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Breeding for micronutrients in staple food crops from a human nutrition perspective

Ross M. Welch[1,*] and Robin D. Graham[2]

[1] *USDA-ARS, US Plant, Soil and Nutrition Laboratory, Cornell University, Tower Road, Ithaca, NY 14853-0001, USA*

[2] *University of Adelaide, Waite Campus, Glen Osmond, 5064, South Australia*

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Abstract

Over three billion people are currently micronutrient (i.e. micronutrient elements and vitamins) malnourished, resulting in egregious societal costs including learning disabilities among children, increased morbidity and mortality rates, lower worker productivity, and high healthcare costs, all factors diminishing human potential, felicity, and national economic development. Nutritional deficiencies (e.g. iron, zinc, vitamin A) account for almost two-thirds of the childhood death worldwide. Most of those afflicted are dependent on staple crops for their sustenance. Importantly, these crops can be enriched (i.e. 'biofortified') with micronutrients using plant breeding and/or transgenic strategies, because micronutrient enrichment traits exist within their genomes that can to used for substantially increasing micronutrient levels in these foods without negatively impacting crop productivity. Furthermore, 'proof of concept' studies have been published using transgenic approaches to biofortify staple crops (e.g. high β-carotene 'golden rice' grain, high ferritin-Fe rice grain, etc). In addition, micronutrient element enrichment of seeds can increase crop yields when sowed to micronutrient-poor soils, assuring their adoption by farmers. 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 or decreasing antinutrient substances (e.g. phytate, polyphenolics, etc) that inhibit micronutrient bioavailability, are both options that could be pursued, but the latter approach should be used with caution. The world's agricultural community should adopt plant breeding and other genetic technologies to improve human health, and the world's nutrition and health communities should support these efforts. Sustainable solutions to this enormous global problem of 'hidden hunger' will not come without employing agricultural approaches.

Key words: Agricultural intervention, food-based approach, human health, iron, malnutrition, minerals, nutritional quality, vitamins, sustainability, trace elements, zinc.

The global micronutrient crisis

Humans require at least 49 nutrients to meet their metabolic needs (Table 1). Inadequate consumption of even one of these nutrient will result in adverse metabolic disturbances leading to sickness, poor health, impaired development in children, and large economic costs to society (Branca and Ferrari, 2002; Golden, 1991; Grantham-McGregor and Ani, 1999; Ramakrishnan *et al*., 1999). Table 2 lists the required daily amounts for some of these nutrients for adults as reported by the Food and Agricultural Organization, United Nations, and the World Health Organization (FAO/WHO, 2000). Importantly, the primary source of all nutrients for people comes from agricultural products. If agricultural systems fail to provide enough products containing adequate quantities of all nutrients during all seasons, dysfunctional food systems result that cannot support healthy lives. Unfortunately, this is the case for many agricultural systems in many developing nations in the Global South (Graham *et al*., 2001; McGuire, 1993; Schneeman, 2001).

* To whom correspondence should be addressed. Fax: +1 607 255 1132. E-mail: rmw1@cornell.edu

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Table 1. *The 49 known essential nutrients for sustaining human life*[a]

Water and energy	Protein (amino acids)	Lipids–fat (fatty acids)	Macro-elements	Micro-elements	Vitamins
Water	Histidine	Linoleic acid	Na	Fe	A
Carbohydrates	Isoleucine	Linolenic acid	K	Zn	D
	Leucine		Ca	Cu	E
	Lysine		Mg	Mn	K
	Methionine		S	I	C (ascorbic acid)
	Phenylalanine		P	F	B_1 (thiamin)
	Threonine		Cl	B	B_2 (riboflavin)
	Tryptophan			Se	B_3 (pantothenic acid)
	Valine			Mo	Niacin
				Ni	B_6 (pyridoxal)
				Cr	Folate
				V	Biotin
				Si	B_{12} (cobalamin)
				As	
				Sn	
				Co (cobalamin)	

[a] Numerous other beneficial substances in foods are also known to contribute to good health.

Today, over three billion people are afflicted with micronutrient† malnutrition and the numbers are increasing (Mason and Garcia, 1993; Welch *et al*., 1997; WHO, 1999; World Bank, 1994). Nearly two-thirds of all deaths of children are associated with nutritional deficiencies, many from micronutrients deficiencies (Caballero, 2002). Marginal intakes of micronutrients have been shown to contribute to increased morbidity and mortality rates, diminished livelihoods, and adverse effects on learning ability, development, and growth in infants and children (Caballero, 2002; WHO, 1999). Much of childhood stunting has been attributed to the impact of micronutrient deficiencies on children from early foetal stages of development through the fourth year of life (Branca and Ferrari, 2002). By any measure, micronutrient malnutrition is currently of alarming proportions in many developing nations (Mason and Garcia, 1993; WHO, 2002).

Developing micronutrient-enriched staple plant foods, either through traditional plant breeding methods or via molecular biological techniques, is a powerful intervention tool that targets the most vulnerable people (resource-poor women, infants, and children; Bouis, 2000; Combs Jr *et al*., 1996). These tools should be fully exploited by the nutrition and public health communities to combat micronutrient malnutrition (Graham *et al*., 2001). Biofortifying these crops ('biofortification' is a word coined to refer to increasing the bioavailable micronutrient content of food crops through genetic selection via plant breeding) that feed the world's poor can significantly improve the amount of these nutrients consumed by these target populations (Welch and Graham, 1999). Furthermore, it is a sustainable intervention, unlike traditional interventions that depend on supplementation and fortification programmes that have not proved to be

Table 2. *Recommended nutrient intakes for males and females between the ages of 25 and 50 years (data from FAO/WHO, 2000)*

