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ORIGINAL PAPER

Improving human micronutrient nutrition through biofortification in the soil–plant system: China as a case study

Xiao-E. Yang · Wen-Rong Chen · Ying Feng

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Abstract Micronutrient malnutrition is a major health problem in China. According to a national nutritional survey, approximately 24% of all Chinese children suffer from a serious deficiency of iron (Fe) (anemia), while over 50% show a sub-clinical level of zinc (Zn) deficiency. More than 374 million people in China suffer from goiter disease, which is related to iodine (I) deficiency, and approximately 20% of the Chinese population are affected by selenium (Se) deficiency. Micronutrient malnutrition in humans is derived from deficiencies of these elements in soils and foods. In China, approximately 40% of the total land area is deficient in Fe and Zn. Keshan and Kaschin-Beck diseases always appear in regions where the soil content of Se in low. The soil–plant system is instrumental to human nutrition and forms the basis of the ‘‘food chain’’ in which there is micronutrient cycling, resulting in an ecologically sound and sustainable flow of micronutrients. Soil-plant system strategies that have been adopted to improve human micronutrient nutrition mainly include: (1) exploiting micronutrient-dense crop genotypes by studying the physiology and genetics of micronutrient flow from soils to the edible parts of crops; (2) improving micronutrient bioavailability through a better knowledge of the mechanisms of the enhancers' production and accumulation in edible parts and its regulation through soil-plant system; (3) improving our knowledge of the relationship between the content and bioavailability of micronutrients in soils and those in edible crop products for better human nutrition; (4) developing special micronutrient fertilizers and integrated nutrient management technologies for increasing both the density of the micronutrients in the edible parts of plants and their bioavailability to humans.

Keywords Biofortification · Bioavailability · China · Fertilizer management · Micronutrient malnutrition · Plant nutritional strategies

Introduction

The World Health Organization (WHO) has estimated that nearly 3.7 billion people are iron (Fe) deficient, with 2 billion of these so severely deficient in Fe that they can be described as being anemic. In addition, 35% of all children in the world between 0 and 5 years old suffer from Zn- or Fe-deficiencies, 250 million suffer from vitamin A deficiency and 260 million suffer from iodine

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X.-E.Yang (✉) · W.-R. Chen · Y. Feng
MOE Key Lab, Environmental Remediation and Ecosystem Health, Zhejiang University, 310029 Hanghzou, China
e-mail: xyang@zju.edu.cn

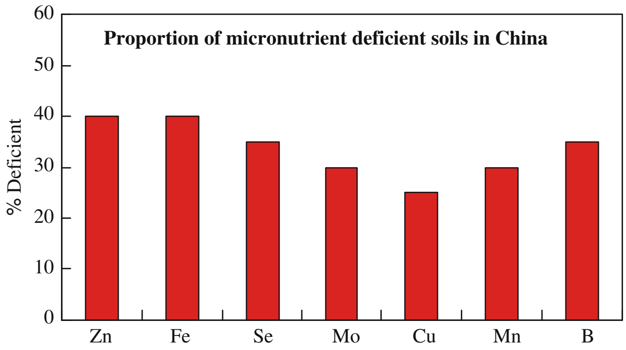
(I) or selenium (Se) deficiencies (Cababallero 2002). Micronutrient deficiencies in humans mainly result from low concentrations and low availabilities of micronutrients in daily diets. Past attempts to solve these dietary deficiencies have included supplementation products and the fortification of food with micronutrients; however, this approach to addressing micronutrient malnutrition has not been ideal due to its high cost and low coverage, even though such programs have been effective in treating severely deficient people. All of the nutrients that humans consume are derived from the soil–plant system, and a new approach to tackling the problem of micronutrient deficiencies in the diet has consisted of increasing the density and bioavailability of micronutrients in edible parts of plants through biofortification. This approach has proved to be sustainable, can be implemented at a relatively low cost, is highly efficacious and has a large coverage, especially in the poorer regions of the world (Bouis 1996; Graham et al. 2001; Welch and Graham 1999, 2002, 2004; Welch 2002; Poletti et al. 2004). In this paper, the micronutrient deficiencies in soils and humans in China, and the plant nutritional strategies that have been adopted for improving human micronutrient nutrition are reviewed.

Micronutrient deficiencies in soils and populations in China

The major soils in China are either calcareous or acidic. Soils deficient in micronutrients, such as boron (B), copper (Cu), Fe, manganese (Mn), molybdenum (Mo), selenium (Se) and zinc (Zn), are widespread in China (Fig. 1). The results of plant-response studies indicate that about 40% of the soils are Zn- and Fe-deficient, and about 30% are Cu-, Mo- and Mn-deficient; B-deficient soils account for 40% of the total land area in China (Liu 1991, 1993, 1994). Iron and Zn deficiencies mainly occur in calcareous soils. Soils with a low Zn content – DTPA-extractable Zn of less than 1.0 mg kg^{-1} – account for more than 50% of the total land in China and are mainly located throughout the provinces or areas of Inner Mongolia, Shanxi, Shaanxi, Henan, Sichun, Hubei, Tibeit, Xingjiang, Anhui and Heilongjiang and near coastal line areas (Fig. 2A). Soils with a low Se content are mainly distributed throughout the provinces of Heilongjiang, Jilin, Liaoning, Hebei, Henan, Yunan, Guizhou, Sichun, Tibeit, Shanxi and Shandong, forming a clear geographical belt from the northeast to the southwest of China (Fig. 2B). Moreover, most of these micronutrient-deficient soils are distributed in highly populated and intensively used agricultural production regions.

