Chapter 6
Rice, Wheat and Maize Biofortification

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Abstract Cereals are the major energy source for humans throughout the world and hold a prominent position in a balanced diet to meet up carbohydrate demand of the body. Most of the cereals are deficient in micronutrient and vitamins and continuous dependency on cereal based diets resulted in human malnutrition. Biofortification is a novel concept defined as the enrichment of micronutrients through conventional plant breeding and modern biotechnology. In this post genomic era, an enormous amount of genetic information is available for staple food crops. This genetic information could be used to improve nutritional quality of the staple food crops to provide nutritional requirements. During last few decades, cereal biofortification research has been significantly contributed to reduce malnutrition around the world. Knowledge of precise phenotyping and genetics of the traits are prerequisite before starting of a genetic biofortification program. The inheritance of major micronutrients and vitamins in rice, wheat and maize were reported to be polygenic and in most of the cases the quantitative trait loci were mapped in the genome. A few commercial cereal cultivars are developed so far using genetic biofortification technique. The ongoing biofortification programs

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are more competitive as newer perspective of food matrix came into picture. In this article we reviewed an overview of current global cereal biofortification efforts, global malnutrition issues, and the promise of biotechnology techniques to improve cereals as a whole food solution to combat global malnutrition issues.

Keywords Biofortification • Bioavailability • Biomolecules • Cereals • Food matrix • Food legumes • Genomics • Malnutrition • Molecular mapping • Nutritional deficiency • Sustainable food systems

Abbreviations

cDNA	Complementary Deoxyribonucleic Acid
CGIAR	Consultative Group on International Agricultural Research
ILs	Introgression Lines
QPM	Quality Protein Maize
QTL	Quantitative Trait Loci
RILs	Recombinant Inbred Lines
SCN	Standing Committee on Nutrition
UN	United Nations
WHO	World Health Organization

6.1 Introduction

Millions of deaths occur every year due to micronutrient malnutrition (Anonymous 2008). Ironically, obesity and diet-related non-communicable diseases mostly are concomitant with micronutrient deficiencies (Bouis and Welch 2010). Chronic, non-communicable diseases including diabetes, heart disease, and cancer result in over 36 million deaths around the world annually (WHO 2005). These chronic diseases, whose development is largely caused by high-energy, micronutrient-poor diet, are not isolated to developed countries, having caused for more deaths than infectious diseases throughout the world (except parts of Africa) in recent years (UN 2011). Both micronutrient deficiencies and associated diseases are by poor nutritional content of staple cereals and food legumes. Therefore, enrichment of micronutrients in staple food crops is of much importance for nutrition security of human beings, especially, in the developing countries.

Anemia is one of the few common nutritional disorders affecting living and survival of humans. According to the World Health Organisation (WHO), around two billion of the world populations are anemic. Anemia is the body condition where hemoglobin concentrations in the blood are below thresholds. The threshold varies between gender and ethnicity. There are generally two groups of causes which may induce anemia: dietary iron deficiency, vitamin B_{12}, vitamin A, and/or folate deficiency and infectious diseases such as malaria, hookworm infections, schistosomiasis, and hereditary disorders such as thalassemia (WHO 2007). Iron deficiency may or may not be accompanied by anemia but always has an important negative impact on human health. The effect of Fe deficiency is more pronounced in case of pregnant women and children (WHO 2007). Sometimes this has severe consequences leading to the mortality of the newly borne children or even the fetus during prenatal stage. Children with acute iron deficiency show mental retardedness, laziness and, in case of working persons, reduced capacity to work (WHO 2007).

Zinc deficiency has a prominent role in affecting human health, particularly in children and mothers. For detailed review, see Gibson (2012). Zinc deficiency is reported to cause child mortality by way of increasing the vulnerability to other diseases like diarrhea and pneumonia (SCN 2004; Jones et al. 2003). Women in the developing countries are also increasingly affected with Zn deficiency and associated illnesses (Caulfield et al. 1998). A clinical trial indicated that Zn supplementation in pregnant women's diet has positive impacts on immune status and morbidity of newborns (SCN 2004; Osendarp et al. 2003).

Maternal vitamin A deficiency has profound effect on maternal mortality risk, infant morbidity, and depressed immunological responses (SCN 2004). The initial findings showing correlation between vitamin supplementation and reduced mortality (SCN 2004; Christian et al. 2000; West et al. 1999) are inspiring and need to be studied in detail.

Populations in developing countries including Southeast Asia and Africa consume mostly cereal-based diets. The 'Green revolution,' which averted the occurrence of famine in many areas, was a major factor leading the current cereal based diet (Bouis and Welch 2010). Cereal diet in most cases provides adequate calories but insufficient quantities of micronutrients to the human body. As a consequence of adopting this cereal based cropping systems, many countries observed a drastic increase in malnutrition cases (Welch and Graham 1999) (Table 6.1).

Table 6.1 Status of micronutrient deficiency in the world

Deficiency	Prevalence in the world	Consequences
Iron	111,000 maternal deaths	Reduced cognitive ability. Anaemia, maternal mortality (SCN 2004)
Vitamin A	140 million preschoolers and 7 million pregnant women suffering from deficiency	Night blindness, Xerophthalmia, Keratomalacia & immune system failure (SCN 2004)
Zinc	2 billion people suffering from deficiency	Infectious diseases, poor child growth, maternal mortality, reduced birth weight (WHO 2005)

6.2 Biofortification – Approach to Reduce Nutrient Deficiency

Traditional plant breeding and crop production technologies have immensely contributed over the past to increase the production of food crops, thereby ensuring the food security around the world. One such example is the Green Revolution. Green Revolution dramatically increased the cereal crop production as in wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), and rice (*Oryza sativa* L.), and this contributed to fulfill the protein and energy requirements of the developing nations (WHO 2013). With time the production of cereal crops increased, whereas the production and the consumption of traditional micronutrient-dense crops like pulses decreased leading to major micronutrient deficiencies such as Fe, vitamin A, and iodine deficiencies among the developing nations (WHO 2013). This example shows that the food security solely may not be sufficient to combat leading nutritional problems. As Chandra (1990) describes, "Taken together, micronutrient deficiencies affect a far greater number of people in the world than protein-energy malnutrition".

Biofortification is the process of enrichment for micronutrients and vitamins in edible parts of staple food crops using traditional plant breeding and modern biotechnology techniques (Welch and Graham 2004). Research shows that consuming biofortified staple food crops significantly increases the target micronutrient concentration in populations (Welch and Graham 1999). Biofortification is identified as a sustainable, less costly, and feasible strategy which aims to reduce micronutrient deficiencies in low-income populations (Welch and Graham 2004; Nestel et al. 2006; Meenakshi et al. 2007). Although agronomic biofortification, is the enrichment of micronutrients through soil amendment, foliar spray or irrigation. Agronomic biofortification though is a common practice it appears to be less effective in the case of some mineral elements such as iron (Fe) and have little promise in reducing deficiencies of such elements in human populations (Bouis and Welch 2010; Tagliavini et al. 2000; Tagliavini and Rombola 2001). On the other hand, genetic biofortification could be a cost-effective way to provide access to nutritional foods to people who are living in the remote underprivileged parts of the world as it requires one time initial investment, and seed multiplication through plant breeding interventions would make it sustainable.