Nutrient	Assessment	Male	Female
Energy (kcal)	AEA[a]	2900	2200
Protein (g)	AEA	63	50
Vitamin A (μg retinol equivalent)	RDA[b]	1000	800
Vitamin D (μg)	RDA	5	5
Vitamin E (mg α-tocopherol equivalent)	RDA	10	8
Vitamin K (μg)	RDA	80	65
Riboflavin (mg)	RDA	1.7	1.3
Niacin (mg niacin equivalent)	RDA	19	15
Thiamin (mg)	RDA	1.5	1.1
Pantothenic acid (mg d^{-1})	ESADDI[c]	4–7	4–7
Vitamin B_6 (mg)	RDA	2	1.6
Vitamin B_{12} (μg)	RDA	2	2
Biotin (μg d^{-1})	ESADDI	30–100	30–100
Folate (μg)	RDA	200	180
Vitamin C (mg)	RDA	90	60
Ca (mg)	RDA	800	800
P (mg)	RDA	800	800
Mg (mg)	RDA	350	280
Na (mg)	MR[d]	500	500
K (mg)	MR	2000	2000
Cl (mg)	MR	750	750
Fe (mg)	RDA	10	15
Zn (mg)	RDA	15	12
Cu (mg)	ESADDIC	1.5–3.0	1.5–3.0
Se (μg)	RDA	70	55
I (μg)	RDA	150	150
Mn (mg)	ESADDI	2–5	2–5
Mo (μg)	ESADDI	75–250	75–250
Cr (μg)	ESADDI	50–200	50–200
F (mg)	ESADDI	1.5–4.0	1.5–4.0

[a] AEA, average energy allowance.
[b] RDA, recommended dietary allowances.
[c] ESADDI, estimated safe and adequate daily dietary intakes.
[d] MR, minium requirement.

† The definition of essential micronutrients used by human nutritionists include both vitamins and essential micronutrient elements. Plant scientists do not include the vitamins in their definition of micronutrients because plants, being autotrophs, synthesize vitamins *in situ* (with the exception of cobalamin, i.e. vitamin B_{12}). In this publication, the use of micronutrients includes both the vitamins and the essential micronutrient elements, thus adopting the human nutritionists definition.

sustainable in many developing nations (Subbulakshmi and Naik, 1999; Yip, 1997). In addition, increasing the micronutrient metals stored in the seeds and grains of staple food crops increases crop productivity when these seeds are sown in micronutrient poor-soils (Welch, 1999). Much of the developing world has significant areas of such soils (White and Zasoski, 1999). Enhancing seeds with micronutrient metals will act as an incentive to farmers cultivating micronutrient-poor soils to adopt the micronutrient-enriched seeds for use on their farms (Graham *et al*., 2001).

Several barriers to metal accumulation in food crops have to be addressed before genetically modifying plants in ways that will increase the density of micronutrient metals in staple seeds and grains (Welch, 1995). Therefore, the physiological processes controlling metal accumulation by plants are discussed briefly below. Furthermore, because plant foods contain substances (i.e. antinutrients and promoters; see Tables 3 and 4 further on) that influence the bioavailability[‡] of these nutrients to humans, it is necessary to demonstrate the efficacy of micronutrient enrichment of plant foods towards improving the nutritional health of targeted populations. This requires that the bioavailability of Fe, Zn, provitamin A carotenoids, and other micronutrients in select micronutrient-enriched genotypes of staple plant foods be demonstrated, in order to assure a human health impact before advancing genotypes in breeding programmes (Graham *et al*., 2001).

The physiology of micronutrient accumulation

The physiological basis for micronutrient efficiency in crop plants and the processes controlling the accumulation of micronutrients in edible portions of seeds are not understood with any certainty (Welch, 1999). Because of the complexity and volume of literature available, these subjects will not be covered in this short review. For an in-depth discussion of these topics the reader is referred to the following references (Graham *et al*., 2001; Graham and Welch, 1996; Marschner, 1995; Welch, 1986, 1995, 1999; Yang and Römheld, 1999). A brief outline of the processes that determine micronutrient concentrations in edible plant tissues is discussed below.

There are several barriers to overcome in genetically modifying plants to accumulate more micronutrient metals (e.g. Fe and Zn) in edible tissues (Welch, 1995). These barriers are the result of tightly controlled homoeostatic mechanisms that regulate metal absorption, translocation, and redistribution in plants allowing adequate, but non-toxic levels of these nutrients to accumulate in plant tissues. The first and most important barrier to micronutrient absorption resides at the root–soil interface (i.e. the rhizosphere). To increase micronutrient metal uptake by roots, the available levels of the micronutrient in the root–soil interface must be increased to allow for more absorption by root cells. This could be enhanced by changing root morphology and by stimulating certain root-cell processes that modify micronutrient solubility and movement to root surfaces, such as by stimulating the rate of root-cell efflux of H^+, metal-complexing compounds, and reductants, and by increasing the root absorptive surface area such as the number and extent of fine roots and root hairs. Second, absorption mechanisms (e.g. transporters and ion channels), located in the root-cell plasma membrane, must be sufficiently active and specific enough to allow for the accumulation of micronutrient metals once they enter the apoplasm of root cells from the rhizosphere. Third, once taken up by root cells, the micronutrients must be efficiently translocated to and accumulated in edible plant organs. For seeds and grains, phloem sap loading, translocation and unloading rates within reproductive organs are important characteristics that must be considered in increasing micronutrient metal accumulation in edible portions of seeds and grains (Welch, 1986). Finally, to be effective, the micronutrient metal species accumulated in edible portions must be bioavailable to people that eat the seeds in a meal (Fairweather-Tait and Hurrell, 1996; Welch, 1986, 2001). Unfortunately, current knowledge of all of these processes is very limited and much more basic research is needed before food crops can be genetically modified efficiently in order to accumulate more bioavailable forms of micronutrients in seeds and grains through modern genetic engineering techniques.

Micronutrient enrichment through plant breeding

Recently, the genetic potential for increasing the concentrations of bioavailable Fe, Zn, and provitamin A carotenoids (as well as Se and I) in edible portions of several staple food crops (including rice, wheat, maize, beans, and cassava) has been reviewed (Graham *et al*., 2001). The following section describes the initial criteria used in this on-going global 'biofortification' research effort.

