# **Biofortification of Food Legumes** and Bioavailability of Nutrients

5

Jagdish Singh

#### Abstract

Growing sufficient food will not in itself assure adequate nutrition and healthy productive lives. The diets of over two-thirds of the world's population lack one or more essential mineral elements; as a result, micronutrient malnutrition, the so-called hidden hunger, affects more than one-half of the world's population, especially women and preschool children. In developing countries, the rise in micronutrient deficiencies is linked to the shift in cultivation with dominance by cereals. High cereal productivity, the result of extensive research, has ensured that cereal production is relatively profitable with a relatively low risk of failure due to biotic and abiotic stresses. Food systems dominated by cereals are low in micronutrients. To address micronutrient deficiencies in the comprehensive way, several new approaches are needed simultaneously. Despite past progress in controlling micronutrient decencies through supplementation and food fortification, new approaches are needed to expand the reach of food-based interventions. Biofortification, a new approach that relies on conventional plant breeding and modern biotechnology to increase the micronutrient density of staple crops, holds great promise for improving the nutritional status and health of poor populations in both rural and urban areas of the developing world. Available genetic variation influences the level of micronutrient increments that can be achieved through breeding, but contribution to nutritional status largely depends on factors related to bioavailability that have to be considered when setting nutritional target levels for breeding. Critical information is needed on how much nutrient is retained after storage, processing, and cooking; on micronutrient bioconversion and bioavailability once the nutrient is ingested; and on micronutrient requirements of the target population. Many of these parameters are interrelated in a

J. Singh (🖂)

Division of Basic Sciences, ICAR-Indian Institute of Pulses Research, Kanpur, Uttar Pradesh 208 024, India e-mail: jagdish1959@gmail.com

highly complex manner, since human micronutrient status, dietary composition, and health status affect bioavailability and its components' bioaccessibility, bioconversion, and bioefficacy. The bioavailability of Fe and Zn is associated with the presence of antinutrients and/or the lack of promoter substances for micronutrients. Since an increase in bioavailability translates into a proportional decrease in the nutritional target increment (increasing Fe bioavailability from 5 % to 10 % reduces the target increment by 50 %), breeding strategies for micronutrient density should consider indirect breeding for increased bioavailability, increased retention, or reduced postharvest micronutrient deterioration. Although not well understood, breeding for increased bioavailability offers tremendous potential.

#### Keywords

Food legumes • Micronutrient malnutrition • Phytochemicals • Pulses

### 5.1 Introduction

Micronutrient malnutrition ("hidden hunger") now afflicts over 2 billion people worldwide, resulting in poor health, low work productivity, high rates of mortality and morbidity, increased rates of chronic diseases (coronary heart disease, cancer, stroke, and diabetes), and permanent impairment of cognitive abilities of infants born to micronutrient-deficient mothers. Evidence is growing that our global food systems are failing to deliver adequate quantities of healthy, nutritionally balanced food especially to underprivileged people globally (Mason and Garcia 1993; McGuire 1993). The consequences are affecting human health, well-being, productivity, and livelihood and contributing to stagnating national development efforts in many developing nations. More than 2 billion people worldwide are iron deficient (ACC/SCN 2000). Iron deficiency anemia is by far the most common micronutrient deficiency in the world. Iron deficiency during childhood and adolescence impairs physical growth, mental development, and learning capacity. In adults, iron deficiency anemia reduces the capacity to do physical labor. Iron deficiency increases the risk of women dying during delivery or in the postpartum period. Prevalence among children exceeds 50 % in South and Southeast Asia, where 1.8 billion out of the approximately 4.5 billion people in developing countries live. Prevalence is equally high in Africa, although the number of persons affected is smaller. Prevalence is consistently highest for pregnant women and lowest for adult males. Even in India, about 79 % of the kids between 6 and 35 months of age and 56 % of women between 15 and 49 years of age are anemic. Zinc deficiencies have equally serious consequences for health. For example, metaanalyses of recent randomized controlled trials show that zinc supplementation can reduce morbidity from a number of common childhood infections, especially diarrhea, pneumonia, and possibly malaria, by one-third (Bhutta et al. 2000; Black 1998). In addition, zinc deficiency is an important cause of stunting (Umeta et al. 2000). Over 450 thousand infant deaths in the world during 2004 were ascribed to Zn deficiency. It is estimated that 60-70 % of the population in Asia and sub-Saharan Africa could be at the risk of low Zn intake; in absolute numbers this translates into 2 billion people in Asia and 400 million people in sub-Saharan Africa. Even in India Zn deficiency is a highly relevant health problem and is responsible for a loss of 2.8 million. Childhood stunting may be considered as an indicator of the human Zn nutritional deficiency and about 61 million children under the age of 15 are stunted. Pregnant women are also susceptible to Zn deficiency and a survey in Haryana on 285 pregnant women showed that 65 % of them had Zn deficiency. The primary underlying cause of micronutrient malnutrition is poor quality diets, characterized by high intake of food staples, but low consumption of animal and fish products, fruits, grain legumes, and vegetables, which are rich sources of bioavailable minerals and vitamins. As such, most of the malnourished are those who cannot afford to purchase highquality, micronutrient-rich foods.