Therefore, food security along with nutrient security should be a key consideration in designing modern-day food systems. Traditional plant breeding and modern biotechnology techniques should be exploited, not only to produce high-yielding starchy crops, but also, to produce micronutrient-dense traditional legumes. One such example is the iron biofortification of food crops (Fig. 6.1).

6.3 Traits of Interest

Initial biofortification research efforts have focused on few micronutrients, Fe and Zn. Over time, other micronutrients like Se and different vitamins like Vitamin A, Folic acid are gaining the attention. Generally, it was observed that genetic and

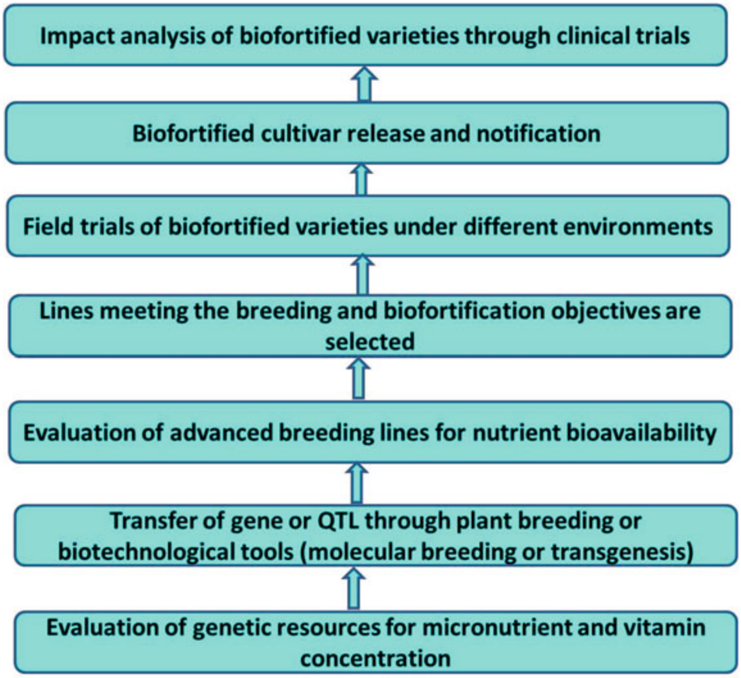

Fig. 6.1 Schematic diagram of multidisciplinary biofortification research. In this flow diagram the entire process of development of biofortified cereal varieties are shown. The process is initiated with selection of lines with high micronutrient or vitamin concentration with optimum food matrix profile. The donor gene or quantitative trait loci is then transferred to the desired line through crossing or marker assisted selection (MAS) or transgenesis. After going through multiple cycle of selections through different filial generations the stabilized advanced breeding line fulfilling the breeding objectives is released and notified for mass cultivation. The impact analysis of released biofortified varieties can be done by clinical trials

environmental interactions are more pronounced in case of secondary metabolites than micronutrients *per se*. These are the traits for which precise phenotyping facility is the prerequisite to improve research conclusions. It has also been observed that these traits mostly have polygenic inheritance and low heritability. This is the reason why molecular breeding tools like marker assisted breeding could be a viable option to breed for these traits. In major cereals like rice, wheat and maize inheritance of micronutrient are today known (Table 6.2). But in case of food legumes more efforts are required to gain genetic knowledge about these traits, particularly number of gene(s)/Quantitative Trait Loci (QTL) controlling these traits and there effects (Thavarajah et al. 2014). The biofortification techniques are separate for traits controlled by single or few genes and by polygenes. Polygenic traits are improved by selecting for region of major importance within plant genome controlling the trait which is known as a major QTL.

Table 6.2 Mapping and tagging of gene(s)/QTL controlling micronutrients in rice, wheat and maize recombinant inbred lines (RILs)/introgression lines (ILs) mapping populations

Crop	Micronutrient	Gene(s)/QTL(s)	Population	Reference
Rice	Fe	7 QTLs for iron concentration	RIL population between two *indica* varieties, Zhengshan 97 × Minghui 63	Lu et al. (2008)
	Fe	1 QTL for Fe concentration	RIL population between, Bala (indica) × Azucena (japonica) cultivars	Norton et al. (2012)
	Fe	2 QTL for Fe concentration	ILs derived from a cross between, Teqing (indica) and the wild rice (*Oryza rufipogon*)	Garcia-Oliveira et al. (2009)
	Zn	6 QTLs for zinc concentration	RIL population between two *indica* varieties, Zhengshan 97 × Minghui 63	Lu et al. (2008)
	Zn	6 QTLs for zinc concentration	RIL population between, Bala (indica) × Azucena (japonica) cultivars	Norton et al. (2012)
Wheat	Fe	2 QTLs for iron concentration	RIL population derived from a cross between wheat cultivars, Xiaoyan 54 and Jing 411.	Xu et al. (2012)
		2 QTLs for iron concentration	RIL population from a cross between *T. boeoticum* accession pau5088 × *T. monococcum* accession pau14087	Tiwari et al. (2009)
	Zn	2 QTLs for zinc concentration	RIL population derived from a cross between wheat cultivars, Xiaoyan 54 and Jing 411.	Xu et al. (2012)
		1 QTL for zinc concentration	RIL population from a cross between *T. boeoticum* accession pau5088 × *T. monococcum* accession pau14087.	Tiwari et al. (2009)
Maize	Fe	3 QTLs for iron concentration and 10 QTLs for iron bioavailability	RIL population between B73 × M17.	Lung'aho et al. (2011)
	Fe	3 QTLs for iron concentration.	RIL population derived from a cross B84 × Os6-2en.	Šimić et al. (2012)
	Zn	Single QTL for zinc concentration	do	Šimić et al. (2012)

In short, knowledge of precise phenotyping and genetics of the traits are prerequisite before starting of a genetic biofortification program.

6.4 Available Genetic Variability for Nutritional Traits

Genetic variation exists for micronutrient concentration (Welch and Graham 2004; Bouis 2003; Graham et al. 2001) and plant breeding tools can improve for β-carotene, iron, zinc, and other micronutrients in food crops by making selection for appropriate genetic material (Nestel et al. 2006). Mostly micronutrient density and yield is positively correlated unlike protein content and yield. Also, it is possible to combine multiple nutrition traits in single cultivar along with high yield (Nestel et al. 2006).