Breeding criteria

Certain criteria must be met before new lines of micronutrient-enriched staple food crops are distributed globally to national agricultural research programmes. Meeting these conditions will ensure that targeted people

[‡] Bioavailability is defined as the amount of a nutrient that is potentially available for absorption from a meal and once absorbed, utilizable for metabolic processes in the body.

at risk of developing micronutrient malnutrition will benefit from such action. These criteria include: (1) crop productivity (i.e. yield) must be maintained or increased to guarantee widespread farmer acceptance; (2) the micronutrient enrichment levels achieved must have significant impact on human health; (3) the micronutrient enrichment traits must be relatively stable across various edaphic environments and climatic zones; (4) ultimately, the bioavailability of micronutrients in enriched lines must be tested in humans to ensure that they improve the micronutrient status of people preparing and eating them in traditional ways within normal household environments; and (5) consumer acceptance must be tested (taste and cooking quality must be acceptable to household members) to ensure maximum impact on nutritional health.

Meeting these conditions will require a new way of thinking and performing research by most agriculturalists, an holistic food systems view of agricultural production. It will necessitate that researchers co-operate with various specialists in disciplines not normally associated with agricultural research, including nutritionists, public health officials, sociologists, political scientists, food technologists, and economists to ensure that their efforts will have meaningful impact on human nutrition and health (Combs Jr *et al*., 1996).

The question of yields

The effects of biofortifying staple plant foods with micronutrients on crop productivity have been addressed in a number of recent reviews (Bouis, 1996; Graham *et al*., 1998, 1999; Graham and Welch, 1996). Briefly, increasing the micronutrient stores in seeds results in more seedling vigour and viability, thus enhancing the performance of seedlings when the seeds are planted in micronutrient-poor soils. This improved seedling vigour is associated with the production of more and longer roots under micronutrient-deficient conditions, allowing seedlings to scavenge more soil volume for micronutrients and water early in growth, an advantage that can lead to improved yields compared with seeds with low micronutrient stores grown on the same soils. For example, Fig. 1 shows the effects of seed micronutrient enrichment on grain yields of wheat grown on farms of Bangladesh (unpublished data from John Duxbury, Department of Crop and Soil Sciences, Cornell University, 2000). In this experiment micronutrient-enriched grain were sown to farmers' fields having low micronutrient stores in three districts in Bangladesh. Seven out of the nine farms had significant increases in wheat grain yield when produced from micronutrient-enriched grain compared with control or farmers' grain not so enriched. These data clearly show the advantage of using biofortified wheat grain on wheat productivity in these regions of Bangladesh.

Many of those countries where micronutrient deficiencies in humans are a problem are also the countries that have large areas of micronutrient-poor/deficient soils (White and Zasoski, 1999). Thus, improving seed vigour with respect to micronutrient stores should be very beneficial to agricultural production in these countries. In addition, disease resistance and stress tolerance are improved in seedlings grown from micronutrient-dense seeds which would also aid agricultural production in target countries (Welch, 1986, 1999). Thus, selecting for these traits in staple food crops is a 'win-win' opportunity. It has potential to enhance crop yields without additional farmer inputs and to improve their nutritional quality simultaneously.

The genetic potential

During the past decade, scientists at several Consultative Group on International Agricultural Research (CGIAR) Centers [the International Rice Research Institute (IRRI), the Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT), the Centro Internacional de Agricultura Tropical (CIAT), and the International Institute of Tropical Agriculture (IITA)] have been collecting data on the potential for breeding to increase the levels of bioavailable Fe, Zn, and provitamin A carotenoids significantly in edible portions of rice, wheat, maize, beans, and cassava (Bouis, 1996; Graham et al., 1998, 1999, 2001; Graham and Welch, 1996; Welch and Graham, 1999). These findings are summarized below.

Phaseolus *bean*: Researchers at CIAT have been studying the degree of genetic variability that exists in Fe and Zn concentrations in seeds of common beans (*Phaseolus vulgaris* L.) (Graham *et al*., 1999). Researchers at CIAT (Beebe *et al*., 2000) evaluated (all genotypes were tested in the field at the same location during the same year) a core collection of over 1000 accessions of common beans, and found a range in Fe concentrations from 34–89 $\mu g\ g^{-1}$ Fe (average=55 $\mu g\ g^{-1}$ Fe) (Graham *et al*., 1999). Zinc concentrations in these same accessions ranged from 21–54 $\mu g\ g^{-1}$ Zn (average=35 $\mu g\ g^{-1}$ Zn). Some bean accessions from Peru were recently found to contain especially high levels of Fe averaging over 100 $\mu g\ g^{-1}$ Fe. The range in seed-Zn concentrations in the core collection was narrower than seed-Fe concentrations ranging from 21 to 54 $\mu g\ g^{-1}$ Zn. Wild types tended to have lower Zn concentrations than common cultivated types. Some seeds from genotypes originating in Guatemala were highest in Zn levels. The data collected suggest that there is sufficient genetic variability to increase significantly Fe (by about 80%) and Zn (by about 50%) concentrations in common beans. Results also indicate that the traits responsible for genetic improvements in Fe and Zn concentrations are stable across bean-growing environments. For both Fe- and Zn-seed concentrations, there were significant location and location×genotype effects demonstrating (as expected) that environments can influence the concentrations

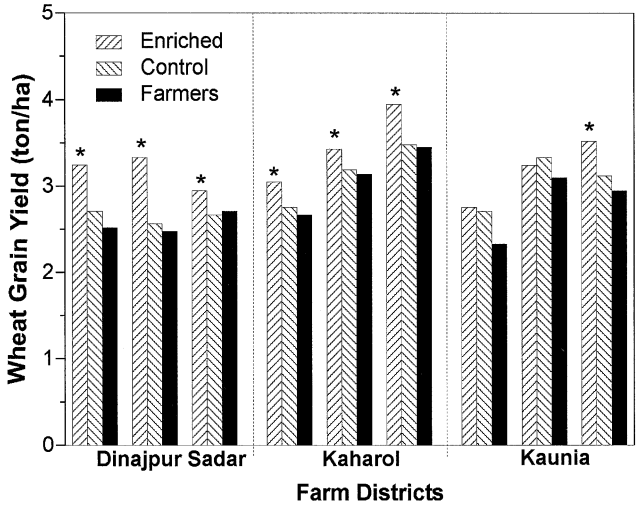

Fig. 1. Effects of micronutrient enrichment of wheat grain on grain yields from nine farms in three districts of Bangladesh. The enriched and control grains were produced on an experimental farm. The enriched grain were produced from foliar applications of micronutrient to mother plants during their reproductive growth stage. Astreisks indicate significant difference form control and farmers' grain (unpublished data from Dr John Duxbury, Department of Crop and Soil Sciences, Cornell University, 2000).

of Fe and Zn in bean seeds. However, high-Fe and high-Zn genotypes will accumulate more of these nutrients when compared to low-Fe and low-Zn genotypes grown at the same location during the same growing season.