Modern agricultural systems are providing calories, but in the process, they have increased "hidden hunger" among the world's poor by displacing acreage allotted to traditional crops such as pulses, making many micronutrient-rich plant foods less available and more expensive to low-income families (Combs et al. 1996). The apparent consequences of "green revolution" cropping systems on micronutrient malnutrition are clearly demonstrated in several world regions. For example, in South Asia, the introduction of modern wheat and rice production practices (which resulted in about 200 % increase in rice production and 400 % increase in wheat production over the past 30 years) is associated with time trends in the growth of iron deficiency anemia among nonpregnant, premenopausal women and negatively related to time trends in the iron density (mg Fe per kcal of available food) of diets. Typically, populations and individuals at risk for iron and Zn deficiency are those that consume high levels of grains such as rice, wheat, and maize as their major source of calories. In regions where the cropping systems have changed and over the past 30 years, the cultivation and consumption of pulse crops have declined because high-yielding grain varieties have displaced them in the farming system. Essentially, the diversity of staple food crops was reduced, and pulse crops, which are relatively high in iron, declined in production and consumption. In addition, processing of grains such as rice and wheat removes much of the iron from these foods, and unfortunately,

many populations prefer the processed products, lowering their consumption even more. Research efforts are now underway to improve the food supply in many countries through a process known as biofortification. Genetic biofortification is a sustainable method of naturally enriching food crops by conventional breeding and modern biotechnology. Biofortification is a focus in a wide range of food crops. In pulses, micronutrients such as iron, zinc, selenium, and folates are more important.

Compounding this problem is the utility or "bioavailability" of essential micronutrients. Many factors influence the degree to which ingested micronutrients are absorbed (bioavailability) and utilized (bioefficacy). Dietary components like phytic acid, polyphenols/ tannins, dietary fiber, and ascorbic acid influence the bioavailability of minerals (Kies et al. 1983). Biofortification will have greater impact if the biofortified nutrients are highly bioavailable.