Micronutrient deficiencies in soils not only limit crop production, but they also have negative effects on human nutrition and health. The WHO has estimated that over 3 billion people in the world suffer from micronutrient malnutrition and that about 2 billion of these have an Fe deficiency. In Asia, about 35% of all children between 0 and 5 years of age suffer from Fe and Zn deficiencies. In China, micronutrient malnutrition is also a serious health problem. According to a National Nutrition Survey carried out in 2002, approximately 15.2% (average) of the Chinese people suffer from severe Fe deficiency (anemia); broken down into gender and age

Fig. 1 The proportion of micronutrient-deficient soils in China as a percentage of the total area of China. Adapted from Liu (1991, 1993, 1994)



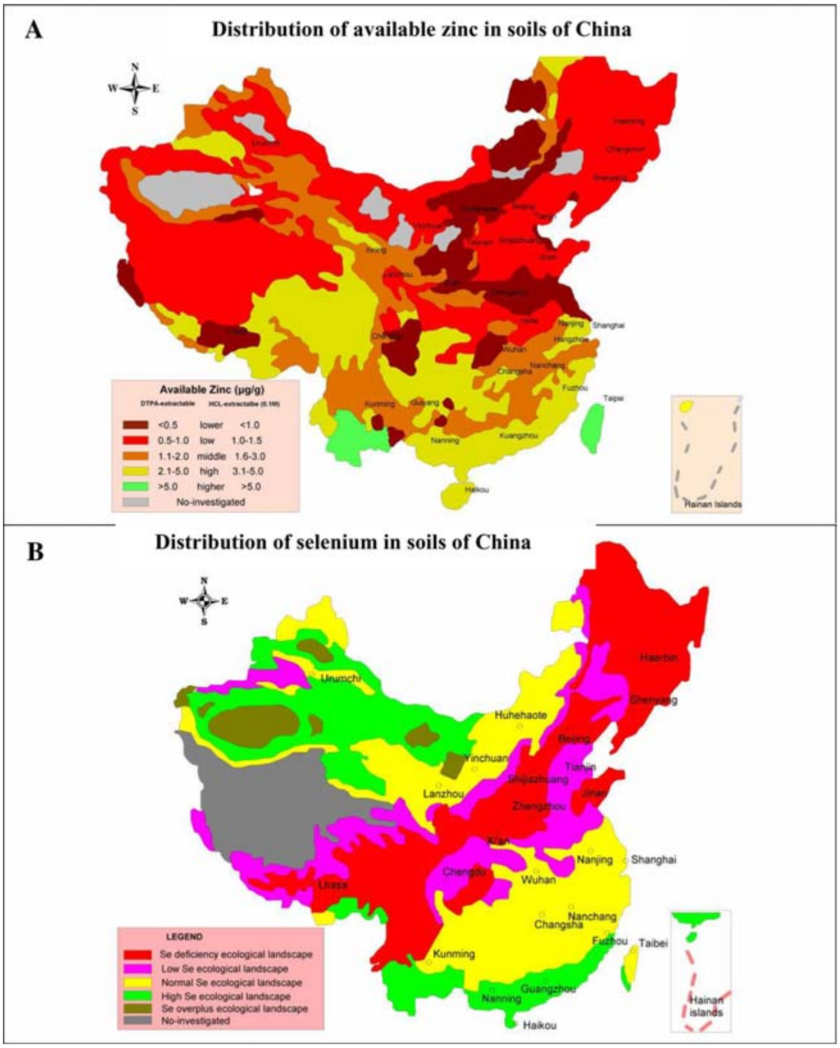


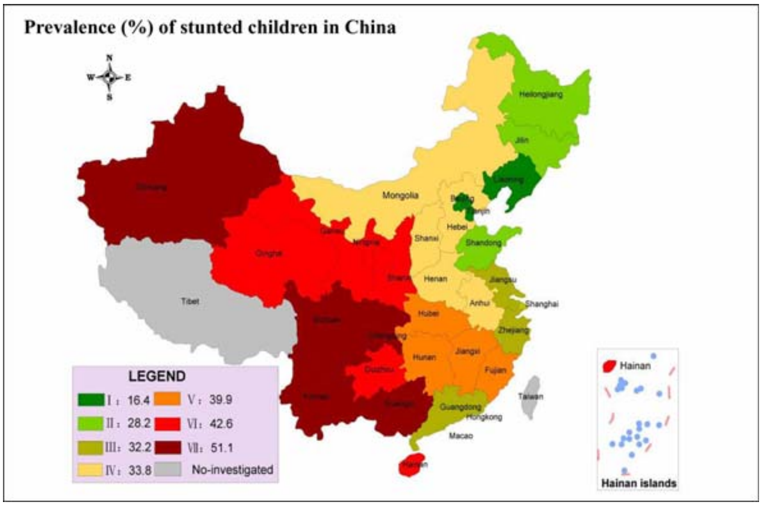
Fig. 2 The distribution of available (DTPA-extractable) Zn in soils in China (**A**) and the distribution of Se-deficient soils in China (**B**). (**A**) Distribution of soil available Zn was adapted from Liu (1994), (**B**) distribution of Se-deficient soils was adapted from Tan (2004)

groups, severe Fe deficiency can be found in 24.2% of the children under 2 years of age, in 21.5% of people elder than 60 years and in 20.6% of all women. In the countryside, 50.5% of all children younger than 6 months have a severe Fe deficiency; this incidence is two-fold higher than that found children of the same age group living in large cities (Chen 2000). Although there is no specific parameter for diagnosing Zn deficiency in the human body, it has been reported that the sub-clinical prevalence of Zn deficiency in children has reached 50–60% or more of the population surveyed in a case study and by Zn status in hairs (Li et al. 1995; Luo et al. 1995; Lao 1999). A survey of Zn-deficient children from 19 provinces and districts in China revealed that 60% of the children suffered from Zn deficiency (Ma and Kou 2003), with those aged 6–10 years having a higher deficiency than those aged 0–5 years (Lao 1999; Ma and Kou 2003). Hu and Gao (2006) reported that the prevalence of Zn deficiency in children of aged 0–1 years was as high as 78% for boys and 74% for girls and that about 30–40% teenagers between 11 and 16 years old suffered from Zn deficiency. A comparison in over 2000 children between 1992 and 1995 showed that the prevalence of Zn deficiency had increased in this period: 52.5 versus 57.8% (Yang and Guo 1995). A comparison of Zn deficiency between female and male children showed higher rates in the latter: 43 versus 65% (Wu et al. 2001). In addition, a survey of micronutrient concentrations in primary school children in Guangzhou showed that the Zn, Fe and Cu deficiencies occurred in 24, 22 and 20% of the children, respectively. Rao et al. (2001) found that Zn deficiency in children was more severe than Fe, Cu and Ca deficiencies. A National Nutritional Survey of China (Chen 2000) found unusually high rates of children with stunted growth (related to low Fe and Zn), especially in children from from the northwest to southeast areas (Fig. 3).