Knowledge of genetic diversity for a particular trait of interest can be a predictive tool for estimating genetic variation in segregating generation or hybrid progeny. For development of molecular markers linked with the high concentration of micronutrient loci initial large scale evaluation of available germplasm sets of different food legumes is essential. After identification of potential genotypes, suitable mapping population can be developed for particular traits (Talukder et al. 2010; Beebe et al. 2000). For the development and advancement of mapping for micronutrient traits state of the art phenotyping facility for micronutrient analysis is required. In case of few studies so far conducted to map and tag the gene(s)/QTL controlling micronutrient status in legumes and model plants mostly found to be having quantitative mode of inheritance and resulting in identification of gene(s)/QTL capable of explaining moderate amount of phenotypic variation for micronutrient concentration. Inheritance of Fe concentration had been identified in major cereals and food legumes, also associated QTL are mapped in the respective genomes; rice (Anuradha et al. 2012), wheat (Xu et al. 2012; Tiwari et al. 2009), maize (Qin et al. 2012; Lung'aho et al. 2011) (for Fe and Zn), Sompong et al. 2012 (for phytic acid in mungbean), Blair et al. 2005; Gelin et al. 2007; Cichy et al. 2009; Blair et al. 2010a, b; (for Fe and Zn in common bean), Sankaran et al. 2009 (for several mineral elements in *Medicago truncatula*), Waters and Grusak 2008 (for several seed mineral contents in *Arabidopsis thaliana*), Walker et al. 2006 (for phytic acid in soybean). Major biofortification projects are undergoing in various laboratories for introgression of such QTL from donor sources to the high yielding backgrounds.

6.4.1 Cereals: Iron, Zinc and Vitamin A

It was quoted many times that biofortification research had been initiated in cereals with the first report of Opaque mutant of maize which later became the source to develop Quality Protein Maize (Taylor et al. 2012); in this review we will be concentrating on micronutrients and vitamins. To have a look into the protein biofortification efforts and effects in maize reader may go through these articles, Hugo et al. 2010; Akalu et al. 2010; Gunaratna et al. 2010.

6.4.1.1 Iron (Fe)

The Fe content ranges from 8.8 to 16.7 mg/kg in milled rice (*Oryza sativa*) (samples included both *indica* and *japonica* types) and from 11.8 to 22 mg/kg in brown rice (Gregorio et al. 2000). Among different accessions of wheat it ranges from 21.26 to 30.59 mg/kg in bread wheat (*Triticum aestivum)*, in macaroni or durum wheat (*Triticum durum*) it ranges from 21.91 to 25.60 mg/kg (Tiwari et al. 2009). *Aegilops ventricosa* accessions were reported up to the concentration of 93.52 mg/kg of Fe (Tiwari et al. 2009). Among the other species and relatives of wheat like *T. boeoticum, T. dicoccoides*, *T. arraraticum, Aegilops longissima, Aegilops Kotschyi, Aegilops peregrine, Aegilops cylindrica, Aegilops ventricosa, Aegilops ovate* Fe concentration ranged from 22.29 to 93.27 mg/kg (Tiwari et al. 2009). The target for plant breeders is to increase the iron concentration in wheat to have a significant effect on nutrition is approximately 30 mg/kg for Fe. This estimate is based on the assumption of daily consumption of 300 g per capita and 90 % of nutrient retention during milling (Cakmak et al. 2010). Fe concentration in maize (Zea mays L.) kernel reported to be between 16.8 and 24.4 mg/kg among the tested late maturing cultivars (Oikeh et al. 2003).

Further, target concentration of iron is highly influenced by factors which affect bioavailability of Fe either positively or antagonistically. Anemia or iron deficiency could be tackled by two ways, either increasing the Fe concentration in grain and or by increasing the Fe absorption in the human gut. For increasing higher gut absorption bioavailability of Fe food matrix becomes the important consideration (Hunt 2003; Plaami 1997; Bravo 1998). It was reported that an increase of 67 % Fe bioavailability in hybrid corn (*Zea mays* L.) over the control (44.2 vs. 26.5 μg ferritin per mg of total protein) was associated with a steep 12 % increase in total Fe concentration, which exhibits the influence of other food matrix factors (Hoekenga et al. 2011). The increase of Fe density in food grain alone is insufficient to address the menace of Fe deficiencies around the world (Lucca et al. 2001). Promoters and inhibitors of Fe absorption within the food matrix should be considered with regard to the bioavailability of Fe in food crops (Cook et al. 1972). Phytic acid or inositol hexakismonophosphate, the primary storage form of phosphorus in plants, is the potential inhibitor of Fe absorption in the human gut (Turnbull et al. 1962). Chelation of $Fe^{2+/3+}$ by phytic acid is the reason of reduced absorption of Fe, on the other hand ascorbic acid antagonist this activity of phytic acid depending on the molar concentrations (Siegenberg et al. 1991). Other important inhibitors are fiber, heavy metals, and certain groups of polyphenols (Glahn et al. 2002). Another important group of promoter is prebiotics (Johnson et al. 2013) which modulates microbiome composition and activity in positive way and thereby increasing the gut absorption of micronutrients. It was reported using *in vitro* study simulating human colon conditions that cereal and legume flours carrying pre-biotics had considerable positive impact on the colonic microbiota (Maccaferri et al. 2012).

Phytoferritin gene family which regulates the Fe uptake and transport within the plant are investigated by many workers (Strozycki et al. 2010; Curie and Briat 2003) and it was reported to be a 24 subunit protein capable of carrying upto 4,500 Fe atoms (Harrison and Arosio, 1996). The expression of Ferritin genes is affected by various stresses and factors; excess iron concentration is one of them. It is also important to mention that expression of plant ferritin gene is controlled at the transcriptional level rather than at translational level as observed in case of animals (Lescure et al. 1991). It was also found that ferritin Fe is less affected (Lönnerdal 2009) with other food matrix factors like phytic acid, ascorbic acid, calcium as studied Caco-2 cells. The above mentioned feature makes phytoferritins as potential candidate for inclusion into the biofortification research. The most studied phytoferritin is soyabean ferritin. It was reported that soyabean ferritin iron is better absorbed in human gut (Lönnerdal 2009). To expression of soyabean ferritin gene in cereal like rice had been made, for example, cDNA clone for soybean ferritin gene as was expressed in rice (*Oryza Sativa*) endosperm tissues caused an increase of two to three fold iron concentration in grain (Goto et al. 1999; Lucca et al. 2001; Qu et al. 2005; Vasconcelos et al. 2003). Similarly in wheat (*Triticum aestivum*) two Ferritin genes (*TaFer1* and *TaFer2*) has been isolated and characterized and it was found that biofortification of wheat is possible through the overexpression of one the ferritin genes in wheat endosperm tissue (Borg et al. 2009).