# 5.2 Biofortification in Pulses: A Sustainable Agricultural Strategy for Reducing Micronutrient Malnutrition

The permanent solution to micronutrient malnutrition in developing countries is a substantial improvement in dietary quality higher consumption of pulses, fruits, vegetables, fish, and animal products, which the poor already desire but cannot presently afford. Meanwhile, breeding staple foods that are dense in minerals and vitamins provides a low-cost, sustainable strategy for reducing levels of micronutrient malnutrition. The prospects of using conventional breeding techniques, biotechnology, and micronutrient-containing fertilizers to improve the micronutrient quality of staple crops and the micronutrient status of the poor have been put forth in recent years; this new strategy is referred to as "biofortification." Biofortification is a relatively recent addition to breeding goals in plants based on improving the nutritional quality of the edible portion of the plant through traditional or transgenic approaches. Biofortification proposes to use agricultural modifications as a public health intervention and, as a result, has the potential to reach more effectively to the rural poor-those who are often at highest risk for micronutrient deficiencies. This strategy is being developed and implemented through the international alliance of HarvestPlus to improve iron, zinc, and vitamin A status in low-income populations. Results from germplasm screening suggest that the iron and zinc content of staple foods can be doubled through conventional breeding. This result, in turn, implies that iron and zinc intakes in poor people's diets can be increased by 50 %. Under HarvestPlus, progress in breeding for biofortified crops has been steady, with promising levels of zinc and provitamin A carotenoids and, to a lesser extent, iron being achieved in several staple food crops, including maize, rice, wheat, pearl millet, potatoes, and bananas or plantains. However, limited pulse biofortification research were also conducted during the last decade which indicates that breeding for micronutrient-rich pulses with high bioavailability may be possible. The use of molecular marker-assisted selection in pulse crop biofortification and research efforts have been initiated in the USA, Canada, and CGIAR around the world. For development of molecular markers linked with high concentration of micronutrient loci, initial large-scale evaluation of available germplasm sets of different food legumes is essential as this in turn is a prerequisite to develop suitable mapping population. Few studies conducted so far to map and tag the gene(s)/QTL(s) controlling the micronutrients status in legumes were mostly found to be having quantitative mode of inheritance and resulting in identification of gene(s)/ QTL(s) capable of explaining moderate amount of phenotypic variation for micronutrient concentration (Sompong et al. 2012) for phytic acid in mung bean and (Blair et al. 2010) for Fe and Zn in common bean.

# 5.3 Exploiting Genetic Variation in Nutrient Composition and Genetics of Nutritional Traits

Germplasm resources can be exploited to improve the nutrient output of food systems in several ways. The simplest way is by designing new cropping systems with better nutrient output, for example, by introducing micronutrient dense pulse species into cereal-dominated production systems. There is an urgent need to reduce the risk to farmers associated with pulse production and to enhance the yield and reliability of most pulses while maintaining or further enhancing their nutrient density. Within any food system, selecting micronutrient dense lines among existing varieties is the first and easiest approach. Not much work has been done in pulse crops as compared to wheat, rice, and maize; however, in beans considerable genetic variation in mineral concentration among wild beans as well as modern cultivars has been demonstrated. The concentration of zinc in beans is one of the highest among vegetable sources. Unlike, wheat and rice, the concentrations of iron are generally even higher than those of zinc, but like the cereals, iron and zinc are positively correlated. However, unlike cereals there is no correlation with calcium. The concentration of iron in most of the beans and in other pulse crops around 70–100 mg  $kg^{-1}$  is much higher than in the cereals, although relatively, the increase that is possible through selection is less. Again, like wheat and rice, the high density traits are fairly stable across environments, although, as in all crops, the environmental effect is also highly significant (Graham et al. 2001). The first genetic study of a micronutrient efficiency factor was conducted by Weiss (1943) on iron efficiency in soybeans, in which it was shown that efficiency was due to a single, major, dominant gene. Since then, several minor additive genes have been discovered to contribute to iron efficiency in this crop (Fehr 1982).

## 5.4 Bioavailability

The total amount of micronutrient in a plant food does not represent the actual micronutrient content of the food that is utilizable by the consumer. In human nutrition terms, the bioavailability is commonly defined as the amount of a nutrient that is absorbable and utilizable by the person concerned. There are multiple interactions occurring between micronutrients in plant foods and other plant substances once the food is consumed. There are several interacting nutrients and chemical substances that can either inhibit (i.e., antinutrients) or enhance (i.e., promotersthat can increase absorption and/or utilization) micronutrient bioavailability (Table 5.1). Thus, determining micronutrient bioavailability is a difficult and a complex issue. Various methods have been developed to determine micronutrient bioavailability in plant foods to humans that encompasses in vitro or in vivo models or combination of both. Unfortunately, none of these models are ideal for all foods, nutrients, and circumstances. Presently Caco-2 cell culture developed from an adenocarcinoma isolated from a human large intestine is being used to screen large number of lines for bioavailable Fe and Zn as it is a human cell model and is relatively inexpensive, rapid, and free of the need of radioisotopes or stable isotope-labeled plants. Caco-2 cell monolayers are morphologically and physiologically very similar to the layer of the mucosal epithelial cells that line the surface of the small intestine and are responsible for most micronutrient absorption from the gut.