Iodine (I) deficiency is an additional micronutrient malnutrition problem in the world and in China as well. Worldwide, there are about 567 million cases of I deficiency, and approximately 374 million of these are in China, where the incidence of endemic goiter disease is over 35 million (ETCAEDEC 1989). However, in contrast to Zn and Fe deficiencies, the distribution of goiter is very extensive, covering 28 provinces and autonomous regions. The seriously affected areas include Da Xing'an mountains and the Changbai mountainous areas of Northeast China, the Yanshan mountainous areas of North China, west Hubei and the Dabie mountanous area of Central China, the Zhejiang and Fujian provinces of Southeast China, the Nanling and Guangxi provinces of South China and the Yunnan-Guizhou plateau of Southwest China (Fig. 4A). The prevalence of endemic goiter is correlated with a low or too high concentration of I in the drinking water, with more women than men suffering from this deficiency. Endemic cretinism occurs in the seriously affected goiter areas, which include all of the provinces and autonomous regions except for Shanghai and Jiangsu (Fig. 4B). Cretinism is considered to be an endemic disease related to low I and Se levels, and worldwide 6 million babies per year are born who suffer from cretinism. In China, the prevalence of cretinism is over 1% in many of the moreseriously affected areas.

Selenium deficiency is also a serious problem in China. Interestingly, the incidences of Keshan and Keshan-beck disease, both of which are considered to be endemic diseases caused primarily by Se deficiency, are evenly distributed throughout a fairly broad belt across China that ranges from the northeast to the southwest (Fig. 5) and which overlaps regions where the soil is deficient in Se. Keshan disease has been recorded in 309 counties of the 15 provinces and autonomous regions of China, including Heilongjiang, Jilin, Liaoning, Inner Mongolia, Hebei, Shandong, Shanxi, Henan, Shaanxi, Gansu, Sichuan, Yunan, Tibet, Hubei and Guizhou. It has also been reported in Korea. Areas with a high incidence of Keshan disease and low Se are mainly hilly areas, ridges and small basins or plains. There is usually a higher prevalence of Keshan disease among women of

Fig. 3 Distribution of prevalence (%) of stunted children in China (Adapted from Chen 2000)



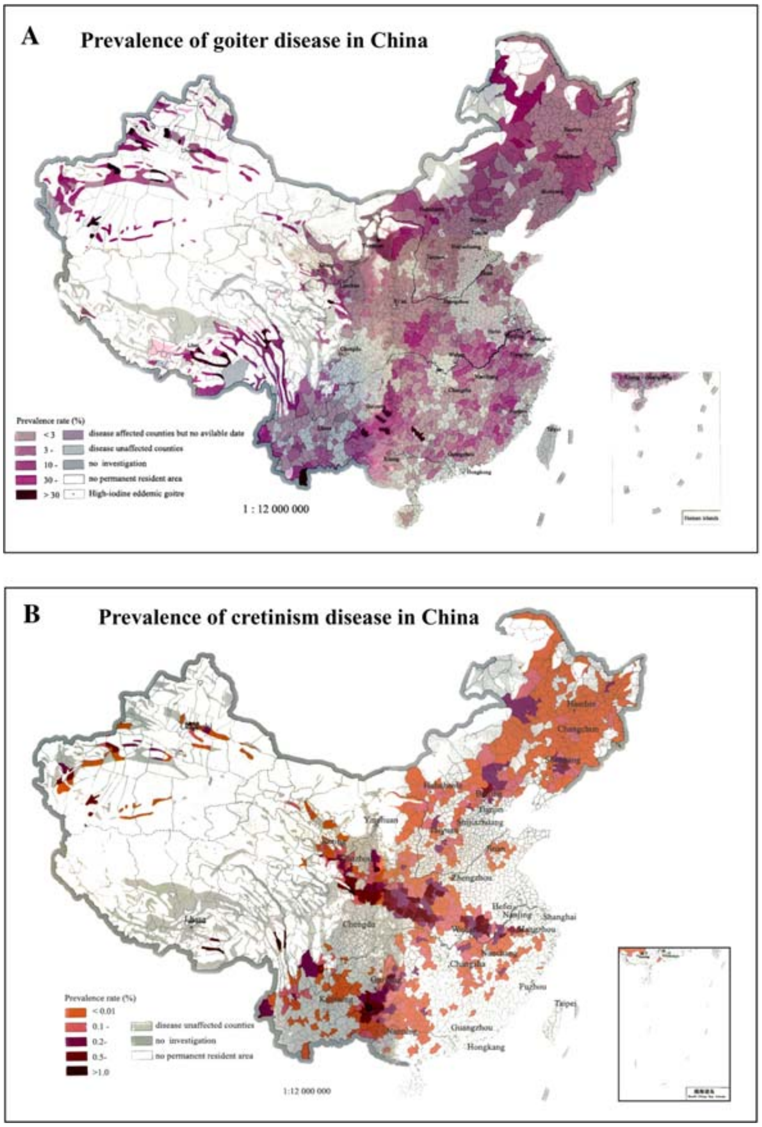


Fig. 4 The distribution of prevalence of goiter (**A**) and cretinism in China (**B**) (adapted from ECAEDEC 1989)

child-bearing age and children under 10 years of age. In recent years, the proportion of affected children has increased. Kecshin-beck disease shows a similar distribution as Keshan disease in China (Fig. 5) and can be found throughout 303 counties of 15 provinces. This disease has also been reported outside of China in both Siberia (Russia) and the northern mountain areas of Korea. The disease mainly occurs in children or teenagers, and the prevalence may be over 50% in children under 10 years of age in seriously affected areas. Farmers consuming locally produced grains as the staple in their diet are the primary potentially candidates for this disease (Tan 2004).