6.4.1.2 Zinc (Zn)

To improve the Zn content of cereal grains, it is important to know the baseline concentration and variability of its deposition in the grains. The Zn concentration ranges from 13.5 to 58.4 mg/kg in milled rice (*Oryza sativa*) (samples included both *indica* and *japonica* types) and from 20.4 to 34.7 mg/kg in brown rice (Gregorio et al. 2000). Over half of the total Zn in rice is present in the endosperm, and polishing will have less severe effect on Zn concentration than other mineral elements (Lu et al. 2013). Zinc ranges from 14.88 to 29.33 mg/kg in bread wheat (*Triticum aestivum*), from 13.68 to 19.60 mg/kg in durum wheat (*Triticum durum*) (Tiwari et al. 2009). *Triticum dicoccoides* accessions were reported up to the concentration of 66.15 mg/kg of Zn (Tiwari et al. 2009). Among other relatives of wheat (*Aegilops longissima, Aegilops Kotschyi, Aegilops peregrine, Aegilops cylindrica, Aegilops ventricosa, Aegilops ovate*) Fe concentration ranged from 22.29 to 58.61 mg/kg (Tiwari et al. 2009). Screening of Zn concentration in wheat cultivars (current and out-of-production) and land races was conducted; wild relatives contained 3–4 times more Zn in some cases (Gregorio et al. 2000; Chhuneja et al. 2006; Tiwari et al. 2009). In maize (Zea mays), it was reported to range from 16.5 to 24.6 mg/kg as forty-nine late maturing cultivars were studied (Oikeh et al. 2003). Bioavailability of zinc is less affected by food matrix factors as compared with iron (HarvestPlus Brief 2006).

While genetic enhancement of seed Zn concentration is a viable approach, it is important to note that the concentration of Zn in grains can be greatly regulated using agronomic practices. Foliar application of ZnSO4 · 7H2O (0.5 %) to commonly grown wheat cultivars nearly doubled the concentration of Zn in the flour. While agronomic biofortification of cereals is successful, genetic biofortification is nevertheless a useful complement in improving Zn concentration in cereal grains.

6.4.1.3 Vitamin A

To combat vitamin A deficiencies, biofortified staple cereals rice and maize have been developed. Vitamin A is synthesized from several provitamin A carotenoids alpha-carotene, beta-carotene, and beta-cryptoxanthin, beta-carotene being the most effectual provitamin, and therefore the most targeted. After its development, 'golden rice' was introduced as the first transgenic rice to provide beta-carotene (Beyer et al. 2002). Using an *Agrobacterium*-mediated transformation, four genes originating from other plant sources were introduced to code for the necessary enzymes in the beta-carotene pathway. Later, it was discovered that one of the genes used coded for the flux-limiting enzyme, and was replaced with the homolog gene *psy* from maize (Paine et al. 2005). This transformation increased the beta-carotene concentration in rice up to 35 μg/g.

Variation in carotenoid concentration in maize exists, allowing for selection through breeding techniques (Harjes et al. 2008). Carotenoids in maize are, from most abundant, lutein, zeaxanthin, beta-carotene, beta-cryptoxanthin, and alpha-carotene. Capturing natural variations in beta-carotene concentrations, provitamin A biofortified lines have been developed (Harjes et al. 2008).

Variability for biofortification related traits such as iron, zinc and vitamins are present among the rice, wheat and maize and selection for these traits among these crops are possible to initiate any genetic biofortification program.

6.5 Biofortified Cultivars Developed

Under the umbrella of HarvestPlus program of Consultative Group on International Agricultural Research (CGIAR) the initial phase of biofortification programs included six food crops, common bean, cassava, maize, rice, sweet potato, and wheat. The initial phase investment resulted in many success stories like orange sweet potato cultivars with high levels of β -carotene rich (over 200 mg/g) (Bouis and Islam, 2012), and beans with improved agronomic traits and grain type and 50–70 % more iron have been bred through conventional breeding (Nestel et al. 2006). Though conventional breeding still encompassing major part of HarvestPlus program research theme transgenic approaches are in some cases necessary and are being used. The most popular and earliest example of success story of transgenic biofortification research is development of Golden Rice or β -carotene rich rice. Golden rice transgenic lines have been under field trial in Philippines (transgenic of RC-28), Bangladesh (transgenic of BRRI Dhan-29) and will certainly helping to fight against iron deficiency. Recently, iron rich high yielding pearlmillet cultivar, ICTP 8203-Fe has been launched as a result of collaborative effort between HarvestPlus program and Nirmal Seeds, a Hyderabad, India based seed company. The ongoing HarvestPlus phase included more food crops and a few more food legumes, especially lentil which is a regular component of everyday diet in major regions in South and South-east Asia. Present biofortification efforts in wheat concentrate on use of higher source of iron and zinc, for example *Triticum spelta*, *T. diccon* × *Aegilops tauschi* derivatives, landraces (Velu et al. 2012). Biofortified wheat lines developed through backcrossing are being evaluated in field trials to observe their performance (Velu et al. 2012).

A few commercial cereal cultivars are developed so far using genetic biofortification technique and presently in almost all major cereals different biofortification programs for different micronutrient and vitamins are ongoing at national and international plant breeding institutes.

6.6 Challenges for the Breeder and Nutritionist

Iron and folate bioavailability of a staple food crop is mainly depends on the food matrix factors. The concentration of the promoter and inhibitor compounds in any food crop is influenced by both genetic and environmental factors. Before breeding for iron or any micronutrient the absorption and within plant transport of micronutrients is important and to be studied in more detail (Fig. 6.2). Modern plant breeding and molecular biology tools now make it possible to reduce antinutrients, such as phytic acid or increase the concentrations of promoter substances, such as beta-carotene, ascorbic acid and phytoferritin in plant foods. Promoters and inhibitors of Fe absorption within the food matrix must be considered with respect to the bioavailability of non-heme Fe in a food crop (Cook et al. 1972). Phytic acid (PA), nearly omnipresent in plants and used as the primary phosphorous (P) storage, inhibits absorption of Fe in the gut (Turnbull et al. 1962). Other inhibitors include fiber, heavy metals, and certain polyphenols and tannins (Glahn et al. 2002).

Enrichment with prebiotics, beta-carotene, ascorbic acid and phytoferritin has been shown to enhance the bioavailability of non-heme Fe in human plant-based diets (Welch 2002). Prebiotics improve Fe bioavailability as a result of biological fermentation of short chain polymers by natural microflora present in the colon (Yeung et al. 2005). Addition of vitamin A or beta-carotene can improve Fe bioavailability from plant-based foods (e.g., rice, wheat, corn) (Garcia-Carsal et al. 2000). For example, analysis of lentil (*Lens culinaris* Medicus) food matrix components, along with cell culture and preliminary human nutrition studies, revealed clear mineral absorption promoter and inhibitor roles in modulating the levels of mineral bioavailability. Lentils contain high levels of Fe absorption promoters, such as prebiotics and beta-carotene, and are low in antinutrients, such as phytic acid and polyphenols (Thavarajah and Thavarajah 2012). It is reported that molar ratios of phytic acid:Fe above ten lead to reduced human Fe bioavailability (Ariza-Nieto et al. 2007).

