Published March, 2010

SYMPOSIA

Biofortification—A Sustainable Agricultural Strategy for Reducing Micronutrient Malnutrition in the Global South

Howarth E. Bouis and Ross M. Welch*

ABSTRACT

Minerals and vitamins in food staples eaten widely by the poor may be increased either through conventional plant breeding or through use of transgenic techniques, a process known as *biofortification*. HarvestPlus seeks to develop and distribute cultivars of food staples (rice [*Oryza sativa* L.], wheat [*Triticum aestivum* L.], maize [*Zea mays* L.], cassava [*Manihot esculenta* Crantz], pearl millet [*Pennisetum americanum* Leeke], beans [*Phaseolus vulgaris* L.], sweet potato [*Ipomoea batatas* L.]) that are high in Fe, Zn, and provitamin A through an interdisciplinary global alliance of scientific institutions and implementing agencies in developing and developed countries. Biofortified crops offer a rural-based intervention that, by design, initially reaches these more remote populations, which comprise a majority of the undernourished in many countries, and then penetrates to urban populations as production surpluses are marketed. Thus, biofortification complements fortification and supplementation programs, which work best in centralized urban areas and then reach into rural areas with good infrastructure. Initial investments in agricultural research at a central location can generate high recurrent benefits at low cost as adapted biofortified cultivars become widely available in countries across time at low recurrent costs. Overall, three things must happen for biofortification to be successful. First, the breeding must be successful—high nutrient density must be combined with high yields and high profitability. Second, efficacy must be demonstrated—the micronutrient status of human subjects must be shown to improve when consuming the biofortified cultivars as normally eaten. Third, the biofortified crops must be adopted by farmers and consumed by those suffering from micronutrient malnutrition in significant numbers.

H.E. Bouis, HarvestPlus, c/o International Food Policy Research Institute, 2033 K St. NW, Washington, DC 20006; R.M. Welch, USDA-ARS, Robert W. Holley Center for Agriculture and Health, Tower Rd., Ithaca, NY 14853-2901. Received 23 Sept. 2009. *Corresponding author (rmw1@cornell.edu).

Abbreviations: CGIAR, Consultative Group on International Agricultural Research; CIAT, International Center for Tropical Agriculture; IBM, intermated B73 × Mol7; IFPRI, International Food Policy Research Institute; LPS, lipopolysaccharides; NILs, near-isogenic lines; PAC, Program Advisory Committee; QTL, quantitative trait loci; RI, recombinant inbred.

Globally, malnutrition, including both overt nutrient deficiencies as well as diet-related chronic diseases (e.g., heart disease, cancer, stroke, and diabetes), is responsible for more deaths than any other cause, accounting for >20 million mortalities annually (Kennedy et al., 2003; WHO and FAO, 2003). Malnutrition also contributes to increased morbidity, disability, stunted mental and physical growth, and reduced national socioeconomic development (WHO and FAO, 2003). Micronutrient malnutrition alone afflicts more than two billion people, mostly among resource-poor families in developing countries, with Fe, I, Zn, and vitamin A deficiencies most prevalent (Kennedy et al., 2003). More than five million childhood deaths occur from micronutrient malnutrition every year (Anonymous, 2007). Leading global economists have identified investing in strategies to reduce malnutrition as the most cost-effective investments governments can make (Anonymous, 2008).

What causes malnutrition? Dysfunctional food systems that cannot supply all the nutrients and health-promoting factors

Published in Crop Sci. 50:S-20–S-32 (2010).
doi: 10.2135/cropsci2009.09.0531
Published online 22 Jan. 2010.

required for human life in sustainable ways are responsible. However, food systems that feed the disadvantaged are very complex (Sobal et al., 1998). Therefore, dysfunctions in numerous interacting factors can result in inadequate supplies of nutrients reaching the most vulnerable populations (World Bank, 2007). Importantly, because food systems are dependent on agricultural products as their source of most nutrients, agricultural systems must be contributing to this worldwide quandary in public health (Welch, 2001).

Unfortunately, agricultural systems have never been explicitly designed to promote human health and, instead, mostly focus on increased profitability for farmers and agricultural industries. Agriculture met the challenge of feeding the world's poor during the "Green Revolution," focusing primarily on three staple crops—rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), and maize (*Zea mays* L.). These crops provided enough energy to prevent widespread famines in many developing nations. An unforeseen consequence of that agricultural revolution was the rapid rise in micronutrient malnutrition in many nations that adopted the cropping systems that prevented large-scale starvation (Welch and Graham, 1999). Agriculture must now formulate new policies that not only provide enough calories to meet the energy needs of the poor but also deliver all the essential nutrients needed for adequate nutritional health.

Sustainable solutions to malnutrition will only be found by closely linking agriculture to nutrition and health and by formulating agriculture, nutrition, and health policies to reflect this need (Graham et al., 2007; Hawkes and Ruel, 2006; Rouse and Davis, 2004; World Bank, 2007). It is shortsighted if the world once again focuses only on delivering the energy needs of resource-poor people during the current food crisis (Casey and Lugar, 2008; Zarocostas, 2009) without also giving those affected the crops and other agricultural products needed for adequate nutrition required for healthy and productive lives.

Humans require at least 44 known nutrients in adequate amounts and consistently to live healthy and productive lives (Table 1). Many agricultural tools (e.g., diversification, crop selection, fertilizers, cropping systems, soil amendments, small livestock production, aquaculture, etc.) could be used to increase the nutrient output of farming systems (Graham et al., 2007). Biofortification (developing food crops that fortify themselves) is the first agricultural tool now being employed to address micronutrient malnutrition worldwide. Conventional breeding has been the primary focus of programs to enhance staple food crops with sufficient levels of Fe, Zn, and provitamin A carotenoids to meet the needs of at-risk populations in the Global South (Hotz et al., 2007; White and Broadley, 2009).

The biofortification strategy is a feasible means of reaching rural families that only have limited access to markets and healthcare facilities needed to provide fortified foods and nutritional supplements because it is targeted at this population. Once implemented, biofortification will lower the number of micronutrient-deficient people requiring interventions dependent on supplementation and fortification programs (see Fig. 1). Thus, biofortification complements other interventions and is a means to provide micronutrients to the most vulnerable people in a comparatively inexpensive and cost-effective way, using an agricultural intervention that is sustainable (Bouis, 1999; Nestel et al., 2006; Pfeiffer and McClafferty, 2007; Qaim et al., 2007).