Taken as a whole, these results indicate that a large proportion of the Chinese population suffers from multi-micronutrient deficiencies. The economic loss due micronutrient malnutrition alone is considerable. For example, Chen (2000) reported that the economic loss resulting from Fe and Vitamin A deficiencies in children only accounted for 2.9% of Chinese gross domestic product (GDP).

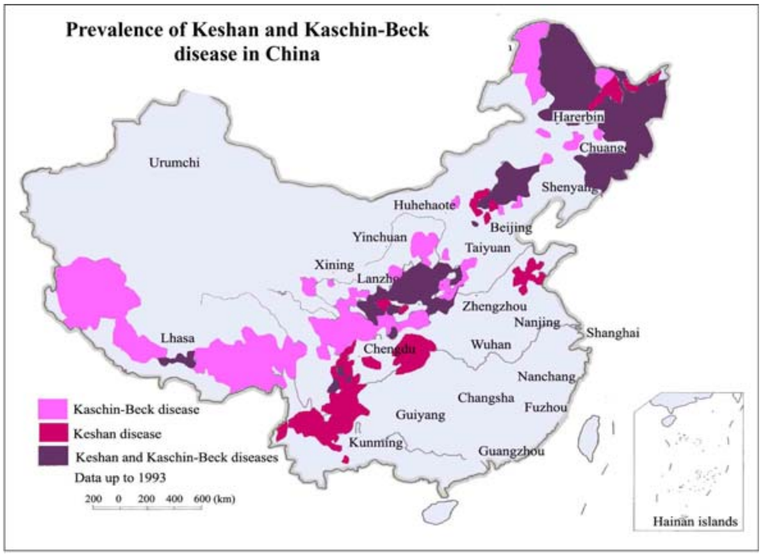


Fig. 5 The distribution of prevalence of Keshan and Kaschin-beck disease in China (adapted from Tan 2004)

Sustainability of improving human micronutrient nutrition through biofortification

The problems associated with micronutrient malnutrition have attracted both the concern and attention of human nutritionists and governments; however, past efforts have mainly involved micronutrient supplementation and/or food fortification/enrichment. A vast variety of Fe, Zn, and Se supplementation products have been developed on Chinese markets, such as ‘‘Red K’’, ‘‘Baby smile’’, ‘‘Enriched-Se and health’’, ‘‘Zn addition’’, among others. Programs involving the enrichment of wheat flour and soy source with Fe have been implemented in some provinces of China, such as Shaanxi, Guizhou, and Yunan. Although the causal pathogenic agent of Keshan disease has not been definitively identified, there is a general consensus that Keshan disease is an endemic disease caused mainly by Se deficiency; as such, the government has made it mandatory to fortify salt with sodium selenite in an attempt to treat and prevent the disease. However, those piecemeal approaches can not solve the whole micronutrient malnutrition problem in China. As approximately 60% of the population are farmers who are also among the most malnourished groups and given the fact that most Chinese are used to eating a relatively high plant-based diet, China needs to implement cheaper and sustainable approaches to combat micronutrient malnutrition in its large population. Micronutrient biofortification in the soil–plant system has been considered to be one of the alternative new approaches that may be able to minimize micronutrient malnutrition on a larger scale than is presently possible with supplementation approaches, especially in terms of reaching larger numbers of the poorer segment of the Chinese population (Bouis 1996; Welch and Graham 2004). Biofortification was firstly defined by Bouis (1996) as increasing the density of micronutrients in crops through breeding. Micronutrient biofortification in the soil–plant system can be defined as increasing the density and bioavailability of micronutrients in the edible parts of crop plants through both plant biotechnology and nutritional management of the soil–plant system with the aim of improving human nutrition and health. From an ecological point of view, such an approach is more sustainable as it involves an adjustment of the flow of micronutrients from soils to humans in order to secure better ecological cycling and environmental effects. The quality of the plant products is also critical for enhancing micronutrient balance in the soil–plant–animal–human food chain. Plant foods such as staple food crops and vegetables comprise the largest part of the diet in China, and the biofortification of both of these with micronutrients is an important step in improving human nutrition and health. The soil–plant system is instrumental to human nutrition based on the "food chain", and improvements in this system resulting in better nutrient cycling will contribute towards a better ecological environment (Fig. 6). Research on Se-enriched crops and Se-enriched food has increased in recent years, and many plant products, such Se-enriched wheat, Se-enriched rice, Se-enriched tea, among others, have been produced and developed (Lu et al. 2000). In addition, private sector enterprises have started shipping and selling Se-enriched plant products from the high soil-Se areas in low soil-Se regions. Zhang et al. (1996) reported that Se-enriched wheat or Se-enriched corn were equally effective in increasing the Se level in the plasma and red blood cells and increasing GSH-px activity in red blood cells and that the prevalence of Keshan disease in children decreased greatly with the consumption of such enriched plant products. However, the production and application of micronutrient-dense crop products is still practiced only on a small scale. The national and many provincial governments have recently started to pay great attention to the breeding of micronutrient-dense crops, and the International HarvestPlus program is promoting research on micronutrient biofortification and its potential impact in China.