Interestingly, CIAT researchers also found a very highly significant positive correlation of 0.52 between the concentrations of Fe and Zn across different genotypes. Thus, genetic factors for increasing Fe are co-segregating with genetic factors increasing Zn. Therefore, selecting for a higher Fe level in bean seeds will also tend to select for increased Zn levels in the seeds.

Rice: Since 1992, researchers at IRRI (Gregorio *et al*., 2000) have been evaluating the genetic variability of Fe concentration in rice grain. In 1995 the research was expanded to include Zn (Graham *et al*., 1999). The range in Fe and Zn concentrations within the six sets of genotypes (n=939) tested in one study were 7.5 μg g^{-1} to 24.4 μg g^{-1} for Fe, and 13.5 μg g^{-1} to 58.4 μg g^{-1} for Zn. Again, all genotypes were tested in the field at the same location during the same year. Thus, within those genotypes tested, there was about a 4-fold difference in Fe and Zn concentrations suggesting some genetic potential to increase the concentrations of these micronutrients in rice grain.

Among the varieties with the highest grain-Fe concentrations (i.e. ranging from about 18 μg g^{-1} to 22 μg g^{-1}) found were in a number of aromatic rice varieties including Jalmagna, Zuchem, and Xua Bue Nuo. In addition, these same aromatic lines also contained the highest grain-Zn concentrations (ranging from about 24 μg g^{-1} to 35 μg g^{-1}). Further research using F_2-derived populations demonstrated that the aromatic trait was not pleiotropic for grain-Fe or grain-Zn concentrations, so although aroma might conceivably be used to screen for high Fe and Zn levels in rice grain, the linkage is only weak.

Several studies were carried out at IRRI to examine the effect of soil and climatic factors (i.e. the environment) on grain-Fe and grain-Zn concentrations among genotypes. Factors studied included wet season–dry season, normal–saline soils, acid–neutral soils, and N-supply. The data from these various studies demonstrated that high-Fe and high-Zn grain traits are expressed in all rice environments tested, although there is some evidence of significant genotype×environment interactions that can ultimately affect Fe and Zn concentrations in extreme environments (Graham *et al*., 1999).

These IRRI results indicate that there is significant genetic diversity in the rice genome to increase Fe and Zn concentrations substantially in the rice grain. However, the effects of rice grain processing on Fe and Zn levels in the edible product (i.e. polished and parboiled rice grain), as well as the bioavailability of the Fe and Zn in the grain to humans still await final results from continuing evaluations of these factors.

Wheat: A wide range of wheat germplasm is being studied at CIMMYT (Monasterio and Graham, 2000) with respect to the concentration of Fe and Zn in the whole grain and environmental interactions on their concentrations. In one study, the ranges in Fe and Zn concentrations (dry weight basis) in wheat grain from plants grown at a field location in El Batan, Mexico in 1994 were 28.8–56.5 μg g^{-1} (mean=37.2 μg g^{-1}; SD=4.10 μg g^{-1}; n=132) for Fe and 25.2–53.3 μg g^{-1} for Zn (mean=35.0 μg g^{-1}; SD=4.99 μg g^{-1}; n=132) (Graham *et al*., 1999). Clearly, enough genetic variation exists within the wheat germplasm to increase Fe and Zn concentrations substantially in wheat grain. Among all wheat germplasm studied, the species *Triticum dicoccum* Schrank had the highest concentrations of Fe and Zn, which warrants further study.

There was a high correlation between grain-Fe and grain-Zn concentrations in the wheat lines studied. While there was significant genotype×environmental interactions obtained for Fe and Zn grain concentrations, there was still a strong genetic component to Fe and Zn accumulation in the grain. This finding indicates that it should be possible to improve Fe and Zn levels in wheat grain simultaneously through plant breeding. Additional research has also shown that there is no negative linkage between grain yield and Fe and Zn density in the grain.

Maize: Current data being collected by CIMMYT suggests that the range in Fe and Zn concentrations in maize kernels is not as great as that found for other cereal crops although

more data are needed to confirm this finding. A Southern African germplasm collection containing 20 lines was evaluated at several field locations near Harare, Zimbabwe in 1996–97. The range in Fe and Zn concentrations was 16.4–22.9 μg g^{-1} for Fe (mean of 19.6 μg g^{-1}), and 14.7–24.0 μg g^{-1} for Zn (mean of 19.8 μg g^{-1}) (Bunziger and Long, 2000). Scientists at the IITA have also screened a number of early-maturing lines of maize for their kernel-Fe and -Zn concentrations from plants grown in several agroecological zones in West Africa.. Genotypic differences (averaged across locations) in Fe and Zn concentrations (μg g^{-1}, dry weight) in the mature maize kernels ranged from 15.5–19.1 for Fe and from 16.5–20.5 for Zn (S Oikeh, IITA, Ibadan, Nigeria, unpublished data). These small ranges found for Fe and Zn in maize kernels clearly indicate that some breeding, possibly combined with genetic modifications, should be pursued to increase the kernel concentrations of these important micronutrients in maize varieties being developed for Africa. This could greatly aid in improving maize productivity on the extensive area of micronutrient-poor soils found in some regions of Africa and in improving the nutritional quality of this important staple for the resource-poor people of this region.