HarvestPlus is the CGIAR's Biofortification Challenge program. It is directed at using plant breeding as an intervention strategy to address micronutrient malnutrition by producing staple food crops with enhanced levels of bioavailable essential minerals and vitamins that will have measurable impact on improving the micronutrient status of target populations, primarily resource-poor people in the developing world. Impressive progress has been made at meeting the goals of the HarvestPlus program set forth at its inception in 2003, but much remains to be done (Bouis et al., 2009).

Three primary issues have been identified that are required to make biofortification successful: (i) a biofortified crop must be high yielding and profitable to the farmer, (ii) the biofortified crop must be shown to be efficacious and effective at reducing micronutrient malnutrition in humans, and (iii) the biofortified crop must be acceptable to both farmers and consumers in target regions where people are afflicted with micronutrient malnutrition. The HarvestPlus program has addressed all of these issues (Hotz et al., 2007). This program has been able to assemble a multi-CGIAR Centers team along with collaborators from numerous universities, nongovernmental organizations, in-country agencies, and international institutions comprising plant scientists, plant breeders, food scientists, nutritionists, economists, and communication and behavioral specialists to tackle these issues. The program model developed by HarvestPlus has been successful in developing transdisciplinary team-research programs among CGIAR Centers and across diverse disciplines (see Web site at http://www.harvestplus.org/ [verified 22 Dec. 2009]).

CONVENTIONAL BREEDING TO BIOFORTIFY STAPLE FOOD CROPS

The task of plant breeders attempting to biofortify staple food crops is to increase the micronutrient level in the edible product of a staple food crop to have measurable impact on improving the nutritional health of individuals at high risk of developing micronutrient malnutrition. For this to be accomplished, plant breeders must work closely with food scientists and nutritionists to develop target micronutrient levels for their breeding programs. Considerations must include not only micronutrient

Table 1. The known essential nutrients for human life[†].

Air, water, and energy	Protein (amino acids)	Lipids–Fat (fatty acids)	Macrominerals	Essential trace elements	Vitamins
Oxygen	Histidine	Linoleic acid	Na	Fe	A (retinol)
Water	Isoleucine	Linolenic acid	K	Zn	D (calciferol)
Carbohydrates	Leucine		Ca	Cu	E (α-tocopherol)
	Lysine		Mg	Mn	K (phylloquinone)
	Methionine		S	I	C (ascorbic acid)
	Phenylalanine		P	F	B_1 (thiamin)
	Threonine		Cl	Se	B_2 (riboflavin)
	Tryptophan			Mo	B_3 (niacin)
	Valine			Co (in B_{12})	B_5 (pantothenic acid)
				B	B_6 (pyroxidine)
					B_7 (biotin)
					B_9 (folic acid, folacin)
					B_{12} (cobalamin)

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

concentrations in the edible portions of crops, but also the amount of the nutrient that can be absorbed by the consumer, after processing and cooking, when eaten in a traditional diet for the target population. This can be a difficult task. Numerous genes may be involved in controlling the amount of a mineral element that is absorbed by roots, translocated to shoots, remobilized from vegetative tissues, and deposited in edible portions of seeds and grains in forms that are utilizable in the person eating the crop (Welch, 1986, 1995). Further, environmental factors and cultural practices (e.g., edaphic, climatic, agronomic, etc.) can interact with plant-gene expression to influence the amount of a micronutrient accumulated in a seed or storage organ. Additionally, various dietary factors can interact to determine how much of a micronutrient can be absorbed and utilized by people eating the biofortified staple plant food (i.e., the bioavailable amount) (Hotz et al., 2007; Ortiz-Monasterio et al., 2007; Welch, 2001).

The HarvestPlus program has set needed levels for Fe, Zn, and provitamin A carotenoids in target crops after addressing these issues. Table 2 list these target levels and assumptions used to set levels for target populations in the developing world (Bouis et al., 2009). These target levels are very conservative estimates and are estimates and will be changed if deemed necessary as new data and information merits adjustment. Figure 2 and Table 3 summarize the progress being made in the HarvestPlus program to develop biofortified crops. Once high-yielding biofortified crop cultivars are developed that meet target nutrient levels, they will be disseminated widely.

HarvestPlus will disseminate the biofortified seeds through established partnerships with country agencies for delivering biofortified seeds to farmers and, ultimately, to the consumer. The HarvestPlus program will do this in several stages. First, national agricultural research and extension programs will multiply the seeds and test the biofortified lines at multiple locations in trials throughout the target country to determine environmental × genetic interactions on expression of the high-micronutrient traits in the biofortified crops. Selected promising lines from these

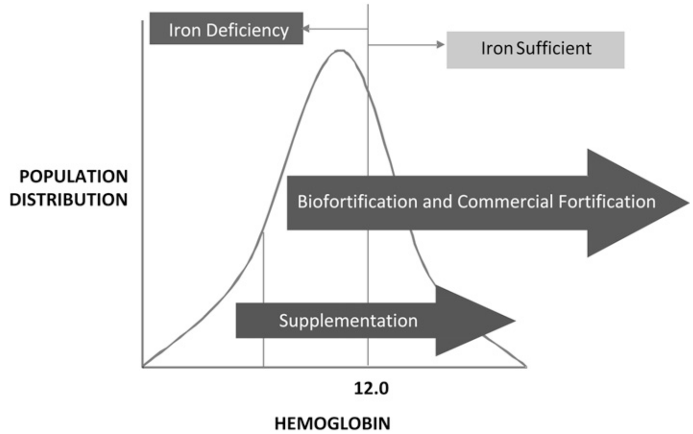

Figure 1. Frequency distribution of Fe adequacy in a population. Biofortification improves status for those less deficient and maintains status for all at low cost. Iron adequacy for a population is indicated as 12.0 mg dL^{-1} on the plot. Biofortification will shift the population into a more Fe-sufficient range.

trials will be formally submitted to the Varietal Release Committees for further testing and, once approved, will be officially released within the target country. This process may take up to 8 yr to complete. Once implemented, baseline nutritional studies will be compared to postdissemination impact and effectiveness studies in both control and intervention locations to establish if biofortified crops can improve the micronutrient status of people in target populations. To facilitate seed dissemination, market chain analysis, production capacity for seed increases, consumer acceptance studies, and development of a favorable policy environment for the production of biofortified crops will also be required for successful and sustainable implementation of the biofortification strategy.

USING FERTILIZERS TO ENHANCE MICRONUTRIENT ELEMENTS IN STAPLE FOOD CROPS

Both macronutrient fertilizers containing N, P, K, and S, and certain micronutrient fertilizers (e.g., Zn, Ni, I, Co, Mo, and Se) can have significant effects on the

Table 2. Information and assumptions used to set target levels for micronutrient content of biofortified staple food crops.