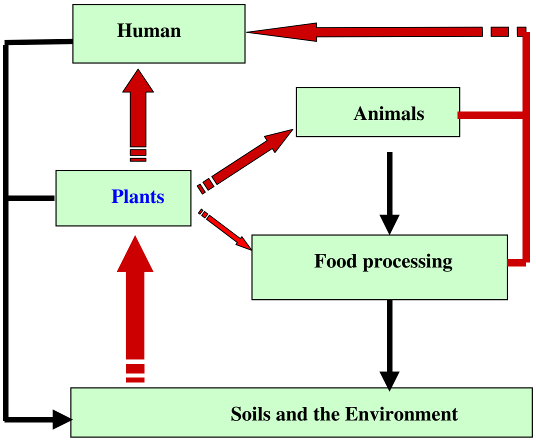


Fig. 6 Flow of micronutrients from soils through human food chains and their ecological cycling

Strategies of micronutrient fortification in soil-plant system

Exploiting micronutrient-dense crop genotypes by understanding the physiology and genetics of micronutrient nutrition in plants

Plant genetic specificity in micronutrient accumulation

Plant species differ greatly in terms of the concentrations of micronutrients in their edible parts or seeds. Leafy vegetables normally contain higher levels of Ca and Fe, and the edible parts of roots/tuber crops and fruit vegetables normally contain lower levels of Zn. The seeds of the cereals have lower Ca and higher phytic acid contents, which in turn may reduce the bioavailability of Fe and Zn, whereas legume seeds contain higher Ca and ascorbic acid levels, both of which are enhancers of nutrient element bioavailability. Large genotypic differences in the micronutrient contents of the edible parts have been observed within same plant species, such as in rice, wheat, corn, bean, among others (Bouis 1996; Graham et al. 1998, 1999, 2001; Senadhira and Graham 1999; Welch and Graham 1999, 2004; Yang et al. 1998). The extent of these genotypic differences with respect to Fe and Zn concentrations in the grains of several cereal crops suggest that it is feasible to increase the Fe/Zn density through breeding (Table 1). In the last few years, germplasm screening of Fe-dense rice, corn, wheat and other crops has been undertaken in China. Considerable genotypic differences in the concentrations of Zn, Fe, Cu and Mn in polished rice grains have been found, with the differences being as high as ten-fold for Zn and seven-fold for Fe (Yang et al. 1998). Several good candidates of Fe-dense cultivars have been selected for breeding programs. It is possible to enrich one crop with several micronutrients, and the characteristics of the micronutrient-dense genetically manipulated rice crops maintain relatively stable in different environments. The micronutrient enrichment has no conflict with high yield; moreover, the micronutrient-dense varieties often have a higher yield than the

Table 1 Genotypic differences in Fe and Zn contents in staple crops and the Food and Agriculture Organization's (FAO) recommended daily intake

Crop species	Genotypes examined (no.)	Ranges of content (mg kg^{-1})		Reference
		Fe	Zn	
Rice	939	13.5–58.4	7.5–24.4	Graham et al. 1999
	238	2.32–30.65	8.66–96.71	Yang et al. 1998
	1138	6.3–24.4	13.5–58.4	Graham et al. 1999
Wheat	132	25.2–53.3	28.8–56.5	Monasterio and Graham 2000
	47	24–74	4.5–37	Batten 1994
	12	12.94–88.13	10.7–57	Wang 1999
Maize	126	13–160	11–95	Chavez et al. 2000
	1814	9.6–63.2	12.9–57.6	Bänziger and Long 2000
Common bean	>1000	21–54	34–89	Graham et al. 1999
Cassava	162	3–48	4–18	Chavez et al. 2000
FAO recommended daily intake (mg)		10 (male) 18 (female)	15	FAO (1980)

non-dense ones in micronutrient-deficient areas because the high micronutrient content of the seeds of the former may increase seeding growth vigor and resistance to harsh environments and diseases. Once the plants have been enriched with micronutrients, the vitality and the nutrient quality of the plant crop can be improved simultaneously (Welch 2002).

Physiological and genetic bases of micronutrient density in edible parts of crops

Great advances has been made in recent years towards gaining an understanding of plant adaptation to micronutrient-deficient stresses – i.e. the efficiency of plants for acquiring micronutrients from soils and utilizing them (Welch 1995; Yang and Römheld 1999; Ghandilyan et al. 2006). The characteristics of micronutrient uptake, translocation and utilization as well as their accumulation in the seeds or storage tissues are under genetic control. The physiological and genetic bases responsible for the uptake of some micronutrients, especially Fe uptake, in crop plants is now much better understood. The response/adaptation of dicot and nongraminaceous plant species to an Fe-deficient soil is mainly to increase Fe reduction, whereas the response of graminaceous plant species is to enhance the synthesis and release of phytosiderophores and the uptake of Fe (III)PS. The genes or clones related to Fe reduction have been identified in tomato, maize, pea and a number of other plant crops, while chromosomes or clones related to the biosynthesis of mugeneic acids (MAs) or Fe-MA transporters have also been identified in barley, wheat, rye (see review by Yang and Römheld 1999). Eide et al. (1996) were the first to clone the Fe^{3+}-transporter gene IRT1 in the dicotyledon *Arabidopsis*. Robinson et al. (1999) separated and cloned Fe^{3+}-reductase (froh family) and Fe^{3+}-chelate reductase (*FRO2*) from *Arabidopsis*. In addition, three other FRO genes (froh gene family) have been identified, and their expression may play a role in the transportation of Fe in the different organs or special cells (Robinson et al. 1997, 1999). Yet another advancement is the discovery of the iron transport protein IRT1 (iron-regulated transporter) in *Arabidopsis thaliana*, which is expressed in roots and induced by Fe deficiency (Eide et al. 1996). This protein is a metal transporter, and it has been suggested that it mediates the uptake and transport of Fe, Mn, Zn, and Co in roots (Korshunova et al. 1999). Ishimaru et al. (2006) recently demonstrated that rice contains Fe^{2+} transporters (OsIRT1 and OsIRT2) in root cells and may play a role in directly absorbing Fe^{2+} under submerged conditions. Other studies have proven that the secretion of the MA-like phytosiderophores is controlled by the 52-kDa and 53-kDa peptides (Okumura et al. 1994). The genes controlling the formation of MAs induced by Fe deficiency have been separately identified in wheat (Mori 1997), and the identity of Ids and their genes make the biosynthesis of the Fe^{2+}-carriers feasible. To increase the density of Fe in grains, more research needs to be focused on Fe translocation from the source tissues to the sink part (grain) of a crop plant (Romheld and Schaaf 2005). We have found that Fe-dense rice genotypes are able to translocate more Fe from the roots to the shoots and grains than non-Fe-dense rice genotypes (Hao et al., unpublished data). We also found higher levels of Fe and Zn in the endosperm tissues of the grains of the Fe-dense rice genotypes than the grains of the non-dense genotypes (Hao et al. 2005). This disparity may be related to the expression of specific genes in the phloem cells and grains, especially in the endosperm cells. Vasconcelos et al. (2003) demonstrated that expression of the ferritin gene resulted in an increased Fe and Zn content in transgenic *indica* rice grains, not only in the whole grain but also in the polished grain. These results imply that it is important to gain a detailed understanding of the forms of micronutrient transportation and accumulation in plant tissues, i.e. the physiological and molecular aspects of micronutrient homeostasis in plants.