Cassava: The variation in β-carotene concentration in cassava roots from a CIAT core collection (630 genotypes) from the global cassava germplasm collection (about 5500 genotypes) was reported by Iglesias *et al*. (1997). In addition, the relationship between root colour and heritability as well as the stability of root β-carotene to different root-processing techniques was studied. They reported a range in β-carotene concentrations in fresh roots from 0.1–2.4 mg (100 g) $^{-1}$ (Chavez *et al*., 2000).

The inheritance of β-carotene root-concentration appears to be determined by two genes (one controlling transport of shoot precursors to the roots and one responsible for the biochemical processes affecting the accumulation of β-carotene in the root). Furthermore, visual screening by using the intensity of the orange colour seemed feasible. However, they also stated that there was a need to rely on quantitative screening techniques to increase the efficiency of the screening programme. It is possible that other provitamin A carotenoids could also be responsible for the deep yellow colour observed in accessions that have intermediate β-carotene concentrations.

Iglesias *et al*. (1997) concluded that there is enough genetic variability within the available cassava germplasm that would make it possible to produce cassava roots that contain enough β-carotene to meet the daily requirements of adults (i.e. 6 mg d^{-1} β-carotene) if the β-carotene in cassava roots is bioavailable. The genotypes containing the highest levels of β-carotene were collected from the Amazonian region of Brazil and Colombia where yellow-root lines are preferred by the indigenous farmers. Processing techniques were shown to have a large effect on the final β-carotene content in the food prepared from cassava roots, with some genotypes being more stable to various forms of processing than others. This factor must also be included in any breeding programme to increase β-carotene in cassava roots.

Bioavailability: a complex determining factor

Determining the bioavailability of micronutrients in plant foods to humans is fraught with difficulty (Fig. 2). Numerous factors interact, ultimately to determine the bioavailability of a particular micronutrient to an individual eating a mixed diet within a given environment. Because of this complexity, the data obtained using various bioavailability model systems are always ambiguous (House, 1999; Van Campen and Glahn, 1999). Only data from feeding trials in micronutrient-deficient test populations under free-living conditions can delineate the efficacy of using micronutrient-enriched varieties of plant foods as an intervention tool. Unfortunately, it is impractical to test in this way the bioavailability of selected micronutrients in numerous genotypes of staple plant foods that can be generated in plant breeding programmes (Graham and Welch, 1996). Therefore, one must use a bioavailability model to screen large numbers of promising lines of micronutrient-enriched genotypes identified in such breeding programmes before advancing them within these programmes.

Model systems

Various models have been used to determine the bioavailability of micronutrients in plant foods to humans (House, 1999; Van Campen and Glahn, 1999). Among these are *in vitro* models such as cultured human intestinal cells (i.e. Caco-2 cell model), animal models (e.g. rats, pigs, and poultry), and small-scale human clinical trials (Underwood and Smitasiri, 1999).

Rat and poultry bioavailability models are easy to perform and relatively inexpensive, but the results obtained are limited in their acceptance by the nutrition community (Greger, 1992). Cultured *in vitro* human intestinal cell models (e.g. the Caco-2 cell model) are rapid, inexpensive, and allow for the ranking of selected staple food genotypes with respect to a standard genotype, and can be used in screening large numbers of genotypes for bioavailable Fe (Van Campen and Glahn, 1999). However, further development of the Caco-2 cell model is required before this model can be adapted for use in determining the bioavailability of Zn and provitamin A carotenoids in staple plant foods (personal communication from Dr Ray Glahn, USDA-ARS, US Plant, Soil and Nutrition Laboratory).

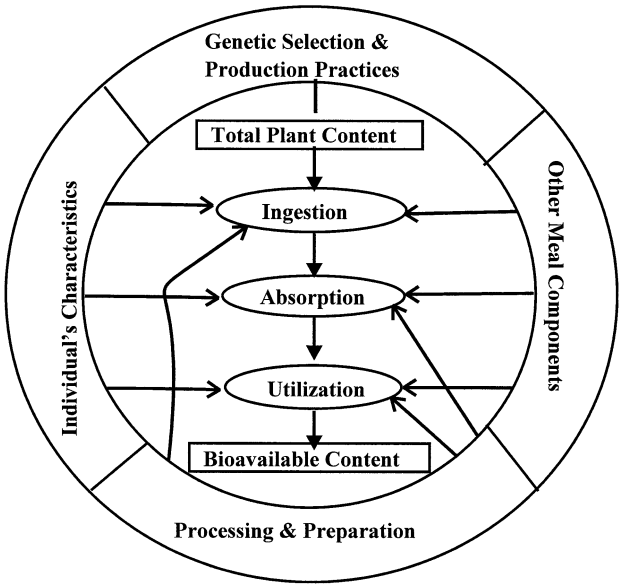

Fig. 2. The complexities of bioavailability in human nutriture (from Graham *et al*., 2001).

The pig model is currently accepted as the most accurate animal model currently available for studying the bioavailability of Fe, Zn, and provitamin A carotenoids in plant foods (Miller and Ullrey, 1987). The results obtained using this model closely parallel those obtained in humans. However, it is relatively expensive compared to *in vitro* models or small animal models and therefore of limited use in screening large numbers of micronutrient-dense staple plant food genotypes that can be identified in plant breeding programmes.

Current breeding efforts to screen large numbers of promising micronutrient-dense lines of staple plant foods (rice, maize, wheat, beans, and cassava) at several CGIAR Centres (IRRI, CIMMYT, CIAT, and IITA) for bioavailable Fe relies on an *in vitro* Caco-2 cell model. Previous bioavailability screens for Fe and Zn were based on a rat model (Welch *et al*., 2000). The determination of bioavailable Zn in promising lines is not currently being directly addressed with Caco-2 cells, but has been evaluated using a rat model. However, it is reasonable to assume that the data obtained for Fe bioavailability will also reflect bioavailable Zn levels in promising genotypes, because most of the factors that inhibit or promote Fe bioavailability also inhibit or promote Zn bioavailability from plant foods (e.g. the antinutrients, phytic acid, polyphenolics and fibres, and several promoters such as 'meat factors'; see subsequent discussion of antinutrients and promoter substances) (Fairweather-Tait and Hurrell, 1996; WHO, 1996).