Increased micronutrient and vitamin content in biofortified cereal cultivars is the ultimate goal in any biofortification program. In recent time's bioavailability of nutrient becoming a more important issue along with increased concentration of nutrients in food. Analysis of food matrix factors in more details will surly convert future bioforfication programs more precise and rewarding.

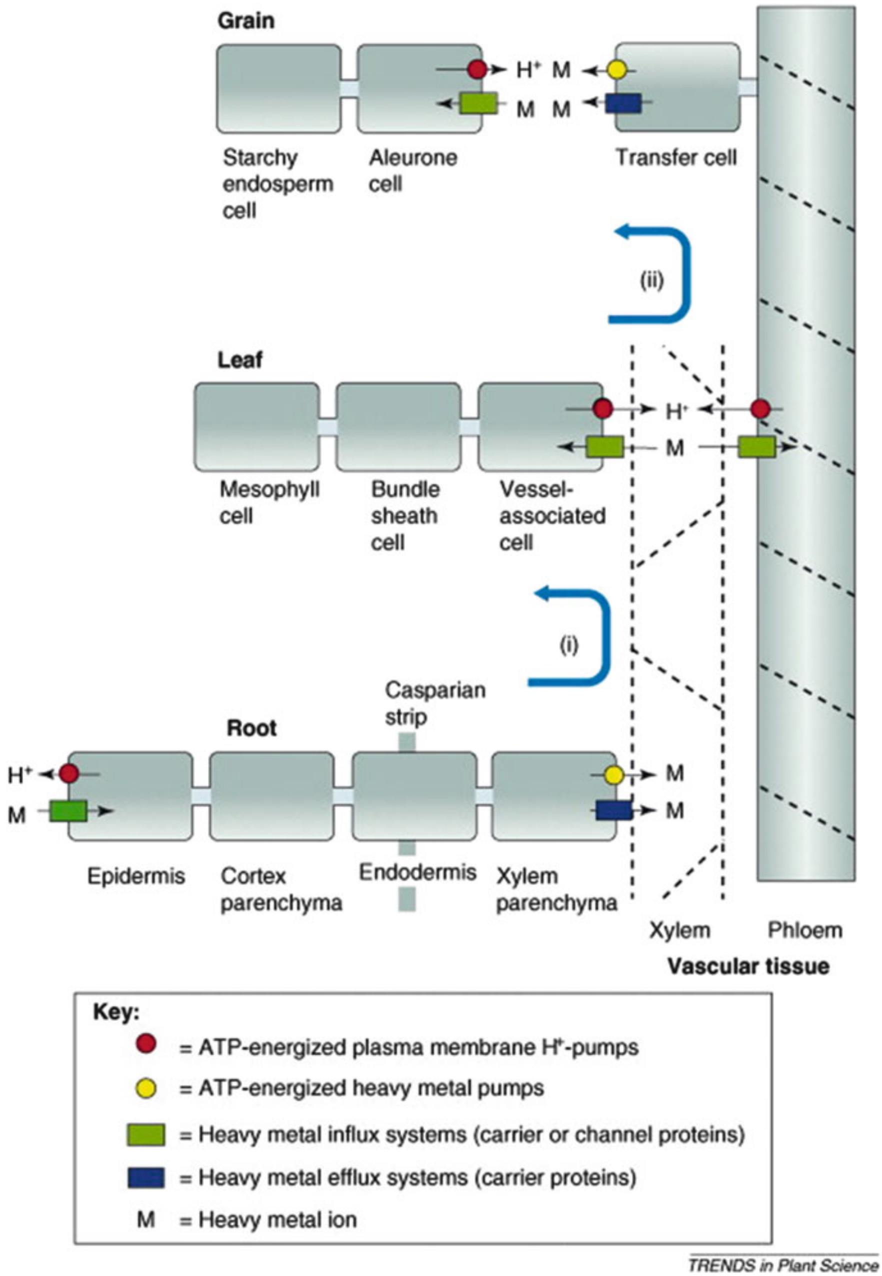

Fig. 6.2 Overview of the transport pathway of a metal ion from soil to grain. To reach the grain, nutrients enter and leave the symplastic continua of cells within the plant several times. For a divalent cation such as Zn, the bottlenecks that limit efficient transport seem to be the control points where Zn has to exit living cells. Plant roots contribute to making metal ions more available for uptake by two strategies. First, depending on the nutrient status of the soil, roots acidify the rhizosphere through

6.7 Conclusion

Since initial years of biofortifcation research have been concentrating on the major micronutrients but coming years would also include various secondary metabolites like vitamins and other important micronutrients, for example, Folic acid and Selenium (Se). Folic acid is the synthetic oxidized form of naturally occurring folates. It consists of a p-aminobenzoic molecule linked to a pteridine ring and one molecule of glutamic acid. Food folates, which exist in various forms, contain additional glutamate residues, making them polyglutamates (Bailey and Gregory 2006). Folate is a water soluble B vitamin, usually participates in numerous biochemical reactions involving one carbon transfer, for example, purine and pyrimidine synthesis as well as amino acid interconversions (Krumdieck 1990), prevention of chromosome breakage and hypomythylation of DNA (Fenech 2001). This role of folates reduces the risk factors leading to cancer and also plays critical role in regulating homocysteine status, an important risk factor for cardiovascular ailments (Pancharuniti et al. 1994). Lower levels of plasma folate are correlated with various health risks, neural tube defects is the prime one with a few other congenital defects (Berry et al. 2000). This is more important to mention that in many cases folate deficiency is also associated with macrocytic anemia (Boushey et al. 1995). There were reports of strong association of anemia due to folate deficiency along with iron deficiency (WHO 2007). The effective evaluation of plant materials for these many nutritional traits model cell culture or animal study would be a prerequisite as far as bioavailability or food matrix factors are concerned. Caco-2 cell culture or *Galus galus* or Pig model (*in vitro* and *in vivo* models) as well as various biotechnological tools would certainly contribute to our better understanding of the role of micronutrients and vitamins in human nutrition in coming days.