Amount eaten or nutrient	Criteria	Rice (polished)	Wheat (whole)	Pearl millet (whole)	Beans (whole)	Maize (whole)	Cassava (fresh wt.)	Sweet potato (fresh wt.)
Per capita consumption	Adult women (g/d)	400	400	300	200	400	400	200
	Children 4–6 yr (g/d)	200	200	150	100	200	200	100
Fe	% of EAR† to achieve				~30			
	EAR, nonpregnant, nonlactating women (µg/day)				1460			
	EAR, children 4–6 yr (µg/d)				500			
	Micronutrient retention after processing (%)	90	90	90	85	90	90	90
	Bioavailability (%)	10	5	5	5	5	10	10
	Baseline micronutrient content (µg/g)	2	30	47	50	30	4	6
	Additional content required (µg/g)	11	22	30	44	22	11	22
	Final target content (µg/g)	13	52	77	94	52	15	28
	Final target content as dry wt. (µg/g)	15	59	88	107	60	45	85
Zn	% of EAR to achieve				~40			
	EAR, nonpregnant, nonlactating women (µg/d)				1860			
	EAR, children 4–6 yr of age (µg/d)				830			
	Micronutrient retention after processing (%)	90	90	90	90	90	90	90
	Bioavailability (%)	25	25	25	25	25	25	25
	Baseline micronutrient content (µg/g)	16	25	47	32	25	4	6
	Additional content required (µg/g)	8	8	11	17	8	8	17
	Final target content (µg/g)	24	33	58	49	33	12	23
	Final target content as dry wt. (µg/g)	28	38	66	56	38	34	70
Provitamin A	% of EAR to achieve				~50			
	EAR, nonpregnant, nonlactating women (µg/d)				500			
	EAR, children 4–6 yr of age (µg/d)				275			
	Micronutrient retention after processing	50	50	50	50	50	50	50
	Bioavailability ratio (µg:RE‡)	12:1	12:1	12:1	12:1	12:1	12:1	12:1
	Baseline micronutrient content (µg/g)	0	0	0	0	0	1	2
	Additional content required (µg/g)	15	15	20	30	15	15	30
	Final target content (µg/g)	15	15	20	30	15	16	32
	Final target content as dry wt. (µg/g)	17	17	23	34	17	48	91

† EAR, estimated average requirement.

‡ RE, retinyl esters.

accumulation of nutrients in edible plant products (Allaway, 1986; Grunes and Allaway, 1985). Other micronutrient fertilizers have very little effect on the amount of the micronutrient accumulated in edible seeds and grains when they are applied to soils or when used as foliar sprays (Welch, 1986). This is especially true for those micronutrient elements with limited phloem sap mobility such as Fe (Welch, 1999). Some examples of the effects of fertilizer practices on the micronutrient concentrations in edible plant parts are given below. For more detailed information concerning the effects of fertilization practices on micronutrient accumulation in plant foods, refer to R.M. Welch's "Importance of seed mineral nutrient reserves in crop growth and development" (Welch, 2001).

For certain essential micronutrient elements (e.g., Zn, Ni, I, and Se), increasing soil-available supply to food crops can result in significant increases in their concentrations in edible plant products (Graham et al., 2007; Welch, 1995). For example, increasing the supply of Zn to pea (*Pisum sativum* L.) plants at levels in excess of that required for maximum yield has been shown to increase the concentration of bioavailable Zn in seeds (Peck et al., 1980; Welch et al., 1974). Furthermore, increasing the supply of Zn and Se to wheat improved the amount of bioavailable Zn and Se in wheat grain (Cakmak, 2008; Haug et al., 2008; House and Welch, 1989). Increasing Zn levels via Zn fertilization has also been shown for navy beans (*Phaseolus vulgaris* L.), as well as other crops (Moraghan, 1980; Welch, 1986). For Fe, providing more to plants than required for maximum yield does little to further increase the Fe in edible seeds and grains. Interestingly, the micronutrient I, supplied in irrigation water, can greatly increase the levels of I in edible portions of food crops, alleviating the debilitating disease cretinism, as well as other I-deficiency disorders in populations dependent on irrigated food crops grown on low-I soils (Cao et al., 1994; Ren et al., 2008). In Finland,

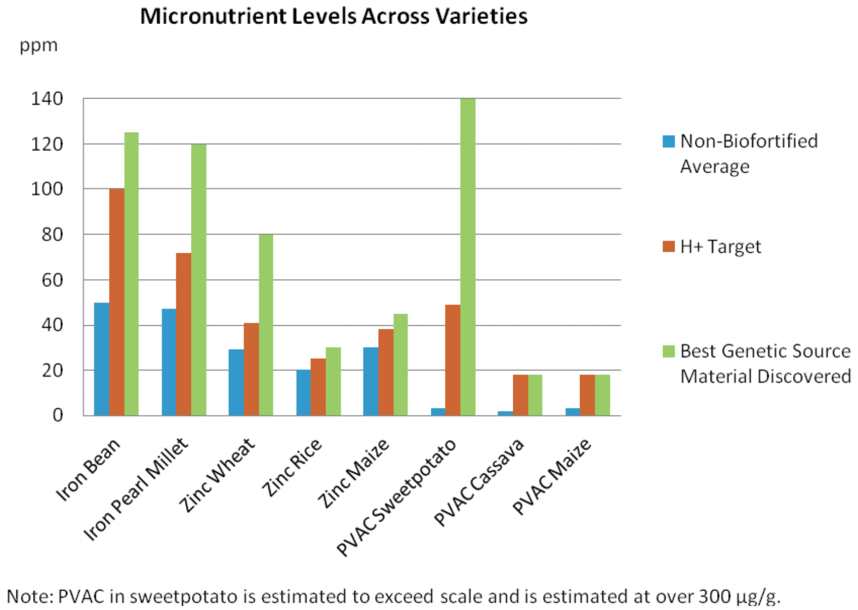

Figure 2. Micronutrient content of staple crops, across varieties from HarvestPlus screening activities. PVAC = provitamin A carotenoids.

Table 3. Breeding progress as of 2007–2008 (iron, zinc, provitamin A expressed as percent of breeding target in lines at indicated stage of breeding).