Intrinsic verses extrinsic labelling of test meals

Extrinsic (i.e. adding isotope 'tags' to test meals) labelling of plant foods has been frequently used to test the bioavailability of Fe and Zn in humans because it is a rapid and less expensive method compared with producing intrinsically isotope-labelled plant foods (i.e. growing test plants in isotopically labelled nutrient solutions) (House, 1999). However, extrinsically labelled plant foods provide equivocal bioavailability data that can lead either to under- or overestimates of Fe or Zn bioavailability from test meals under some circumstances (House, 1999). This is so because some naturally occurring chemical forms of Fe and Zn in plant foods are not readily exchangeable with added extrinsic isotope labels (e.g. the Fe in ferritin, monoferricphytate, haemoglobin, etc) (Welch, 1993). Thus, intrinsic isotope labelling is the only unequivocal means of determining micronutrient bioavailability in plant foods to humans.

Micronutrient status of test subjects in human trials

The Fe and Zn nutritional status of subjects used in Fe and Zn bioavailability studies can greatly affect the amount of these nutrients that are absorbed and utilized from a meal (Welch, 1993;WHO, 1996; Wienk *et al*., 1999). This results because of tightly regulated processes that control the homeostasis and homeorhesus of these nutrients within the body. Subjects that are deficient in these nutrients 'up-regulate' the cellular processes (e.g. absorption from the gut, transfer to body tissues and storage pools) that are responsible for absorbing, transporting, and utilizing these nutrients within the body and thereby maximize the ability of the body to acquire these nutrients from a test meal. These same processes are 'down-regulated' under nutrient adequate conditions, which minimizes the amount of these nutrients that can be absorbed from a meal and stored in the body. Therefore, to maximize the response of a subject to a test meal requires that Fe and Zn-depleted test subjects be used. Marginal deficiency states (with micronutrient stores depleted) are preferred over severely deficient states in test subjects, because when the deficiency is too severe, intestinal malabsorption, attributable to the deficiency state alone, can occur which would result in artifactual data that is not relevant to the effects of the test meal on Fe or Zn bioavailability in more normal individuals (House, 1999; Van Campen and Glahn, 1999).

Micronutrient inhibitor and promoter substances

Plant foods (especially seeds and grains) contain various antinutrients (Table 3) in amounts depending on both genetic and environmental factors that can reduce the bioavailability of dietary non-haem Fe, Zn, and other nutrients to humans (Welch and House, 1984). Some dietary substances that promote the bioavailability of Fe and Zn in the presence of antinutrients are also known (Table 4). Their levels are also influenced by both genetic and environmental factors. Current plant molecular biological and genetic modification approaches now make it possible to reduce or eliminate antinutrients from staple

Table 3. *Antinutrients in plant foods that reduce Fe and Zn bioavailability, and examples of major dietary sources (from Graham* et al.*, 2001)*

Antinutrients	Major dietary sources
Phytic acid or phytin	Whole legume seeds and cereal grains
Fibre (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
Haemagglutinins (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 4. *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
Haemoglobin	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

plant foods, or to increase the levels of promoter substances significantly in these foods (Forssard *et al*., 2000; Welch, 2001). Given these options (i.e. to decrease antinutrients or increase promoters in staple plant foods) which is the wisest path to pursue?

Plant breeders could breed for genotypes that contain lower concentrations of antinutrients or molecular biologists could alter plant genes in ways that reduce or even eliminate antinutrients from plant food meals. However, doing so is not without risk and should be done with caution because many antinutrients are major plant metabolites that may play important roles in plant metabolism, in plant stress resistance, and in plant resistance to crop pests or pathogens. In addition, some of the antinutrients, such as phytate and polyphenols, may play important beneficial roles in human diets by acting as anticarcinogens or by promoting health in other ways such as in decreasing the risk of heart disease or diabetes (Shamsuddin, 1999; Saied and Shamsuddin,1998; Zhou and Erdman Jr, 1995). Thus, plant breeders and molecular biologists should be aware of the possible negative consequences of changing antinutrients in major plant foods before they attempt to alter food crops in this fashion (Graham and Welch, 1996).

Other factors, as shown in Table 4, promote the bioavailability of micronutrients in plant foods to humans even in the presence of antinutrients in those foods. Many of these compounds are normal plant metabolites and only small changes in their concentration may have significant effects on the bioavailability of micronutrients (Welch and House, 1995). Therefore, it is highly recommended that plant breeders and molecular biologists closely scrutinize the strategy of increasing promoter substances in food crops when attempting to improve food crops as sources of micronutrients for people (Graham *et al*., 2000).

Importance of diet composition

The bioavailability of micronutrients in plant foods can be greatly affected by the composition of the diet used to test the bioavailability (House, 1999; van het Hof *et al*., 2000; Wienk *et al*., 1999). Various food processing techniques, meal components, and meal preparation techniques can modify plant foods in ways that either promote or reduce the amount of bioavailable micronutrients in plant foods (Michaelsen and Friis, 1998). For example, eating some animal protein (e.g. beef, fish, pork, poultry) with plant foods high in antinutrients, such as phytic acid, can ameliorate the negative effects of the antinutrients on Fe and Zn bioavailability (Mulvihill and Morrissey, 1998; Welch, 1993; Welch and House, 1995). The mechanisms responsible for this are still a mystery as is the actual identity of the 'meat factors', although the sulphur-containing amino acid, cysteine, has been implicated (Fig. 3). In addition, food preparation techniques, such as seed germination and fermentation before consumption, can be used to reduce the level of antinutrients, such as phytate, in staple plant food meals (Gibson, 1997).