Bioavailability issues must be addressed when employing plant breeding and/or transgenic approaches to reduce micronutrient malnutrition. Enhancing substances (e.g., ascorbic acid, S-containing amino acids, etc.) that promote micronutrient bioavailability and decreasing antinutrient substances (e.g., phytate, polyphenolics, etc) that inhibit micronutrient bioavailability (Table 5.2) are both options that could be pursued. Given this complexity, three breeding sub-strategies may be applied individually or in various combinations. These are (1) increasing the mineral and vitamin content, (2) reducing the level of antinutrients in food staples that inhibit the bioavailability of minerals and vitamins, and (3) increasing the levels of nutrients and compounds that promote the bioavailability of minerals and vitamins. For example, phytates and tannins inhibit iron and zinc absorption. With respect to compounds that promote bioavailability, certain amino acids (such as cysteine) enhance iron and/or zinc bioavailability (Hallberg et al. 1989). These amino acids occur in many staple foods, but their concentrations are lower in grain legumes than those found in animal products. A modest increase in the concentrations of promoter amino acids in grain

Production practices	Meal components	Processing	Individual's characteristics
Nutritional efficiency	Antinutrients	Raw	Age
Fertilizer practices	Promoters	Cooking	Sex
Soil amendments	Protein quality	Fermentation	Ethnicity
Cropping system	Quantity of other interacting major and micronutrients	Malting	Economic status
Variety	Supplements	Extraction	Physiological status
Soil fertility and health		Soaking	Nutritional status
Other interacting elements		Drying	Disease status
		Polishing	Education and awareness
		Milling	Genetic propensity

**Table 5.1** Various factors affecting micronutrient bioavailability

Source: Graham et al. (2001)

Antinutrients	Promoters	
Phytic acid	Some organic acids (ascorbate, fumarate, malate, citrate)	
Fibers (cellulose, hemicellulose, lignin, cutin, suberin, etc.)	Phytoferritin (plant ferritin)more bioavailable Fe	
Tannins and other polyphenols	Some free amino acids (methionine, cysteine, histidine, lysine)	
Oxalic acid	Long-chain fatty acids (palmitic acid) promotes Zn bioavailability	
Hemagglutinins (lectins)	Inulin (oligofructose, $\beta$ -2, 1-fructooligosaccharides) promotes calcium bioavailability	
Goitrogens	Riboflavin (FAD) promotes bioavailability of iron and Zn	
Toxic heavy metals (Cd, Hg, Pb, Ag)	Selenium promotes bioavailability of iodine	

**Table 5.2** Examples of dietary substances that inhibit (antinutrients) or enhance (promoters) the bioavailability of nutrients to humans

Source: Graham et al. (2001)

legumes may have a positive effect on iron and zinc bioavailability in humans. Iron and zinc occur only in micromolar amounts in plant foods, so only micromolar increases in the amounts of these amino acids may be required to compensate for the negative effects of antinutrients on iron and zinc bioavailability. These amino acids are essential nutrients for plants as well as for humans, so relatively even small increase in their concentrations in plant tissues should not have adverse consequences on plant growth (Graham et al. 2001). While most vitamins are very well absorbed, most essential minerals are not. Normally absorption of minerals ranges from less than 1 % to over 90 %.

### 5.4.1 Bioavailability of Iron from Grain Legumes

Iron bioavailability typically ranges from 5 % to 15 % of total iron intake (FAO/WHO 2002). Direct measurement of iron absorption from meals representing different types of largely plant-based diets suggests that the bioavailability of iron can be even less than 5 % (FAO/WHO 2002). This presents a particular challenge for the biofortification strategy, because a very large amount of additional iron would be needed to compensate for its very low bioavailability. Phytate is also a potent inhibitor of iron bioavailability. Whereas, for zinc the dose-response inhibitory effect of phytate is more gradual, for iron it is more acute, so that even relatively small amounts of phytate can have a substantial inhibitory effect on iron bioavailability (Hallberg et al. 1989). Polyphenolic compounds are also strong inhibitors of iron bioavailability. Studies using in vitro models of iron bioavailability have shown that even after reduction of the phytate content of staple foods such as sorghum and millet, iron bioavailability remains low in the presence of polyphenolic compounds (Rao and Deosthale 1988; Lestienne et al. 2005).