Fig. 6.2 (continued) plasma membrane H^+-ATPases, proton pumps in the cell membrane (Palmgren 2001). An increased proton concentration in the soil results in cation exchange and release of divalent metal ions that are tightly bound to soil particles. Second, roots actively secrete low-molecular-weight compounds that can function as metal chelators in the soil. Depending on species, these include organic acids and phytosiderophores (Romheld and Marschner 1986; Takahashi et al. 1999). Secreted phytosiderophores are known to facilitate uptake of Fe by graminaceous plants, and recently, strong evidence has pointed to a similar role of these compounds in Zn uptake (Suzuki et al. 2008; Suzuki et al. 2006). (i) After metal ions have entered root cells by transport-protein-mediated processes, they migrate by diffusion to xylem parenchyma cells, from where they are actively transported out of the symplast into the dead xylem. In the shoot, they are again taken up by vessel-associated cells in the leaves. (ii) During the period of grain filling, metal ions are remobilized in leaves, from where they are exported and transported via the phloem to the fruit. Here they are exported by transfer cells from the mother plant and are subsequently taken up into the developing seed by specialized metal transport proteins (Reproduced with permission from Palmgren et al. (2008)). N.B. H^+-ATPases are the proton (H^+) translocating ATPases; required for uptake of most metabolites in plants, fungi and many protists. ATPases are the ATP hydrolyzing enzymes

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References

Akalu G, Taffesse S, Gunaratna NS, De Groote H (2010) The effectiveness of quality protein maize in improving the nutritional status of young children in the Ethiopian highlands. Food Nutr Bull 31:418–430

Anonymous (2008) The Copenhagen Consensus 2008—Results. The Copenhagen Consensus Cent., Frederiksberg, pp 1–6. Available at http://www.copenhagenconsensus.com/default.aspx?ID=953. Accessed 24 Apr 2015

Anuradha K, Agarwal S, Rao YV, Rao KV, Viraktamath BC, Sarla N (2012) Mapping QTLs and candidate genes for iron and zinc concentrations in unpolishedrice of Madhukar × Swarna RILs. Gene 508:233–240

Ariza-Nieto M, Blair MW, Welch RM, Glahn RP (2007) Screening of iron bioavailability patterns in eight bean (*Phaseolus vulgaris* L.) genotypes using the Caco-2 cell in vitro model. J Agric Food Chem 55:7950–7956

Bailey LB, Gregory JFR (2006) Folate. In: Bowman B, Russell R (eds) Present knowledge in nutrition, vol 1. International Life Sciences Institute, Washington, DC, pp 278–301

Beebe S, Gonzalez AV, Renfigo J (2000) Research on trace minerals in common bean. Food Nutr Bull 21:387–390

Berry RJ, Li Z, Erickson JD, Li S, Moore CA, Wang H, Mulinare J, Zhao P, Wong LYC, Gindler J, Hong S-X, Correa A (2000) Prevention of neural-tube defects with folic acid in China. N Engl J Med 341:1485–1490

Beyer P, Al-Babili S, Ye X, Lucca P, Schaub P, Welsch R, Potrykus I (2002) Golden rice: introducing the β-carotene biosynthesis pathway into rice endosperm by genetic engineering to defeat vitamin A deficiency. J Nutr 132:506S–510S

Blair MW, Astudillo C, Beebe S (2005) Analysis of nutritional quality traits in an andean recombinant inbred line population. Bean Improv Coop 48:52–53

Blair MW, Knewtson SJB, Astudillo C, Li C, Fernandez AC, Grusak MA (2010a) Variation and inheritance of iron reductase activity in the roots of common bean (*Phaseolus vulgaris* L.) and association with seed iron accumulation QTL. BMC Plant Biol 10:215

Blair MW, Medina JI, Astudillo C, Rengifo J, Beebe SE, Machado G, Graham R (2010b) QTL for seed iron and zinc concentration and content in a Mesoamerican common bean *(Phaseolus vulgaris* L.) population. Theor Appl Genet 121:1059–1070

Borg S, Brinch-Pedersen H, Tauris B, Holm PB (2009) Iron transport, deposition and bioavailability in the wheat and barley grain. Plant Soil 325:15–24

Bouis HE (2003) Micronutrient fortification of plants through plant breeding: can it improve nutrition in man at low cost? Proc Nutr Soc 62:403–411

Bouis HE, Welch RM (2010) Biofortification—a sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. Crop Sci 50:S-20

Bouis H, Islam Y (2012) Delivering nutrients widely through biofortification: building on orange sweet potato. Focus 19, Brief 11. http://www.ifpri.org/sites/default/files/publications/focus19_11.pdf

Boushey CJ, Shirley AAB, Omenn GS, Motulsky AG (1995) A quantitative assessment of plasma homocysteine as a risk factor for vascular disease: probable benefits of increasing folic acid intakes. JAMA 274:1049–1057

Bravo L (1998) Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutr Rev 56:317–333

Cakmak I, Pfeiffer WH, McClafferty B (2010) Biofortification of Durum wheat with zinc and iron. Cereal Chem 87(1):10–20

Caulfield LE, Zavaleta N, Shankar A, Merialdi M (1998) Potential contribution of maternal zinc supplementation during pregnancy for maternal and child survival. Am J Clin Nutr 68:S499–S508

Chandra RK (1990) Micronutrients and immune functions. Ann New York Acad Sci 587:9–16

Christian P, West KP Jr, Khatry SK, Katz J, LeClerq SC, Kimbrough-Pradhan E, Dali SM, Shrestha SR (2000) Vitamin A or ß -carotene supplementation reduce symptoms of illness in pregnant and lactating Nepali women. J Nutr 130:2675–2682

Chhuneja P, Dhaliwal HS, Bains NS, Singh K (2006) *Aegilops kotschyi* and *Aegilops tauschii* as sources for higher levels of grain iron and zinc. Plant Breed 125:529–531

Cichy KA, Caldas GV, Snapp SS, Blair MW (2009) QTL Analysis of seed iron, zinc, and phosphorus levels in an andean bean population. Crop Sci 49:1742–1750

Cook JD, Layrisse M, Martinez-Torres C, Walker R, Monsen E, Finch CA (1972) Food iron absorption measured by an extrinsic tag. J Clin Invest 51:805–815

Curie C, Briat JF (2003) Iron transport and signaling in plants. Annu Rev Plant Biol 54:183–206

Feil B, Fossati D (1995) Mineral composition of triticale grains as related to grain yield and grain protein. Crop Sci 35:1426–1431

Fenech M (2001) The role of folic acid and vitamine B12 in genomic stability of human cells. Mutat Res 475:56–67

Garcia-Casal MN, Leets I, Layrisse M (2000) Ý-carotene and inhibitors of iron absorption modify iron uptake by caco-2 cells. J Nutr 130:5–9

Garcia-Oliveira AL, Tan L, Fu Y, Sun C (2009) Genetic identification of quantitative trait loci for contents of mineral nutrients in rice grain. J Integr Plant Biol 51(1):84–92

Gelin JR, Forster S, Grafton KF, McClean P, Rojas-Cifuentes GA (2007) Analysis of seed-zinc and other nutrients in a recombinant inbred population of navy bean (*Phaseolus vulgaris* L.). Crop Sci 47:1361–1366

Gibson RS (2012) Zinc deficiency and human health: etiology, health consequences, and future solutions. Plant and Soil 361(1–2):291–299