Enriching diets in β-carotene has recently been shown to enhance the bioavailability of non-haem Fe in staple plant food diets fed to humans (Garcia-Casal *et al*., 2000;

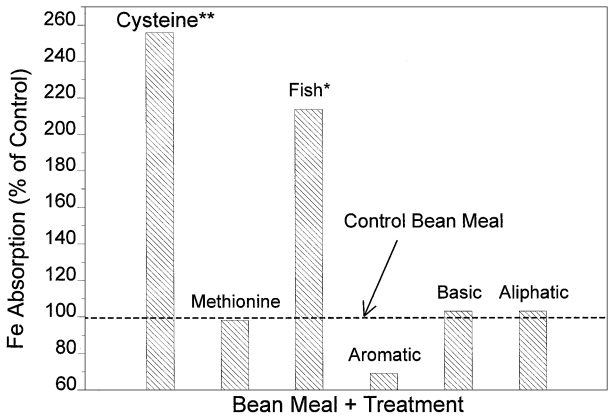

Fig. 3. The effects of amino acids and fish on Fe bioavailability to humans fed intrinsically ^{59}Fe-labelled black bean seed meals. Fe absorption was determined by 'whole-body'counting of subjects fed the intrinsically labelled bean meals. Single asterisk, significant at P=0.05; double asterisk, significant at P=0.01. Data from Martinez-Torres and Layrisse (1970).

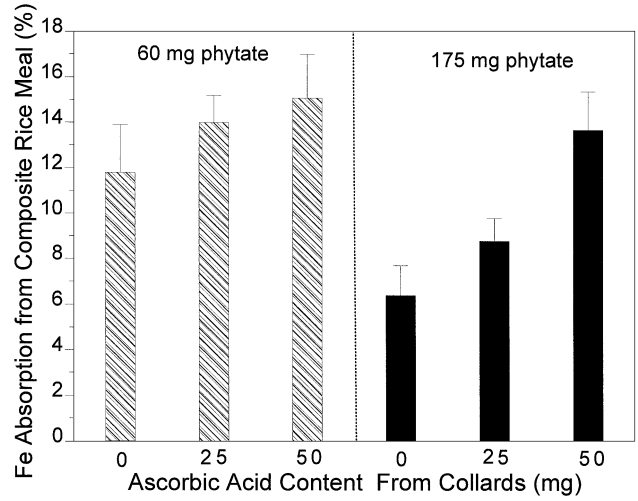

Fig. 4. The effects of increasing ascorbic acid from collard greens on Fe absorption by humans fed basal meals of pork, cooked cabbage and rice extrinsically radio-labelled with both ^{55}Fe and ^{59}Fe. Fe absorption was determine by radioassay of red blood cells 2 weeks after the meals were fed. The meals contained two levels of phytate, 60 mg or 175 mg from rice. Error bars represent standard error of the means. Data from Tuntawiroon *et al*. (1990).

Garcia-Casal and Layrisse, 1999; Mwanri *et al*., 2000; Ncube *et al*., 2001). Again, the underlying reason for this promotion of Fe bioavailability by β-carotene or vitamin A is not understood. However, a recent report suggests that the effects of vitamin A on Fe bioavailability may not be the result of improved Fe solubility through a soluble vitamin A–Fe complex, but may be the result of an indirect effect of vitamin A on Fe absorption and/or incorporation into red blood cells in subjects with marginal vitamin A status (Walezyk *et al*., 2003).

Eating foods rich in the non-haem Fe bioavailability promoter, ascorbic acid (i.e. vitamin C), can also reduce the negative effects of certain antinutrients, such as phytate, on non-haem Fe bioavailability to humans. For example, Fig. 4 shows the results of a study where eating collard greens, rich in ascorbic acid, ameliorated the negative effects of phytate on Fe absorption in humans (Tuntawiroon *et al*., 1990). Increasing ascorbate levels to 50 mg in the composite rice meals overcame the negative effects of 175 mg of phytate in the meals on Fe bioavailability (as determined by 'whole body' radioassay and by total retention of radio-iron in red blood cells).

The bioavailability conundrum

Anomalies in some research, directed at the study of various dietary factors on micronutrient bioavailability, suggest that some aspects of the current dogma concerning the mechanisms of action of some antinutrients in plant foods on Fe and Zn bioavailability are not fully understood. Under some experimental conditions, antinutrients, such as phytic acid, do not have large negative effects on Fe and Zn bioavailability in human subjects. The reasons for these anomalous findings are unknown. For example, Table 5 lists some data reported by Morris and Ellis (1982) from a study with humans fed either low or high phytate muffin diets. As expected, the subjects fed the dephytinized muffins remained in positive Fe balance for the entire period they were fed the dephytinized muffins. Interestingly, subjects fed the high phytate diet were in negative Fe balance (as expected) during the first 5 d, but, by the tenth day of the study, these same subjects demonstrated positive Fe balances (which was not expected) suggesting that there must have been some adaption to the high phytate meals in these test subjects. Others have reported similar results from balance studies to those of Morris and Ellis (Apte and Venkatachalam, 1962).

Another example can be found in a recent human study on soybean Fe bioavailability. Historically, the Fe in soybean seeds has been thought to be a poor source of Fe because of the high levels of phytic acid accumulated in the mature soybean seed. Interestingly, recent research reported by Murray-Kolb *et al*. (2003) demonstrates that this long-held dogma concerning the bioavailability of Fe in soybean seeds is erroneous, because their findings demonstrate that the bioavailability of Fe in soybean seeds is not always low. They grew soybeans in ^{55}Fe radio-labelled nutrient solutions and prepared soy soup and soy muffin meals from the intrinsically labelled seeds. The seeds were fed in a single repast to non-anaemic (blood haemoglobin's averaging 131 g l^{-1}) human subjects previously selected to have low-Fe stores (plasma ferritin averaging 11.2 μg l^{-1}). The mature soybeans contained very high phytic acid concentrations (mean=2.1% dry weight) which should have greatly inhibited Fe bioavailability according to previous studies. The average % absorption (±SE) of Fe from the soy soup (calculated from radio-iron incorporated into red blood cells 14 d after

Table 5. *Apparent iron absorption in men fed whole bran muffins or dephytinized bran muffins; data from Morris and Ellis (1982)*