There are several plant food components that interact with iron and zinc during digestion to alter their bioavailability. For iron, staple food components that enhance the bioavailability of nonheme iron include ascorbic acid, and those that inhibit iron bioavailability include phytate and some polyphenolic compounds. The bioavailability of Fe is not only a question of the Fe concentration in a given food, but also whether the Fe is plant or animal derived and whether other biochemical factors are present within the food matrix (Hunt 2003; Bravo 1998). For example, an increase of 67 % Fe bioavailability in hybrid corn (Zea mays L.) over the control was associated with a mere 12 % increase in total Fe concentration, thus demonstrating the impact of other food matrix factors (Hoekenga et al. 2011). 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). The iron and zinc from vegetarian diets are generally less bioavailable than from non vegetarian diets because of more phytic acid and other plant-based inhibitors. Phytic acid (PA) myoinositol 1,2,3,4,5,6-hexakisphosphate, nearly ubiquitous in plants and used as the primary phosphorus (P) storage, inhibits absorption of Fe in the gut (Turnbull et al. 1962). Inhibition is achieved by chelation of Fe  $^{2+/3+}$  by PA, but this action can be prevented by ascorbic acid (AA), depending on dosage (Siegenberg et al. 1991). Other notable inhibitors include fiber, heavy metals, and certain polyphenols and tannins (Welch and Graham 2004). Iron absorption from vegetarian diets can likely be somewhat improved by the consumption of ironcontaining foods concurrently with sources of ascorbic acid-containing foods while limiting low-phytate foods or the use of preparation methods that reduce phytic acid (Gibson et al. 1997). Although a vegetarian diet is likely to contain iron in amounts equivalent to amounts in a non vegetarian diet, the iron from a vegetarian diet is likely to be substantially less available for absorption because of differences in the chemical form of iron and the accompanying constituents that enhance or inhibit iron absorption. The chemical form of iron is an important factor affecting the iron availability. Heme iron is better absorbed ( $\approx$ 15–40 %) than non-heme iron ( $\approx 1-15$  %) (11–15). The Dietary Reference Intakes recently proposed for iron (Food & Nutrition Board 2001) suggest that vegetarians need to increase dietary iron by 80 % to compensate for an estimated lower iron bioavailability of 10 % from a vegetarian diet.

#### 5.4.2 Bioavailability of Zinc

The main staple food component that inhibits the bioavailability of zinc is phytate. The proportion of zinc that is absorbed from typical diets appears to range from about 18 % to 34 %, where lower bioavailability is associated with a higher molar ratio of phytate: zinc in the diet. Plant foods rich in zinc, such as legumes, are also high in phytic acid, an inhibitor of zinc bioavailability (Harland and Oberleas 1987). Because the phytate content of whole grains and legumes tends to be very high, it

is expected that breeding for lower phytate content would lead to improved zinc bioavailability and hence increase dietary zinc adequacy. A World Health Organization publication (WHO 1996) categorized phytate-zinc molar ratios of 5–15 as moderate in zinc bioavailability (30–35 % absorption). In comparison, high-zinc bioavailability (50–55 % absorption) diets were described as refined, low in cereal fiber, with a phytate-zinc molar ratio of <5, and with adequate protein. Low-zinc-bioavailability diets (15 % absorption) were listed as phytate-zinc ratio >15. Bioavailability of zinc is enhanced by dietary protein (Sandström et al. 1980), but plant sources of protein are also generally high in phytic acid.

The bioavailability of zinc from vegetarian diets is also likely to be less than that of nonvegetarian diets. High levels of calcium fortification may also reduce zinc bioavailability (WHO 1996). The description of the new Dietary Reference Intakes for zinc (Food and Nutrition Board 2001) suggested that, because of lower absorption of zinc, those consuming vegetarian diets, especially with phytate-zinc molar ratios >15, may require as much as 50 % more zinc than nonvegetarians.