	Screening	Crop improvement			G × E[†] testing	Launch
Crop	Screening gene/ trait identification validation	Early development parent building	Intermediate product development	Final product development	Performance G × E testing in target countries	Release prelaunch seed multiplication
Sweet potato			NARS[‡] Uganda Program		Introduction	NARS Uganda
Breeding	Provitamin A	100% target	100%	100%	100%	100%
Fast-track	Uganda, Mozambique				100%	100%
Maize						
Breeding	Provitamin A	100% target	60%	50%	NA[§]	
Cassava						
Breeding	Provitamin A	100% target	>75%	>75%	50%	≥30%
Fast-track	Democratic Republic of Congo					NA
Bean						
Breeding	Fe	100% target	60%	40–50%	40–50%	
Fast-track	Rwanda					40–50%
Rice, polished						
Breeding	Zn	100% target	100%	75–100%	75–100%	≥30%
Wheat						
Breeding	Zn	100% target	100%	≥30%	≥30%	
Pearl millet						
Breeding	Fe	100% target	100%	75–100%	50–75%	

[†]G × E, genotype × environment interaction.

[‡]NARS, National Agricultural Research Systems.

[§]NA, not applicable.

Se added to fertilizers and applied to soils increased the Se status of the entire Finnish population (Mäkelä et al., 1993). Thus, fertilizers can be used as an effective agricultural tool to improve the nutritional health of people in the developing world. Graham et al. (2007) discuss such food system strategies in detail.

THE BIOAVAILABILITY ISSUE

Increasing the concentrations of micronutrients in staple food crops is only the first step in making these foods richer sources of these nutrients for humans. As stated previously, this is because not all of the micronutrients in plant foods are bioavailable to humans who eat these foods. Plant foods can contain substances (i.e., antinutrients) that interfere with the absorption or utilization of these nutrients in humans (Welch and Graham, 1999). In general, staple food crop seeds and grains contain very low bioavailable levels of Fe and Zn (i.e., about 5% of the total Fe and about 25% of the total Zn present in the seed is thought to be bioavailable). Increasing the bioavailable amounts of Fe from 5 to 20% would be equivalent to increasing the total Fe by fourfold. Using conventional breeding, it should be genetically much easier to greatly improve the bioavailability of Fe and Zn compared with increasing their total content by this magnitude. Antinutrients that depress Fe and Zn bioavailability (such as phytate and certain polyphenolics) or promoter substances (such as ferritin) have fewer genes involved in their biosynthesis and metabolism compared with the uptake, transport, and deposition of Fe and Zn in edible seeds and grains (e.g., >4000 genes have been shown to be up-regulated or down-regulated in controlling Fe homeostasis in higher plants). The fewer the genes needed to breed for makes the job of breeding for the trait easier.

Determining the bioavailability of micronutrients in plant foods to humans is pervaded with numerous complexities. A myriad of factors interact to ultimately determine the bioavailability of a particular micronutrient to an individual eating a mixed diet within a given environment (Fairweather-Tait and Hurrell, 1996; Graham et al., 2001; House, 1999; Van Campen and Glahn, 1999). Because of this complexity, the data obtained using various bioavailability model systems are always equivocal and dependent on the experimental design used to obtain the data. Only data obtained on reducing the prevalence of micronutrient deficiencies among those afflicted using feeding trials in test populations under free-living conditions can delineate the actual effectiveness of using micronutrient-enriched cultivars of plant foods as an intervention tool. However, 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 programs (Graham and Welch, 1996; Graham et al., 2001). Thus, model bioavailability systems must be used for crop screening purposes but ultimately tested in target populations.

IDENTIFYING MOLECULAR MARKERS IN CEREAL CROPS TO ENHANCE BIOAVAILABLE IRON CROPS

An integrated genetic, physiological, and biochemical strategy can be used to identify molecular markers for improving Fe bioavailability in cereal crops. The intermated B73 × Mo17 (IBM) recombinant inbred (RI) maize population can be employed to identify these markers (Lee et al., 2002). The RI populations are maintained mapping populations, developed for plant breeders. The maize IBM population is a valuable resource for the analysis of quantitative traits and is the maize breeders' community standard for genetic mapping, as it has a large number of members (302), extensive recombination, and an extensive number of molecular genetic markers (Falque et al., 2005; Sharopova et al., 2002). B73, a parent from the IBM mapping population, was also used in the Maize Genome Sequencing Project and this facilitates molecular genetic analyses. Scientists at the USDA-ARS Robert W. Holley Center for Agriculture and Health at Cornell University in Ithaca, NY, collected a data set using RIs to find genetic links to improving Fe bioavailability from mature maize kernels using an in vitro Caco-2 cell model. These data were then analyzed using single-marker analysis to identify quantitative trait loci (QTL) that regulate this trait.

The Caco-2 cell line bioassay identified genetic loci in this breeding population associated with increased Fe bioavailability. The identified loci were on six chromosomes and explained 54% of the variance observed in RIs from a single year–location. Three of the largest Fe bioavailability QTL were successfully isolated in near-isogenic lines (NILs). The NILs are lines that are >90% genetically identical to each other; yet this population contained significant differences in the levels of kernel-Fe bioavailability. The NILs were grown 3 yr after the initial RI population used in the first Caco-2 cell screening experiment. These findings confirm the identification of the QTL from the first screening. This is the first genetic analysis for seed-Fe bioavailability and an excellent example for Fe biofortification in a staple food crop. The magnitude of improvement in Fe bioavailability observed in the NILs was comparable to that reported for the highest transgenic events (Drakakaki et al., 2005). This preliminary study was a proof-of-concept study showing the power of using genetic tools to determine which factors in plant foods impact bioavailable Fe from staple food crops. This breeding strategy shows great promise as a tool for plant breeders in the future. However, animal models–human trials should be conducted to substantiate these Caco-2 cell model findings before attempting to breed biofortified maize crops using the identified markers. Preliminary data comparing Caco-2 cell model data with data from a poultry model Fe bioavailability study using high- and low-bioavailable maize kernel recombinant inbred lines is very encouraging (see Fig. 3).

