range of reports and survey studies, large genetic variation exists for Zn and Fe concentration in various countries in cereals. This implies that sufficiently enough genetic variation exists to substantially increase grain Fe and Zn concentration. By exploring the genetic variation in existing varieties and in germplasm collections, genes and QTLs affecting mineral contents have recently been identified in several staple food crops, including rice, maize and wheat. Anti-nutrient factors could be minimized to enhance micronutrient bioavailability.

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