#### 5.4.3 Bioavailability of Other Trace Elements

Much less information is available about the bioavailability of other trace elements besides iron and zinc. Because plant foods are generally good source of elements such as copper and manganese, vegetarian diets are likely to provide greater amounts of these trace elements than non vegetarian diets. Plasma copper was not significantly different between vegetarians and nonvegetarians in a cross-sectional study (Krajcovicova-Kudlackova et al. 1995). As assessed by monitoring the fecal excretion of a stable copper isotope, copper was absorbed less efficiently from the vegetarian diet, but more total copper was absorbed because of the greater copper content of a vegetarian diet, compared with a nonvegetarian diet (Hunt and Vanderpool 2001). The selenium content of foods varies greatly with the selenium in the soil where the food is grown. Furthermore, the retention and use of selenium from the diet likely depends on the chemical form of selenium in foods. By analysis, the total selenium content of vegetarian diets was similar or lower than that of nonvegetarian diets (Drobner et al. 1997); in contrast, dietary selenium increased by about 40 % when subjects switched to a vegetarian diet (Srikumar et al. 1992). In those healthy subjects who switched to self-selected vegetarian diets, plasma selenium decreased by 11 %.

# 5.5 Bioavailability and Bioefficacy of Folate and Folic Acid

Folates represent an important group among the B vitamins, participating in one-carbon transfer reactions required within the cell, especially for purine and pyrimidine biosynthesis (DNA and RNA) and amino acid interconversions. Health benefits of folates regarding their prevention of neural tube defects in babies and occlusive vascular diseases caused by elevated plasma homocysteine, their link to mental fitness, and possibly certain forms of cancer have already been recognized. Low folate intake causes mild hyperhomocysteinemia, which is a potential risk factor for cardiovascular disease (Danesh and Lewington 1998). Supplementation with folic acid leads to a significant reduction in plasma homocysteine concentrations in healthy subjects, even at low doses (Ward et al. 1997). An increased intake of dietary folate lowers plasma homocysteine concentrations (Brouwer et al. 1999).

The analyses of food folates are tedious because of a lack of validated methods for characterization and quantitation of the great number of native folate forms but also due to a lack of adequate methods for sample pretreatment. Therefore, the assessment of folate losses through industrial and household food processing is still incomplete, as well as knowledge on folate bioavailability in humans.

The bioavailability and bioefficacy of dietary folate appears to be less than those of folic acid

(Sauberlich et al. 1987). Bioavailability is defined as the fraction of folate that is absorbed and can be used for metabolic processes or storage as measured by changes in folate status, whereas bioefficacy, or more correctly functional bioefficacy, is defined as the fraction that has a positive effect on a functional parameter, for the lowering of homocysteine instance. concentrations (Brouwer et al. 2001). Folate functions as a coenzyme in single-carbon transfers in the metabolism of nucleotides and amino acids. It is essential for the formation of thymidylate (TMP) for DNA synthesis, so that without folate, living cells cannot divide. The need for folate is higher when cell turnover is increased, such as in fetal development. It is also involved in purine synthesis, in the generation of formate, and in amino acid interconversions. The bioavailability of folate has been a topic of active investigation for many years. Much of the interest in this area originated from reports by Tamura and Stokstad (1973) that showed a wide range of bioavailability of endogenous folate in a wide variety of common foods. It is widely recognized that for typical mixed diets, the bioavailability of naturally occurring folate is incomplete. The results of a long-term controlled dietary study with human subjects by Sauberlich et al. (1987) indicated that the bioavailability of folate in a typical mixed diet was not more than 50 %. Cuskelly et al. (1996) conducted a similar study with free-living subjects and observed that fortified foods and folic acid in supplements were substantially more effective than highfolate foods provided to the subjects.