The chromosomes associated with Zn uptake efficiency have been reported in wheat, rye and rice, and those associated with Mn and Cu efficiency have been reported in rye (see review by Yang and Römheld 1999). Much progress has been made in our understanding of the molecular mechanisms of Cu, Fe, and Zn transport, including aspects of uptake, distribution, chelation and/or sequestration (Grotz and Guerinot 2006). Ishimaru et al. (2005) revealed that OsZIP4 is a Zn transporter that is expressed in the shoots and roots, especially in phloem cells, and which may be responsible for Zn translocation within the rice plant. Our studies have shown that several ZIP genes in rice are expressed in a tissue-specific manner, such as *OsZIP1* (expressed only in root), *OsZIP5* (panicle), *OsZIP8* (root and panicle), *OsZIP9* (root and shoot), *OsZIP13* (root and panicle), *OsZIP14* (shoot) and *OsZIP15* (root and panicle) (Chen et al. 2007). For seeds and grains, phloem sap loading, translocation and unloading rates within the reproductive organs are important characteristics that must be considered when the aim is to increase micronutrient accumulation in edible parts of the seeds and grains (Welch and Graham 2004; Welch 1986). Therefore, the efficiency of the plant in retranslocating micronutrients to grains or vegetative stores may directly control micronutrient density and be under separate genetic control. Cooperative research projects between plant breeders and plant nutritionists are highly desirable as such an approach would result in the creation of new types of plant genotypes with both a high micronutrient efficiency and a high density of micronutrients in major food crops.

Improving micronutrient bioavailability by understanding its regulation in the soil-plant system

Technology for evaluating micronutrient bioavailability in food crops

The currently popular manner for evaluating micronutrient density in grains is through their concentrations. However, the actual concentration of a micronutrient may not always closely correlate with their respective bioavailability. A variety of approaches have been used to evaluate the bioavailability of micronutrients in plants to the human consumer. Among these are in vitro systems such as cultured human intestinal cells (i.e. Caco-2 cell system), animal systems (e.g. rats, pigs and poultry) and small-scale human clinical trials (Underwood and Smitasiri 1999; Garcia-casal et al. 2000; Welch and Graham 2004; Haas et al. 2005). However, all of these systems have the shortcoming of being either inaccurate or time-consuming, or both. Sharp (2005) reviewed the benefits and limitations of using Caco-2 cells as a predictive tool of human micronutrient bioavailability. Glahn et al. (2002) compared Fe bioavailability among 15 genotypes using the Caco-2 cell mode system and showed large genotypic differences; however, they were unable to determine the underlying mechanisms. High Fe levels in plants are not always positively correlated with a high bioavailability, which suggests that Fe/Zn bioavailability largely depends on the presence of enhancers and inhibitors in rice grains. Oikeh et al. (2003) reported on Fe/Zn bioavailability among maize genotypes using the Caco-2 cell model system and observed significant genotype × environment interactions on Fe/Zn bioavailability in maize grains. The Caco-2 cell system has been used to evaluate the effect of retinol on Fe bioavailability from Iranian bread (Gargari et al. 2006)and to investigate the influence of protein and mineral interactions on the transport and uptake of Ca, Cu, Fe, and Zn in Spanish school children (Camara et al. 2006). However, in China, little information has been obtained to date on the micronutrient bioavailability in edible parts of crops using the Caco-2 cell system.

As the digestion, absorption and metabolism of nutrients in the pig are similar to those in man and it is much cheaper and easier to control animal feeding trials than human clinical trials, piglet model system has recently been used to assess the bioavailability and biological effects of micronutrients in feeds and crops. Miller and Ullrey (1987) used the pig model to evaluate the bioavailability of Fe, Zn, and provitamin-A carotenoids in plant foods. This model is believed to be the most accurate one, but it is relatively costly to carry out. Young pig models have been used to evaluate the effects of meat on enhancing Fe absorption and the effects of phytase on improving Fe bioavailability for hemoglobin synthesis (Stahl et al. 1999). Schaffer et al. (2004) have used early-weaned piglets to study the biological effects of using high-Fe rice as feed. The weanling pig model has also been used to study the effect of Zn supplements and microbial phytase on the utilization of Zn and other minerals (Revy et al. 2004). There are special piglet species available in Yunan province of China that are not only small in weight but also relatively pure with respect to their genetics. Thus, the establishment of an effective piglet model for use in evaluating micronutrient bioavailability and biological effects is feasible in China.