INHIBITOR AND PROMOTER SUBSTANCES

Plant foods (especially staple seeds and grains) contain various antinutrients (Table 4) in differing amounts, depending on both genetic and environmental factors that can reduce the bioavailability of dietary nonheme Fe, Zn, and other micronutrients to humans (Welch, 2001; Welch and House, 1984). Dietary substances that promote/enhance the bioavailability of micronutrients in the presence of antinutrients are also known whose levels are controlled by genes but also influenced by environmental factors (Table 5). Current plant molecular, biological, and genetic modifications, combined with plant breeding approaches, now make it possible to reduce or eliminate

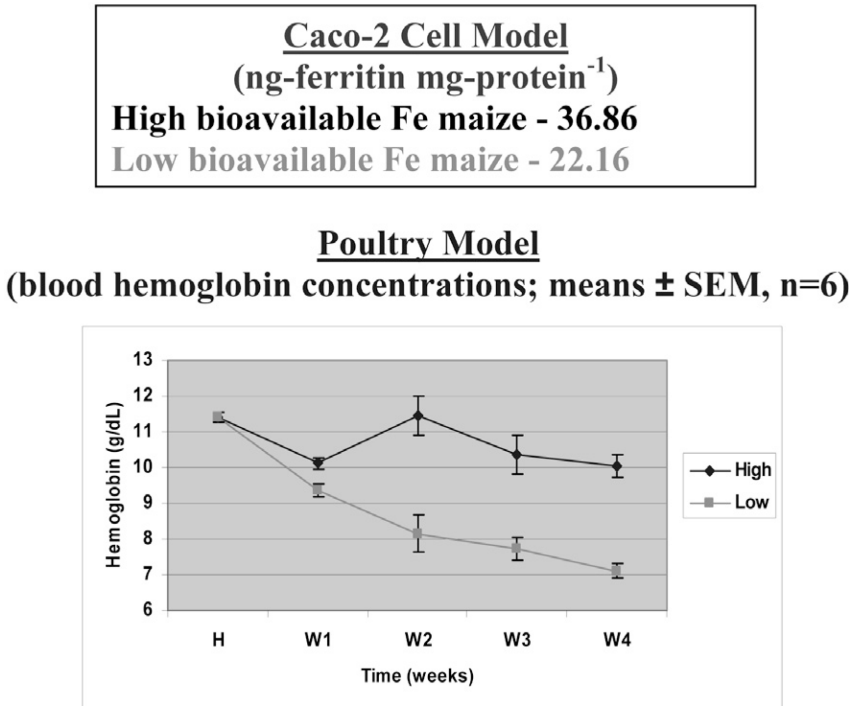

Figure 3. Bioavailable Fe in maize kernels from two recombinant inbred lines (RILs) of maize determined using either the in vitro Caco-2 cell model (cell ferritin level was used as a proxy for Fe bioavailability) or using a poultry model and blood hemoglobin as a measure of Fe bioavailability (R.P. Glahn and E. Tako, unpublished data, 2009). Total dietary Fe and kernel Fe levels were about equal for both high- and low-Fe-bioavailability maize RILs used in the poultry model.

Table 4. Examples of antinutrients in plant foods that reduce the bioavailability of essential trace elements and examples of major dietary sources (modified from Graham et al., 2001).

Antinutrients	Essential micronutrient metal inhibited	Major dietary sources
Phytic acid or phytin	Fe, Zn, Cu, Ni	Whole legume seeds and cereal grains
Certain fibers (e.g., cellulose, hemicellulose, lignin, cutin, suberin)	Fe, Zn, Cu	Whole cereal grain products (e.g., wheat, rice, maize, oat, barley [*Hordeum vulgare* L.], rye [*Secale cereale* L.])
Certain tannins and other polyphenolics	Fe	Tea [*Camellia sinensis* (L.) Kuntze], coffee (*Coffea arabica* L.), beans, sorghum [*Sorghum bicolor* (L.) Moench]
Hemagglutinins (e.g., lectins)	Fe	Most legumes and wheat
Goitrogens	I	*Brassica*s and *Allium*s
Heavy metals (e.g., Cd, Hg, Pb)	Fe, Zn	Contaminated leafy vegetables and roots

antinutrients from staple plant foods, or to significantly increase the levels of promoter substances in these foods (Becker and Frei, 2004; Forssard et al., 2000; Genc et al., 2005; King, 2002; Theil et al., 1997; Welch, 2002; White and Broadley, 2009). Given these options (i.e., to decrease antinutrients or to 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 abiotic stress resistance, and in plant resistance to crop pests or pathogens (Graham et al., 2001). Additionally, 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 (Anonymous, 1996; Saied and Shamsuddin, 1998; Shamsuddin, 1999; Zhou and Erdman, 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).

Some promoter compounds are normal plant metabolites. Only a few genes control their levels in plants and only small changes in their concentration may have significant effects on the bioavailability of micronutrients. Thus, breeding for increased levels of these promoters should be relatively easy compared with breeding for higher levels

Table 5. Examples of substances in foods reported to promote Fe and Zn bioavailability and examples of major dietary sources (modified from Graham et al., 2001).

Substance	Trace element	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)	Fe and/or Zn	Animal meats
Long-chain fatty acids (e.g., palmitate)	Zn	Human breast milk
Se	I	Seafoods, tropical nuts
β-carotene	Fe	Green and orange vegetables
Inulin and other nondigestible carbohydrates (prebiotics)	Fe, Zn	Chicory (*Cichorium intybus* L.), garlic (*Allium sativum* L.), onion (*Allium cepa* L.), wheat, Jerusalem artichoke (*Helianthus tuberosus* L.)

of Fe and Zn, which involves numerous genes and their interactions with the environment. 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., 2007; Welch and Graham, 1999, 2004).

PREBIOTICS AS PROMOTERS OF MICRONUTRIENTS

Which known plant food promoter substances should be targeted for increasing in staple plant foods through biofortification to improve Fe and Zn bioavailability? Unfortunately, there is a dearth of knowledge concerning Fe and Zn promoters in staple plant foods. The well-known Fe promoter and antioxidant ascorbate could be increased in staples, although it is not stable because it can be oxidized to dehydroascorbate during storage, food preparation, and cooking, losing its promoter properties (Combs, 2008). Thus, ascorbate may not be a good target promoter for plant breeding. The amino acid cysteine is also known to promote Fe and Zn bioavailability. Breeding for higher levels of cysteine-rich peptides and proteins could be achieved (Lucca et al., 2001; White and Broadley, 2009). However, cysteine also is prone to oxidation to the disulfide cystine during processing and cooking, potentially losing its promotion properties by oxidation of its metal-binding sulfhydryl functional group. The Fe stored as phytoferritin (a 450,000-Da protein) in seeds is a bioavailable source of Fe in staple food crops. It protects up to 4500 ferric-Fe atoms stored in its Fe cage from binding to antinutrients such as phytate (Lonnerdal, 2009). Breeding for enhanced levels of phytoferritin in staple food crops appears to be a viable strategy if genetic engineering approaches are used (Lucca et al., 2006; White and Broadley, 2009), although the genetic diversity in seed-phytoferritin accumulation in the genomes of the major staple food crop seeds is not known. If enough genetic diversity existed for this trait in these genomes, then conventional breeding could be used to increase phytoferritin in these crops.