Glahn RP, Wortley GM, South PK, Miller DD (2002) Inhibition of iron uptake by phytic acid, tannic acid, and ZnCl2: Studies using an in vitro digestion/Caco-2 cell model. J Agric Food Chem 50:390–395

Goto F, Yoshihara T, Shigemoto N, Toki S, Takaiwa F (1999) Iron fortification of rice seed by the soybean ferritin gene. Nature Biotechnol 17:282–286

Graham RD, Welch RM, Bouis HE (2001) Addressing micronutrient malnutrition through enhancing the nutritional quality of staple foods: principles, perspectives and knowledge gaps. Adv Agron 70:77–142

Gregorio GB, Senadhira D, Htut T, Graham RD (2000) Breeding for trace mineral density in rice. Food Nutr Bull 21:382–386

Gunaratna NS, De Groote H, Nestel P, Pixley KV, McCabe GP (2010) A meta-analysis of community-based studies on quality protein maize. Food Policy 35:201–210

Harjes CE, Torbert RR, Ling B, Thomas PB, Catherine BK, Stephen GS, Ann ES et al (2008) Natural genetic variation in lycopene epsilon cyclase tapped for maize biofortification. Science 319:330–333

Harrison PM, Arosio P (1996) The ferritins: molecular properties, iron storage function and cellular regulation. Biochim Biophys Acta 1275:161–203

HarvestPlus Brief Breeding Crops for Better Nutrition (2006). Washington, DC

Hoekenga OA, Lung'aho MG, Tako E, Kochian LV, Glahn RP (2011) Iron biofortification of maize grain. Plant Genet Res 9:327–329

Hugo De Groote, Gunaratna N, Ergano K, Friesen D (2010) Extension and adoption of biofortified crops: quality protein maize in East Africa. Presented at the Joint 3rd African Association of Agricultural Economists (AAAE) and 48th Agricultural Economists Association of South Africa (AEASA) Conference, Cape Town, 19–23 Sept 2010

Hunt JR (2003) Bioavailability of iron, zinc, and other trace minerals from vegetarian diets. Am J Clin Nutr 78:633–639

Johnson CR, Thavarajah D, Combs GF, Thavarajah P (2013) Lentil (*Lens culinaris* L.): a prebiotic-rich whole food legume. Food Res Int 51:107–113

Jones G, Steketee RW, Black RE, Bhutta ZA, Morris SS (2003) How many child deaths can we prevent this year? Lancet 362:65–71

Krumdieck C (1990) Folic acid. In: Brown ME (ed) Present knowledge in nutrition. ILSI, Washington, DC, pp 179–188

Lescure AM, Proudhon D, Pesey H, Ragland M, Theil EC, Briat JF (1991) Ferritin gene transcription is regulated by iron in soybean cell cultures. Proc Natl Acad Sci U S A 88:8222–8226

Lönnerdal B (2009) Soybean ferritin: implications for iron status of vegetarians. Am J Clin Nutr 89:1680S–1685S

Lu K, Li L, Zheng X, Zhang Z, Mou T, Hu Z (2008) Quantitative trait loci controlling Cu, Ca, Zn, Mn and Fe content in rice grains. J Genet 87:305–310

Lu L, Tian S, Liao H, Zhang J, Yang X, Labavitch JM, Chen W (2013) Analysis of metal element distributions in rice (*Oryza sativa* L.) seeds and relocation during germination based on X-ray fluorescence imaging of zn, fe, K, ca, and mn. PLoS One 8(2)

Lucca P, Hurrell R, Potrykus I (2001) Genetic engineering approaches to improve the bioavailability and the level of iron in rice grains. Theor Appl Genet 102:392–397

Lung'aho MG, Mwaniki AM, Szalma SJ, Hart JJ, Rutzke MA et al (2011) Genetic and physiological analysis of iron biofortification in maize kernels. PLoS One 6(6):e20429. doi:10.1371/journal.pone.0020429

Maccaferri S, Klinder A, Cacciatore S, Chitarrari R, Honda H, Luchinat C, Bertini I, Carnevali P, Gibson GR, Brigidi P, Costabile A (2012) In vitro fermentation of potential prebiotic flours fromnatural sources: impact on the human colon microbiota and metabolome. Mol Nutr Food Res 56:1342–1352

Meenakshi JV, Nancy J, Manyong V, De Groote H, Javelosa J, Yanggen D, Naher F, Garcia J, Gonzales C, Meng E (2007) How cost-effective is biofortification in combating micronutrient malnutrition? An ex ante assessment. HarvestPlus Working Paper #2. Washington, DC: International Food Policy Research Institute

Nestel P, Bouis HE, Meenakshi JV, Pfeiffer W (2006) Biofortification of staple food crops. J Nutr 136:1064–1067

Norton GJ, Duan G-L, Lei M, Zhu Y-G, Meharg AA, Price AH (2012) Identification of quantitative trait loci for rice grain element composition on an arsenic impacted soil: Influence of flowering time on genetic loci. Ann Appl Biol 161:46–56

Oikeh SO, Menkir A, Maziya-Dixon B, Welch R, Glahn RP (2003) Genotypic differences in concentration and bioavailability of kernel-iron in tropical maize varieties grown under field conditions. J Plant Nutr 26:2307–2319

Osendarp SJM, West CE, Black RE (2003) The need for maternal zinc supplementation in developing countries: an unresolved issue. J Nutr 133:817S–827S

Paine JA, Shipton CA, Chaggar S, Howells RM, Kennedy MJ, Vernon G, Wright SY, Hinchliffe E, Adams JL, Silverstone AL, Drake R (2005) Improving the nutritional value of Golden Rice through increased pro-vitamin A content. Nat Biotechnol 23:482–487

Palmgren MG (2001) Plant plasma membrane H+-ATPases: powerhouses for nutrient uptake. Annu Rev Plant Physiol Plant Mol Biol 52:817–845

Palmgren et al (2008) Zinc biofortification of cereals: problems and solutions. Trends Plant Sci 13(9):464–473

Pancharuniti N, Lewis CA, Sauberlich HE, Perkins LL, Go RC, Alvarez JO, Macaluso M, Acton RT, Copeland RB, Cousins AL (1994) Plasma homocyst (e) ine, folate, and vitamin B-12 concentrations and risk for early-onset coronary artery disease. Am J Clin Nutr 59:940–948

Plaami S (1997) Myoinositol phosphates: analysis, content in foods and effects in nutrition. Food Sci Technol 30:633–647

Qin H, Liu YCZ, Wang G, Wang J, Guo Y, Wang H (2012) Identification of QTL for zinc and iron concentration in maize kernel and cob. Euphytica 187:345–358

Qu LQ, Yoshihara T, Ooyama A, Goto F, Takaiwa F (2005) Iron accumulation does not parallel the high expression level of ferritin in transgenic rice seeds. Planta 222:225–233