Any assessment of the impact of micronutrient-dense food crop products on bioavailability and human health requires scientific data from human clinical trials. Haas et al. (2005) evaluated the biological effects of high-Fe rice on preventing anemia in a Filipino population and found that Fe-biofortified rice could improve the Fe stores of non-anemic Filipino women. In China, human trials have been adopted to test the effectiveness of Fe-fortified food. Chen (2003) ran ahuman trial on preschool children and found that NaFeEDTA-fortified soy source could prevent Fe deficiency in Quizhou children older than 3 years of age. As a human clinical trial is the most costly research approach, it is not suitable for pre-screening the bioavailability of micronutrient-fortified crop foods. Consequently, a methodology for determining micronutrient bioavailability accurately, quickly and cheaply is urgently needed. In addition to the Caco-2 cell model, the stable isotope tracing technique seems to be a promising option. Stable isotope methods have been widely used for studying micronutrient metabolism in human (Fairweather-Tait and Dainty 2002). Hou et al. (2003) used the stable ^{54}Fe and ^{58}Fe tracing method to compare the use efficiency of $FeSO_4$– and NaFeEDTA-fortified Fe in a Chinese population. However, the methodology is still lacking for tracing stable isotopic Fe or Zn into the soil–plant–human system as the means for evaluating their bioavailability for human nutrition.

Factors controlling micronutrient bioavailability in crops for human nutrition

The bioavailability of Zn and Fe in grains, such as phytate to Zn molar ratios or the phytate and/or Ca to Zn ratio, has also been found to vary greatly among different crop species and genotypes (Cakmak et al. 1999). This variability makes it possible to improve or develop plant genotypes containing high micronutrient concentrations and an improved bioavailability of these micronutrients, thereby providing the means for improving human nutrition and health. The edible parts of a plant contain various amounts of antinutrients (Table 2), with the amounts being dependent on both genetic and environmental factors that can reduce the bioavailability of dietary Fe, Zn and other nutrients. Phytic acid, which is widespread in plants, can fix Zn and Fe in plants, making it hard to digest and absorb and, consequently, decreasing its bioavailability. On the other hand, plants also contain substances, mostly metabolic products, that promote the bioavailability of Zn

Table 2 Antinutrients in plant foods that reduce Fe and Zn bioavailability, and examples of major dietary sources (from Graham et al. 2001)

Antinutrient	Major dietary food resources
Phytic acid or phytin	Whole legume seeds and cereal grains
Fiber (e.g. cellulose, hemicellulose, lignin, cutin, suberin, etc.)	Whole cereal grain products (e.g. wheat, rice, maize, oat, barley, rye)
Certain tannins and other polyphenolics	Tea, coffee, beans, sorghum
Oxalic acid	Spinach leaves, rhubarb
Hemagglutinins (e.g. lectins)	Most legumes and wheat
Goitrogens	*Brassicas* and *Alliums*
Heavy metals (e.g. Cd, Hg, Pb,etc.)	Contaminated leafy vegetables and roots

Table 3 Examples of substances in foods that promote Fe, Zn and vitamin A bioavailability, and major dietary sources (from Graham et al. 2001)

Substance	Nutrient	Major dietary sources
Certain organic acids (e.g. ascorbic acid, fumarate, malate, citrate)	Fe and/or Zn	Fresh fruits and vegetables
Hemoglobin	Fe	Animal meats
Certain amino acids (e.g. methionine, cysteine, histidine, and lysine)	Fe and/or Zn	Animal meats
Long-chain fatty acids (e.g. palmitate)	Zn	Human breast milk
Fats and lipids	Vitamin A	Animal fats, vegetable fats
Selenium	I	Sea foods, tropical nuts
Iron, Zinc	Vitamin A	Animal meats
β-carotene	Fe, Zn	Green and orange vegetables
Inulin and other non-digestible carbohydrates (prebiotics)	Ca, Fe(?), Zn(?)	Chicory, garlic, onion, wheat, Jerusalem artichoke

and Fe (Table 3). Slight improvements in the concentration of these metabolic products would greatly increase the bioavailability of the micronutrients. Gargari et al. (2006) in a study on an Iranian population found that retinol (vitamin A) enhanced Fe absorption in bread by 2.6-fold, while Camara et al. (2006) in a study on Spanish children found that protein was able to significantly increase Fe absorption but not Zn absorption. A recent investigation by Miller et al. using the pig model (2006, personal communication) demonstrated that inulin was able to enhance Fe bioavailability and absorption.

Regulation of micronutrient bioavailability in the soil-plant system

Reducing the level of antinutrients in the edible parts of crops, such as reducing the level of phytic acid by modifying the phytase activity and/or decreasing the concentration of phytin in the edible plant parts, can increase micronutrient bioavailability and improve human Fe nutrition (Raboy 2002). However, many antinutrients are major plant metabolites that may play important roles in plant metabolism, plant stress resistance and plant resistance to crop pests or pathogens. For example, phytin is a source of P, energy and minerals for the seedlings, and any decrease in the level of phytin may reduce the seedling's vitality, especially in nutrient-poor soil. The removal of phytate from plant seeds is likely to reduce seed germination and early growth. In addition, some antinutrients, such as phytate and polyphenols, may play important beneficial roles in human diets by acting as anticarcinogens or by promoting good health in other ways, such as by decreasing the risk of heart disease or diabetes (Shamsuddin 1999). Thus, plant breeders should be aware of the possible negative consequences of changing the levels ofantinutrients in staple crops (Welch and Graham 2004).