One very promising area related to improving the bioavailability of Fe and other micronutrients in staple food crops is the role of nondigestible carbohydrates as enhancers of micronutrient bioavailability. Within the past decade, numerous studies have reported promoter effects of various nondigestible carbohydrates on Ca, Mg, Fe, Cu, and Zn absorption in animal models and in humans, even when consumed in diets containing high amounts of antinutrients from staple food crops. Much of this research has focused on fructans, the fructo-oligosaccharides including inulin. The mode of action of fructans is the result of their promoting the growth of beneficial microbiota primarily within the caecum and colon, which has systemic effects on improving micronutrient absorption and utilization. These carbohydrates are classed as prebiotics—substances that significantly promote the growth of beneficial bacteria (i.e., probiotics) in the distal small intestine and the large intestine. Increases in probiotic bacteria in the intestine have been shown to have beneficial systemic effects on a number of metabolic pathways in the human body. Research into human gut microbiota and their effects on human nutrition and health is in its infancy. Yet, it is clear that the effect of our intestinal microbiota on our ability to utilize food, nutrients, and phytochemicals is immense (Dethlefsen et al., 2007; FAO and WHO, 2006; Manning and Gibson, 2004). With respect to Fe nutriture, probiotics may play a critical role in Fe absorption from the diet and this is discussed below.

THE HUMAN “SUPERORGANISM”—THE BODY, ITS MICROBES, AND THEIR ROLE IN IRON BIOAVAILABILITY

The human intestine contains more bacteria than the eukaryotic cells of the body (i.e., at least 10 trillion microbial cells compared with about one trillion body cells). The metabolic activity of these organisms is equal to that of the body’s vital organs and can account for 60% of the dry weight of feces (Steer et al., 2000). Studies have shown that host–microbe interactions are essential to normal mammalian physiology, including metabolic activity and immune homeostasis (Dethlefsen et al., 2007). Their activity provides energy from undigested food substrates, trains the immune system, prevents growth of pathogens, transforms certain nutrients and beneficial phytochemicals into utilizable substrates, synthesizes certain vitamins, defends against certain diseases, stimulates cell growth, prevents some allergies, improves mineral absorption, produces anti-inflammatory effects, and improves gut health in general.

Low-grade inflammation (i.e., systemic inflammation) can occur because of changes in the bacteria populations colonizing the intestine from certain dietary habits. For example, high fat intake has been reported to increase the proportion of gram-negative to gram-positive bacteria in the intestine (Cani et al., 2008). Gram-negative bacteria contain the endotoxin lipopolysaccharides (LPS) in their cell walls; gram-positive bacteria contain no LPS. Endotoxemia, resulting from intestinal epithelium exposure to cell-wall LPS from gram-negative bacteria, causes a cellular immune signaling cascade that results in the inflammatory response (Bensinger and Tontonoz, 2008; Schiffrin and Blum, 2002). Inflammation can lead to up-regulation of the genes encoding the biosynthesis of the Fe-regulation peptide hormone hepcidin. Injection of humans with LPS dramatically increased serum IL-6 and urinary hepcidin within 6 h and reduced serum Fe concentrations by 57% within 22 h (Kemna et al., 2005). Hepcidin is primarily produced in the liver. It is translocated to intestinal enterocytes where it suppresses the induction of Fe deficiency response genes in the apical and basal membranes of mucosal cells, lowering their ability to absorb and utilize Fe from the diet and to transfer Fe across their basolateral membrane into the blood. This can lead to the anemia of inflammation even when diets contain adequate levels of bioavailable Fe, as a host defense mechanism to inhibit the growth of infectious bacteria.

Changes in the bacterial profile of the gut to a higher gram-positive (e.g., Firmicutes bacteria) to a gram-negative bacteria (e.g., Proteobacteria) ratio has been shown to result in reduced inflammation and lower LPS levels in the intestine and an improvement in mucosal barrier function (Cani et al., 2008; Wang et al., 2006). Furthermore, prebiotics, such as fructans, stimulate the growth of beneficial gram-positive (probiotic) bacteria at the expense of gram-negative bacterial growth (Bouhnik et al., 2007; Salminen et al., 1998). Beneficial gram-positive bacteria, such as bifidobacteria, do not degrade intestinal mucous glycoproteins, which promote a healthier microvillus environment by reducing intestinal permeability to gram-negative bacteria. This results in less LPS entering the blood (Cani et al., 2007; Griffiths et al., 2004; Teitelbaum and Walker, 2002). Therefore, changes in the ratio of gram-positive to gram-negative bacteria in the intestine and their link to inflammation may provide an Occam's razor explanation for the effects of prebiotics on up-regulating the genes for Fe absorption by enterocytes in the intestine.

Changing the gut microbiota populations to more gram-positive bacteria may also have enhancing effects on Zn absorption, but little experimental evidence exists. Providing prebiotics may overcome the negative effects of antinutrients on Fe and Zn bioavailability because many bacteria in the gut can degrade antinutrients, such as phytate and polyphenols, releasing their bound metals (such as Fe and Zn) and allowing them to be absorbed by enterocytes lining the intestine. Probiotics' systemic effects on inducing the genes controlling the absorption of Fe and other metals from the intestine may enhance the bioavailability of these micronutrients. Of equal and possibly more importance is the role of prebiotics on improving gut health and the intestine's ability to absorb and utilize numerous nutrients, regulate the immune system, and protect against invasion by pathogenic organisms. Thus, increasing the levels of prebiotics in staple food crops is an extremely important strategy to enhance the nutrition and health of malnourished people everywhere, especially resource-poor families with poor gut health living in less sanitary environments.

DEVELOPMENT IMPACT

As briefly summarized above, reducing micronutrient malnutrition improves cognitive ability, reduces morbidity and mortality, and improves work productivity.

In an analysis of commercial fortification, Horton and Ross (2003) estimate that the present value of each annual case of Fe deficiency averted in South Asia is approximately US$20.2. Consider the value of 1 billion cases of Fe deficiency averted in 16–25 yr after a biofortification research and development project was initiated (100 million cases averted per year in South Asia). The nominal value of US$20 billion (1 billion cases × a value of US$20 per case) must be discounted because of the lags involved between the times that investments are made in biofortification and when benefits are realized. At a 3% discount rate the present value would be approximately US$10 billion, and at a 12% discount rate the present value would be approximately US$2 billion. This benefit is far higher than the cost of breeding, testing, and disseminating high-Fe and high-Zn cultivars of rice and wheat for South Asia (more than US$100 million in nominal costs).