#### 5.6 Conclusion

Most of the agricultural systems in the developing world do not provide enough micronutrients to meet the human needs. Even though micronutrients are required in minute quantities, they have tremendous impact on human health. Insufficient dietary intake of these nutrients impairs the functions of the brain, the immune and reproductive systems, and energy metabolism. These deficiencies result in learning disabilities, reduced work capacity, serious illnesses, and even death. Micronutrient malnutrition is a serious global problem that limits the work capacity of people and seriously hinders economic development. Finding sustainable solution to this developing global nutritional crisis and to address micronutrient deficiencies in the comprehensive needs several way approaches. In the past, supplementation and fortification programs have shown some positive results in treating the symptoms of micronutrient malnutrition. Breeding staple foods that are dense in minerals and vitamins provides a low-cost, sustainable strategy for reducing levels of micronutrient malnutrition. Conventional breeding techniques, biotechnology and micronutrient-containing fertilizers to improve the micronutrient quality of staple crops and the micronutrient status of the poor have been put forth in recent years; this new strategy is referred to as "biofortification." Genetic biofortification is a sustainable method of naturally enriching food crops by conventional breeding and modern biotechnology. "Bioavailability" of essential micronutrients is another important aspect which needs attention. Iron bioavailability typically ranges from 5 % to 15 % of total iron intake, whereas the proportion of zinc that is absorbed from typical diets appears to range from about 18 % to 34 % and lower bioavailability is associated with a higher molar ratio of phytate: zinc in the diet. The bioavailability issues must be addressed when employing plant breeding and/or transgenic approaches to reduce micronutrient malnutrition.

## References

- ACC/SCN (UN Administrative Committee on Coordination, Subcommittee on Nutrition) (2000) Fourth report on the world nutrition situation. Geneva: ACC/SCN in collaboration with the International Food Policy Research Institute
- Bhutta ZA, Bird SM, Black RE, Brown KH, Gardner JM, Hidayat A, Khatun F, Martorell R, Ninh NX, Penny ME, Rosado JL, Roy SK, Ruel M, Sazawal S, Shankar A (2000) Therapeutic effects of oral zinc in acute and persistent diarrhoea in children in developing countries: pooled analysis of randomized controlled

trial. Zinc investigators' collaborative group. Am J Clin Nutr 72:1516–1522

- Black RE (1998) Therapeutic and preventive effects of zinc on serious childhood infectious diseases in developing countries. Am J Clin Nutr 68:S476–S479
- Blair MW, Knewton SJB, Astudillo C, Li C, Fernandez AC, Grusak MA (2010) 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
- Bravo L (1998) Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutr Rev 56:317–333
- Brouwer IA, van Dusseldorp M, West CE et al (1999) Dietary folate from vegetables and citrus fruit decreases plasma homocysteine concentrations in humans in a dietary controlled study. J Nutr 129:1135–1139
- Brouwer IA, van Dusseldorp M, West CE, Steegers-Theunissen RPM (2001) Bioavailability and bioefficacy of folate and folic acid in humans. Nutr Res Rev 14:267–293
- Combs GF, Welch RM, Duxbury JM, Uphoff NT, Nesheim MC (1996) Food-based approaches to preventing micronutrient malnutrition: an international research agenda. Cornell International Institute for Food, Agriculture, and Development, Cornell University, Ithaca
- Cook JD, Layrisse M, Martinez-Torres C, Walker R, Monsen E, Finch CA (1972) Food iron absorption measured by an extrinsic tag. J Clin Investig 51:805–815
- Cuskelly GJ, McNulty H, Scott JM (1996) Effect of increasing dietary folate on red-cell folate: implications for prevention of neural tube defects. Lancet 347:657–659
- Danesh J, Lewington S (1998) Plasma homocysteine and coronary heart disease: systematic review of published epidemiologic studies. J Cardiovasc Risk 5:229–232
- Drobner C, Röhrig B, Anke M, Thomas G (1997) Selenium intake of adults in Germany depending on sex, time, living area and type of diet. In: Fischer PWF, L'Abbé MRL, Cockell KA, Gibson RS (eds) Trace elements in man and animals: proceedings of the ninth international symposium on trace elements in man and animals. NRC Press, Ottawa, pp 158–159
- FAO/WHO (2002) Food and Agriculture Organization/ World Health Organization-Human vitamin and mineral requirements. Report of a joint FAO/WHO expert consultation, Bangkok
- Fehr WR (1982) Control of iron deficiency chlorosis in soybeans by plant breeding. J Plant Nutr 5:611–621
- Food and Nutrition Board (2001) Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. National Academy Press, Washington, DC
- Gibson RS, Donovan UM, Heath AL (1997) Dietary strategies to improve the iron and zinc nutrition of