	Whole bran muffin		Dephytinized bran muffin	
	5 d (Fe, mg d^{-1})[a]	10 d (Fe, mg d^{-1})[a]	5 d (Fe, mg d^{-1})[a]	10 d (Fe, mg d^{-1})[a]
Subjects 1–5[b]	–3.0±1.1	2.2±0.4	1.3±2.5	2.8±1.1
Subjects 6–10[b]	–0.3±2.5	3.5±0.9	0.0±1.6	1.1±1.8
All subjects	–1.6±1.6	2.9±0.7	0.7±1.6	2.0±1.1

[a] Intake minus fecal excretion, means ±SD, 10 subjects total.
[b] Subjects 1–5 consumed whole bran muffins for the first 15 d then consumed dephytinized bran muffins. Subjects 6–10 consumed the muffins in the reverse sequence.

feeding) was 25±1.8% (22 subjects) and from the soy muffins 30±1.5% (18 subjects). These absorption values are very high, approaching those values reported for Fe absorption by humans fed highly bioavailable haem-Fe sources (i.e. the majority of Fe found in animal meats), which average about 47% absorption in Fe-depleted humans (Viteri, 1998). These findings contrast dramatically from some earlier studies, that reported low bioavailable Fe to humans fed soybean meals (Benito and Miller, 1998; International Nutritional Anemia Consultative Group, 1982; Sayers *et al*., 1973; and references cited therein), and certainly brings into question the previous wisdom concerning the bioavailability of Fe in soybeans. Apparently, there are unknown factors controlling the bioavailability of Fe from soybeans and further research is needed to delineate what these factors are and their mechanisms of action.

One possible explanation for these contradictory results may be because the Tokyo variety of soybean, used in the Murray-Kolb *et al*. (2003) study, contained high levels of Fe in the form of phytoferritin. Possibly, this protein-bound Fe was responsible for the highly bioavailable Fe in the soybeans, but further research is required to be certain, because not all of the Fe in the Tokyo soybeans was in the phytoferritin form (i.e. about 50% of the total Fe in the soybean seed was in the phytoferritin form).

Figure 5 also presents some recent findings reported by Manary *et al*. (2000) that cannot be explained using the current dogma concerning phytate action on Zn bioavailability in the human gut (i.e. precipitation of Zn as a Zn–phytate complex in the lumen of the small intestine making it unavailable for absorption). They studied the effects of phytate on Zn homeostasis in two groups of children, i.e. those recovering from tuberculosis, or those that were well, but in the hospital for elective surgery and other treatments (Manary *et al*., 2000). As expected, in the recovering children fed meals high in phytate, fractional Zn absorption, total Zn absorption, and net Zn absorption were significantly reduced while endogenous fecal Zn decreased. These findings are in agreement with current phytate dogma concerning Zn–phytate interactions. However, in the well children, feeding a high phytate diet had no affect on fractional Zn absorption, while total

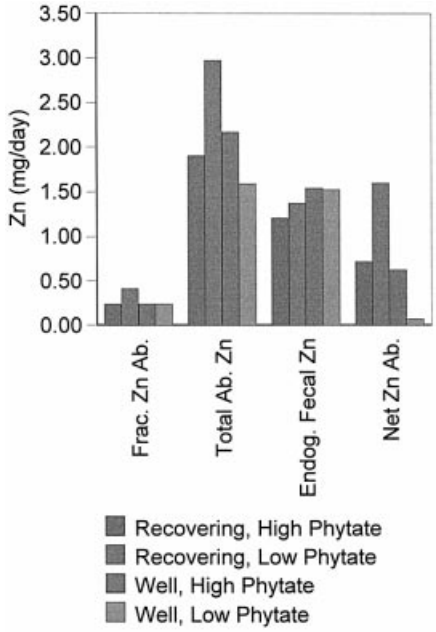

Fig. 5. Effects of dietary phytate reduction (via phytase treatment of corn and soy flours) on Zn bioavailability to either Malawian children recovering from tuberculosis, or in well children fed a corn, soy porridge meal served five times a day. Frac. Zn Ab., fractional absorption of Zn; Total Ab. Zn, total absorbed Zn; Endog. Fecal Zn, endogenous fecal Zn; Net Zn Ab., net absorbed Zn. Oral and intravenous doses of stable isotopes of Zn were used to determine Zn homeostasis values from analyses of urine and stool samples. Data from Manary *et al*. (2000).

Zn absorption and net Zn absorption were higher in the well children fed the high phytate diet compared with the low phytate diet. These finding cannot be explained by current phytate dogma. A major difference between the recovering children and the well children was the fact that the recovering children had received four potent antibiotics for over 60 d while the well children received none. This suggests that the activity of microorganisms in the gut may have a large influence on the effects of phytate in meals on Zn bioavailability. Possibly, certain microorganisms in the gut may have active phytases that hydrolyse phytate, making it inactive towards Zn absorption from the gut. If this is found to be the case, the composition of the diet and how it affects the microorganism population in the gut may

be an important factor in determining the effects of phytate on Fe and Zn bioavailability. Further studies are needed to clarify this possibility.

Conclusions

There are ample compelling global human health and nutritional reasons to encourage plant breeders to pursue improving the micronutrient density of staple food crops as a primary objective in their work targeted for the developing world. Furthermore, accomplishing this goal would improve crop productivity when micronutrient-dense seeds and grains are planted to micronutrient-poor soils, thus assuring farmer adoption of the micronutrient-enriched seeds once they are developed. Current evidence strongly supports the contention that there is enough genetic diversity within the genomes of staple plant foods (with the possible exception of maize) to accomplish this task. Succeeding in doing this would dramatically contribute to improving the health, livelihood, and felicity of numerous resource-poor, micronutrient-deficient people in many developing countries, and would contribute greatly to sustaining national development efforts in these countries. Importantly, finding sustainable solutions to micronutrient malnutrition will not be forthcoming in the foreseeable future if we do not start to adopt agriculturally based tools, such as plant breeding, to attack this important global crisis in human health and well being.

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