ACHIEVABLE GOALS FOR THE SHORT- AND LONG-TERM

HarvestPlus's experience in the dissemination of biofortified crops is limited to orange sweet potato (*Ipomoea batatas* L.), which is very high in provitamin A. A published pilot study in Mozambique showed that (i) behavior can be changed among farmers by switching from production of white to orange cultivars, and change in consumption to orange cultivars by their families; and that (ii) vitamin A deficiency can be improved (Low et al., 2007). As a result, vitamin A deficiency among preschool children in treatment villages declined from 60 to 38%, while vitamin A deficiency remained constant in control villages. HarvestPlus is now concentrating on identifying activities and messages that will effect this same behavior change at the lowest cost possible.

The dissemination strategy for nutrients that are invisible (Fe and Zn) will piggyback on superior agronomic characteristics of the newly introduced cultivars. For example, high-Fe beans that are drought- and heat-tolerant are undergoing national release trials in Africa.

In developing detailed plans for delivery of biofortified crops and to achieve realistic goals for delivery during 2014 to 2019, HarvestPlus management realized that the number of crops being developed under HarvestPlus II would need to be reduced. Given progress to date, HarvestPlus can now anticipate release dates for the biofortified products (Table 6).

NEW MODALITIES FOR RESEARCH COLLABORATIONS—FACTORS AFFECTING RESEARCH COLLABORATION

Interdisciplinary exchange/communication is crucial for the success of HarvestPlus. Such interactions become increasingly productive as experience is gained, that is, over time and at a series of meetings. HarvestPlus has an advantage from experience that was gained by a subset of the collaborating institutions in precursor projects, but many new non-CGIAR collaborators have participated since 2003. To motivate true collaboration, it is important that the collaborating institutions share a common set of shared goals/objectives, which must be jointly discussed and agreed on. Understanding across disciplines is hindered by technical language, which is either not commonly understood or has different connotations to different disciplines. These barriers must be surmounted. This all takes time and the give and take of interacting on repeated occasions.

The optimal situation in terms of team-building is one in which the partner institutions are all known at the start of the planning process. Competitive bidding can hinder this process of team-building in three ways. First, if one does not know that their proposal will be selected, either he/she will be more reluctant to fully buy into the planning process, or may not have been invited to participate in the planning process at all. Second, a winning bidder has agreed to undertake a specific activity. Challenge Programs must be flexible as ongoing research and external circumstances dictate changes in overall plans. Unless fully integrated into a culture of teamwork, the winning bidder may be reticent to alter the terms of reference of the winning bid, which may have taken quite a substantial amount of work to prepare. Third, it is usually expected that competitive bids will be decided only on the basis of technical competence, perhaps also with a value placed on capacity building. However, ability/willingness to collaborate across disciplinary boundaries is essential and difficult to assess in evaluating formal proposals.

GOVERNANCE THROUGH DISTRIBUTED DECISION-MAKING POWER INCREASES TRANSACTION COSTS

Building consensus among collaborating institutions is vital to the success of HarvestPlus. The Program Director reports to a Project Advisory Committee which has ultimate decision-making power over workplans and budgets, as well as the Directors General of CIAT and IFPRI. Such a structure inherently forces consensus-building.

Nevertheless, consensus-building requires considerable transactions costs. The Program Management Team must have flexibility to make operational decisions, subject to Program Advisory Committee (PAC) oversight every 6 mo, within the strategic boundaries set by the PAC. The PAC members do not represent stakeholder institutions (except for minority representation of CIAT and IFPRI), but do represent a broad spectrum of scientific disciplines, career work experiences, and nations around the world. This governance system has worked well.

CONSIDER OUTREACH TO THE PUBLIC AT INCEPTION

We took the decision to change the name of the Biofortification Challenge Program to HarvestPlus as a way to reach out more effectively to the public. We felt that this was important in terms of (i) sustaining donor support for a long-term program, and (ii) meeting one of the goals of the Challenge Programs to raise the public profile of the CGIAR Centers. Not everyone agreed with the decision; several scientists were reticent to use such an "imprecise" title. However, the decision-making process was highly participatory, the decision approved by a large majority, accepted, and behind us. Time has proven that this was a good decision.

SUMMARY

The biofortification strategy seeks to take advantage of the consistent daily consumption of large amounts of food staples by all family members, including women and children who are most at risk for micronutrient malnutrition. As a consequence of the predominance of food staples in the diets of the poor, this strategy implicitly targets low-income households. After a one-time investment in developing seeds that fortify themselves, recurrent costs are low and germplasm may be shared internationally. It is this multiplier aspect of plant breeding across time and distance that makes it so cost-effective. Once in place, production and consumption of nutritionally improved cultivars is highly sustainable, even if government attention and international funding for micronutrient issues fade. Biofortification

Table 6. Schedule of product release for biofortified products.

Crop	Nutrient	Countries of first release	Agronomic trait	Release year†
Sweet potato	Provitamin A	Uganda, Mozambique	High yielding, virus resistance, drought tolerance	2007
Bean	Fe, Zn	Rwanda, Democratic Republic of Congo	Virus resistance, heat and drought tolerance	2010
Pearl millet	Fe, Zn	India	Mildew resistance, drought tolerance	2011
Cassava	Provitamin A	Nigeria, Democratic Republic of Congo	High yielding, virus resistance	2011–2012
Maize	Provitamin A	Zambia	High yielding, disease resistance, drought tolerance	2011–2012
Rice	Zn, Fe	Bangladesh, India	Disease and pest resistance, submergence tolerance	2012–2013
Wheat	Zn, Fe	India, Pakistan	Disease resistance, lodging	2012–2013

†Approved for release by national governments after 2–3 yr of testing.

provides a feasible means of reaching malnourished populations in relatively remote rural areas, delivering naturally fortified foods to people with limited access to commercially marketed fortified foods, which are more readily available in urban areas. Biofortification and commercial fortification, therefore, are highly complementary.

Ultimately, good nutrition depends on adequate intakes of a range of nutrients and other compounds, in combinations and levels that are not yet completely understood. Thus, the best and final solution to eliminating undernutrition as a public health problem in developing countries is to provide increased consumption of a range of nonstaple foods. However, this will require several decades to be realized, informed government policies, and a relatively large investment in agricultural research and other public and on-farm infrastructure.

In conceptualizing solutions for a range of nutritional deficiencies, interdisciplinary communication between plant scientists and human nutrition scientists holds great potential. Human nutritionists need to be informed, for example, about the extent to which the vitamin and mineral density of specific foods, as well as compounds that promote and inhibit their bioavailability, can be modified through plant breeding. Plant breeders need to be aware of both the major influence that agricultural research may have had on nutrient utilization in the past (e.g., the bioavailability of micronutrients in modern cultivars vs. bioavailability in traditional cultivars), and the potential of plant breeding for future improvements in nutrition.