young women following a vegetarian diet. Plant Foods Hum Nutr 51:1–16

- Graham RD, Welch RM, Bouis HE (2001) Addressing micronutrient malnutrition through enhancing the nutritional quality of staple food: principles, perspectives and knowledge gaps. Adv Agron 70:77–142
- Hallberg L, Brune M, Rossander L (1989) Iron absorption in man: ascorbic acid and dose-dependent inhibition by phytate. Am J Clin Nutr 49:140–144
- Harland BF, Oberleas D (1987) Phytate in foods. World Rev Nutr Diet 52:235–259
- Hoekenga OA, Lung'aho MG, Tako E, Kochian LV, Glahn RP (2011) Iron biofortification of maize grain. Plant Genet Resour 9:327–329
- Hunt JR (2003) Bioavailability of iron, zinc, and other trace minerals from vegetarian diets. Am J Clin Nutr 78:633S–639S
- Hunt JR, Vanderpool RA (2001) Apparent copper absorption from a vegetarian diet. Am J Clin Nutr 74:803–807
- Kies C, Young E, McEndree L (1983) Zinc bioavailability from vegetarian diet: influence of dietary fiber, ascorbic acid, and past dietary practices. In: Inglett GE (ed) Nutritional bioavailability of zinc. American Chemical Society, Washington, DC, pp 115–126
- Krajcovicova-Kudlackova M, Simoncic R, Babinska K et al (1995) Selected vitamins and trace elements in blood of vegetarians. Ann Nutr Metab 39:334–339
- Lestienne I, Caporiccio B, Besancon P, Rochette I, Treche S (2005) Relative contribution of phytates, fibers, and tannins to low iron and zinc in vitro solubility in pearl millet (*Pennisetum glaucum*) flour and grain fractions. J Agric Food Chem 53:8342–8348
- Mason JB, Garcia M (1993) Micronutrient deficiency the global situation. SCN News 9:11–16
- McGuire J (1993) Addressing micronutrient malnutrition. SCN News 9:1–10
- Rao Udayasekhara P, Deosthale YG (1988) In vitro availability of iron and zinc in white and coloured ragi (*Eleusine coracana*): role of tannin and phytate. Plant Foods Hum Nutr 38:35–41
- Sandström B, Arvidsson B, Cederblad A, Bjorn-Rasmussen E (1980) Zinc absorption from composite

meals, I: the significance of wheat extraction rate, zinc, calcium, and protein content in meals based on bread. Am J Clin Nutr 33:739–745

- Sauberlich HE, Kretsch MJ, Skala JH, Johnson HL, Taylor PC (1987) Folate requirement and metabolism in non pregnant women. Am J Clin Nutr 46:1016–1028
- 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
- Sompong U, Somta P, Raboy V, Srinivas P (2012) Mapping of quantitative trait loci for phytic acid and phosphorous contents in seed and seedling of mungbean (*Vigna radiata* (L.) Wilczek.). Breed Sci 62:87–92
- Srikumar TS, Johansson GK, Öckerman P, Gustafsson J, Åkesson B (1992) Trace element status in healthy subjects switching from a mixed to a lactovegetarian diet for 12 months. Am J Clin Nutr 55:885–890
- Tamura T, Stokstad ELR (1973) The availability of food folate in man. Br J Haematol 25:513–532
- Turnbull A, Cleton F, Finch CA (1962) Iron absorption. IV. The absorption of hemoglobin iron. J Clin Investig 41:1897–1907
- Umeta M, West CE, Haidar J, Deurenberg P, Hautvast JG (2000) Zinc supplementation and stunted infants in Ethiopia: a randomised controlled trial. Lancet 355:2021–2026
- Ward M, McNulty H, McPartlin J, Strain JJ, Weir DG, Scott JM (1997) Plasma homocysteine, a risk factor for cardiovascular disease, is lowered by physiologic doses of folic acid. Q J Med 90:519–524
- Weiss MG (1943) Inheritance and physiology of efficiency of iron utilization in soybeans. Genetics 28:253–268
- Welch RM, Graham RD (2004) Breeding for micronutrients in staple food crops from a human nutrition perspective. J Exp Bot 55:353–364
- World Health Organization (1996) Trace elements in human nutrition and health. WHO, Geneva