On the other hand, increasing the contents of enhancers, such as pro-vitamin A and ascorbic acid (AA), in the edible parts of crops is a promising approach to obtaining increased micronutrient bioavailability. Lucca et al. (2001) reported that following the transfer of the metallothionein gene into rice grains, the rice grains contained about sevenfold higher cysteine residues and 130-fold greater phytase activity, thus markedly increasing Fe bioavailability. The AA content varies greatly among different cultivars of some plants (Chavez et al. 1999), and this variability also provides possibilities for breeding root and tuber crops and vegetables with a high AA content. The application of nitrogen (N) fertilizers can improve AA content because N is the key element in its biosynthetic pathway. However, an excessive application of fertilizer has negative effects (Mozafar 1993, 1994; Babik et al. 1996). Large differences in protein content exist in rice, wheat and maize; thus, it is possible to select or breed the ideal genotype with both high micronutrient and protein contents. A previous study by our groups revealed that a Zn/Fe-dense native Chinese rice genotype, Biyuzhaonou, also contains a relatively higher protein content (Li et al. 2003). It would therefore be feasible to select for both high Fe/Zn and inulin vegetable genotypes. However, little information is available at the present time on the regulation of genotype × environment interactions on inulin accumulation in edible parts of crops.

Assessment and prediction of soil micronutrient contents and bioavailability for human nutrition

In the past, the assessment of soil micronutrient thresholds was made on the basis crop growth responses. Soil and plant nutritionists have set up a series of critical thresholds for diagnosing and predicting soil micronutrient status according to plant growth and correction factors for micronutrient deficiency in crop plants. However, those soil micronutrient maps and thresholds are only available for some of the plant essential micronutrients, such as B, Cu, Fe, Mo and Zn (Liu 1994). Gaps exist between the micronutrient concentrations required by plants and those required by humans. It is a very complex operation to establish the critical levels of micronutrients in the soil or plant that are ultimately required for promoting human health. In China, both the government and consumers are currently paying a great deal of attention to food quality (including taste and nutritional quality). It is quite evident that large differences exist between locations in terms of producing special good-quality crop products. The high quality of those special crop products is related to their micronutrient composition and density, which in turn is closely associated with soil micronutrient content and availability. Therefore, a system for establishing the relationship between soil micronutrient content and bioavailability and the quality of special crop products for better human nutrition and health is urgently needed. The geospatial distribution of micronutrients in agriculturally-suited soils of northern North Dakota has been precisely investigated by Norvell and Wu (2004). However, little has been done in China to date on establishing thresholds and methods for assessing soil micronutrient status and bioavailability linked to human nutrition. Soil micronutrient maps should foster the discovery of relationships between soil micronutrient content and availability and human health problems (White and Zasoski 1999). Effective methods for assessing soil micronutrient bioavailability in soils are still lacking. Further research should be directed to gaining an understanding of the movement of micronutrients through the soil–plant–human food chain. Applications of advanced technologies, such as like global positioning system (GPS), GIS (geographic information systems), inductively coupled plasma (ICP) and precision agriculture, facilitate soil micronutrient mapping and provide the means for a quantitative assessment and prediction of soil micronutrient status and bioavailability for human nutrition and health.

Developing special micronutrient fertilizers and integrated nutrient management technologies

In China, numerous research projects have been conducted during recent years on the effects of micronutrient fertilizer applications on crop yields, and micronutrient application technologies for increasing crop yields have been established.

Research has recently been focused on gaining an understanding of the effects of fertilizer applications on crop and environmental quality. The uptake of such micronutrients as I, Se and Zn by the plant and its subsequent transport to the edible parts can be increased by suitable fertilizer applications to soils, and the levels of all human essential micronutrients in the plant can be enhanced by proper foliar application. The application of a Zn fertilizer has been found not only to increase yield but also to enhance crop quality in wheat (Haslett et al. 2001; Hu et al. 2003), rice (Zhu et al. 1997; Li et al. 2003a), pea and cowpea (Fawzi et al. 1993). Iron bioavailability in calcareous soil is normally very low and the fertilizer effect is considered to be small, but it was shown that the application of organic–Fe fertilizer increased yield and quality in peanut (Xiao et al. 2000), wheat (Hu et al. 2003) and green pea (Zhang et al. 2006). Fawzi et al. (1993) found that the application of $FeSO_4$ to pea and cowpea increased the Fe content of the seed by 5–8%. In addition, micronutrient accumulation in edible parts of crop plants is influenced by NPK fertilizer management. Gregorio et al. (2000) reported that the N level is a major factor influencing Fe accumulation in rice grains. High P has been found to inhibit Zn uptake by wheat (Rengel et al. 1999), and the uptake and accumulation of Fe/Zn was found to be regulated by application ratios of NPK (Moreno et al. 2003; Li et al. 2003b) and organic substances (Pinto et al. 2004). Foliar application of Se fertilizer has been shown to be effective in increasing the level of Se in grains. A great deal of the research being carried out in China at the present time is focusing on developing special Se fertilizers and their application technology in various crops, such as in wheat (Song et al. 2005) and tea (Li et al. 2005).

The application of fertilizer has been shown to be able to alter plant secondary metabolisms and may influence the contents of the enhancers or the antinutrients in plants. Therefore, it is both important and feasible to develop special micronutrient fertilizers for both soil and foliar application and for integrated nutrient management technologies for enhancing micronutrient density and bioavailability in the edible parts of crop plants. Fertilization with inorganic and organic forms of micronutrients has the potential to increase their concentrations in grains, especially in agricultural soils deficient in micronutrients. However, a number of barriers still exist before this potential can be realized: (1) there is a large temporal and spatial variation in the availability of soil micronutrients; (2) most soil-applied micronutrients are quickly fixed into plant-unavailable forms; (3) most foliar-applied micronutrients are not efficiently transported toward the roots, which may remain deficient; (4) different crops may vary a great deal in terms of the amount of micronutrients absorbed from the soil, and even the same crop may vary in this aspect depending on the geological and climatic conditions. Therefore, an integrated micronutrient application technology for different crops in different regions would appear to be urgently required.

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