Romheld V, Marschner H (1986) Evidence for a specific uptake system for iron phytosiderophores in roots of grasses. Plant Physiol 80:175–180

Sankaran R, Huguet T, Grusak M (2009) Identification of QTL affecting seed mineral concentrations and content in the model legume *Medicago truncatula*. Theor Appl Genet 119:241–253

SCN (2004) 5th report on the world nutrition situation: nutrition for improved development outcomes – March 2004. http://www.unscn.org/layout/modules/resources/files/rwns5.pdf. Accessed 24 Apr 2015

Siegenberg D, Baynes RD, Bothwell TH, Macfarlane BJ, Lamparelli RD, Car NG, MacPhail P, Schmidt U, Tal A, Mayet F (1991) Ascorbic acid prevents the dose-dependent inhibitory effects of polyphenols and phytates on nonheme-iron absorption. Am J Clin Nutr 53:537–541

Šimić D, Drinić SM, Zdunić Z, Jambrović A, Ledenčan T, Brkić J, Brkić A, Brkić I (2012) Quantitative trait loci for biofortification traits in maize grain. J Hered 103:47–54

Sompong U, Somta P, Raboy V, Srinives P (2012) Mapping of quantitative trait loci for phytic acid and phosphorus contents in seed and seedling of mungbean (*Vigna radiata* (L.) Wilczek). Breed Sci 62:87–92

Strozycki PM, Szymanski M, Szczurek A, Barciszewski J, Figlerowicz M (2010) A new family of ferritin genes from *Lupinus luteus*—comparative analysis of plant ferritins, their gene structure, and evolution. Mol Biol Evol 27:91–101

Suzuki M et al (2006) Biosynthesis and secretion of mugineic acid family phytosiderophores in zinc-deficient barley. Plant J 48:85–97

Suzuki M et al (2008) Deoxymugineic acid increases Zn translocation in Zn-deficient rice plants. Plant Mol Biol 66:609–617

Tagliavini M, Rambola AD (2001) Iron deficiency and chlorosis in Orchard and Vineyard Ecosystems. Eur J Agron 15:71–92

Tagliavini M, Abadia J, Rombola A, Abadia A, Tsipouridis C, Marangoni B (2000) Agronomic means for the control of iron deficiency chlorosis in deciduous fruit trees. J Plant Nutr 23:11–12

Takahashi M et al (1999) Cloning two genes for nicotianamine aminotransferase, a critical enzyme in iron acquisition (strategy II) in graminaceous plants. Plant Physiol 121:947–956

Talukdar ZI, Anderson F, Miklas PN, Blair MW, Osorno J, Dilawari M, Hossain KG (2010) Genetic diversity and selection of genotypes to enhance Zn and Fe content in common bean. Can J Plant Sci 90:49–60

Taylor J, Taylor JRN, Kini F (2012) Cereal biofortification: strategies, challenges, and benefits. Cereal Foods World 57(4):165–167

Thavarajah D, Thavarajah P (2012) Lentils (*Lens culinaris L.*): a superior whole food solution to micronutrient malnutrition. In: Adan B, Humberto G (eds) Micronutrients sources, properties, and health effects. Nova Science Publishers Inc, New York, pp 179–191

Thavarajah D, Thavarajah P, Gupta DS (2014) Pulses biofortification in genomic era: multidisciplinary opportunities and challenges. In: Gupta S, Nadarajan N, Gupta DS (eds) Legumes in the Omic Era. Springer Science+Business Media, New York, pp. 207–220

Tiwari VK, Rawat N, Chhuneja P, Neelam K, Aggarwal R, Randhawa GS, Dhaliwal HS, Keller B, Singh K (2009) Mapping of quantitative trait loci for grain iron and zinc concentration in diploid a genome wheat. J Hered 100(6):771–776

Turnbull A, Cleton F, Finch CA (1962) Iron absorption IV. The absorption of hemoglobin iron. J Clin Invest 41:1897–1907

UN (2011) Expert Paper, No. 2011/2- prevention and treatment of chronic diseases in developing countries. http://www.un.org/en/development/desa/population/publications/pdf/expert/2011-2-gaziano.pdf. Accessed 24 Apr 2015

Vasconcelos M, Datta K, Oliva N, Khalekuzzaman M, Torrizo L, Krishnan S, Oliveira M, Goto F, Datta SK (2003) Enhanced iron and zinc accumulation in transgenic rice with the ferritin gene. Plant Sci 164:371–378

Velu G, Singh RP, Huerta-Espino J, Peña-Bautista RJ, Arun B, Mahendru-Singh A, Yaqub Mujahid M, Sohu VS, Mavi GS, Crossa J, Alvarado G, Joshi AK, Pfeiffer WH (2012) Performance of biofortified spring wheat genotypes in target environments for grain zinc and iron concentrations. Field Crops Res 137:261–267

Walker DR, Scaboo AM, Pantalone VR, Wilcox JR, Boerma HR (2006) Genetic mapping of loci associated with seed phytic acid content in CX1834-1-2 Soybean. Crop Sci 46:390–397
Waters BM, Grusak MA (2008) Quantitative trait locus mapping for seed mineral concentrations in two *Arabidopsis thaliana* recombinant inbred populations. New Phytol 179:1033–1047
Welch RM (2002) Breeding strategies for biofortified staple plant foods to reduce micronutrient malnutrition globally. J Nutr 132(3):495–499
Welch RM, Graham RD (1999) A new paradigm for world agriculture: meeting human needs: productive, sustainable and nutritious. Field Crop Res 60:1–10
Welch RM, Graham RD (2004) Breeding for micronutrients in staple food crops from a human nutrition perspective. J Exp Bot 55(396):353–364
West KP Jr, Katz J, Khatry SK, LeClerq SC, Pradhan EK, Shrestha SR, Connor PB, Dali SM, Christian P, Pokhrel RP, Sommer A (1999) NNIPS-2 Study Group. Double blind, cluster randomised trial of low-dose supplementation with vitamin A or ß-carotene on mortality related to pregnancy in Nepal. BMJ 318:570–575
WHO (2005) The world health report- make every mother and child count. World Health Organization, Geneva. http://www.who.org/whr. Accessed 24 Apr 2015
WHO (2007) The world health report- A safer future: global public health security in the 21st century. World Health Organization, Geneva. http://www.who.org/whr. Accessed 24 Apr 2015
WHO (2013) The world health report – research for universal health coverage. World Health Organization, Geneva. http://www.who.org/whr. Accessed 24 Apr 2015
Xu Y, Ana D, Liub D, Zhang A, Hongxing X, Li B (2012) Molecular mapping of QTLs for grain zinc, iron and protein concentration of wheat across two environments. Field Crop Res 138:57–62
Yeung CK, Glahn RP, Welch RM, Miller DD (2005) Prebiotics and iron bioavailability-is there a connection? J Food Sci 70:88–92