REFERENCES

Allaway, W.H. 1986. Soil–plant–animal and human intrrelationships in trace element nutrition. p. 465–488. *In* W. Mertz (ed.) Trace elements in human and animal nutrition. Academic Press, Orlando, FL.

Anonymous. 1996. Viewpoint: The optimal breeding strategy is to increase the density of promoter compounds and micronutrient minerals in seeds; caution should be used in reducing antinutrients in staple food crops. Micronutr. Agric. 1:20–22.

Anonymous. 2007. Global childhood malnutrition. Lancet 367:1459.

Anonymous. 2008. The Copenhagen Consensus 2008—Results. p. 1–6. Available at http://www.copenhagenconsensus.com/default.aspx?ID=953 (verified 21 Dec. 2009). The Copenhagen Consensus Cent., Frederiksberg, Denmark.

Becker, K., and M. Frei. 2004. Improving the nutrient availability in rice—Biotechnology or biodiversity? Agric. Rural Devel. 11:64–65.

Bensinger, S.J., and P. Tontonoz. 2008. Integration of metabolism and inflammation by lipid-activated nuclear receptors. Nature 454:470–477.

Bouhnik, Y., L. Raskine, K. Champion, C. Andrieux, S. Penven, H. Jacobs, and G. Simoneau. 2007. Prolonged administration of low-dose inulin stimulates the growth of bifidobacteria in humans. Nutr. Res. 27:187–193.

Bouis, H.E. 1999. Economics of enhanced micronutrient density in food staples. Field Crops Res. 60:165–173.

Bouis, H.E., C. Hotz, B. McClafferty, J.V. Meenakshi, and W. Pfeiffer. 2009. Biofortification: A new tool to reduce micronutrient malnutrition. *In* Int. Congr. of Nutrition, 19th, Bangkok, Thailand. 4–9 Oct. 2009.

Cakmak, I. 2008. Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? Plant Soil 302:1–17.

Cani, P., A. Neyrinck, F. Fava, C. Knauf, R. Burcelin, K. Tuohy, G. Gibson, and N. Delzenne. 2007. Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. Diabetologia 50:2374–2383.

Cani, P.D., R. Bibiloni, C. Knauf, A. Waget, A.M. Meyrinck, N.M. Delzenne, and R. Burcelin. 2008. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high fat diet–induced obesity and diabetes in mice. Diabetes 57:1470–1481.

Cao, X.Y., X.M. Jiang, A. Kareem, Z.H. Dou, M.R. Rakeman, M.L. Zhang, T. Ma, K. O'Donnell, N. DeLong, and G.R. DeLong. 1994. Iodination of irrigation water as a method of supplying iodine to a severely iodine-deficient population in Xinjiang, China. Lancet 344:107–110.

Casey, R.P., and R.G. Lugar. 2008. A call for a strategic U.S. approach to the global food crisis. p. 1–9. CSIS Press, Washington, DC.

Combs, G.F., Jr. 2008. The vitamins. Third ed. Elsevier Academic Press, San Diego, CA.

Dethlefsen, L., M. McFall-Ngai, and D.A. Relman. 2007. An ecological and evolutionary perspective on human–microbe mutualism and disease. Nature 449:811–818.

Drakakaki, G., S. Marcel, R. Glahn, E. Lund, S. Pariagh, R. Fischer, P. Christou, and E. Stoger. 2005. Endosperm-specific co-expression of recombinant soybean ferritin and *Aspergillus* phytase in maize results in significant increases in the levels of bioavailable iron. Plant Mol. Biol. 59:869–880.

Fairweather-Tait, S.J., and R.F. Hurrell. 1996. Bioavailability of minerals and trace elements. Nutr. Res. Rev. 9:295–324.

Falque, M., L. Decousset, D. Dervins, A.M. Jacob, J. Joets, J.P. Martinant, X. Raffoux, N. Ribiere, C. Ridel, D. Samson, A. Charcosset, and A. Murigneux. 2005. Linkage mapping of

1454 new maize candidate gene loci. Genetics 170:1957–1966.
FAO and WHO. 2006. Probiotics in food. Health and nutritional properties and guidelines for evaluation. World Health Organ./Food and Agric. Organ. of the United Nations, Rome.
Forssard, E., M. Bucher, F. Mächler, A. Mozafar, and R. Hurrell. 2000. Review. Potential for increasing the content and bioavailability of Fe, Zn and Ca in plants for human nutrition. J. Sci. Food Agric. 80:861–879.
Genc, Y., J.M. Humphries, G.H. Lyons, and R.D. Graham. 2005. Exploiting genotypic variation in plant nutrient accumulation to alleviate micronutrient deficiency in populations. J. Trace Elem. Med. Biol. 18:319–324.
Graham, R.D., and R.M. Welch. 1996. Breeding for staple-food crops with high micronutrient density. Int. Food Policy Res. Inst., Washington, DC.
Graham, R.D., R.M. Welch, and H.E. Bouis. 2001. Addressing micronutrient malnutrition through enhancing the nutritional quality of staple foods: Principles, perspectives and knowledge gaps. Adv. Agron. 70:77–142.
Graham, R.D., R.M. Welch, D.A. Saunders, I. Monasterio, H.E. Bouis, M. Bonierbale, S. de Hann, G. Burgos, G. Thiele, R. Liria, C.A. Meisner, S.E. Beebe, M.J. Potts, M. Kadiajn, P.R. Hobbs, R.K. Gupta, and S. Twomlow. 2007. Nutritious subsistence food systems. Adv. Agron. 92:1–74.
Griffiths, E.A., L.C. Duffy, F.L. Schanbacher, H. Qiao, D. Dryja, A. Leavens, J. Rossman, G. Rich, D. Dirienzo, and P.L. Ogra. 2004. In vivo effects of bifidobacteria and lactoferrin on gut endotoxin concentration and mucosal immunity in Balb/c mice. Dig. Dis. Sci. 49:579–589.
Grunes, D.L., and W.H. Allaway. 1985. Nutritional quality of plants in relation to fertilizer use. p. 589–619. *In* O.P. Engelstad (ed.) Fertilizer technology and use. SSSA, Madison, WI.
Haug, A., R.D. Graham, O.A. Christopherson, and G.H. Lyons. 2008. How to use the world's scarce selenium resources efficiently to increase the selenium concentration in food. Microb. Ecol. Health Dis. 19:209–228.
Hawkes, C., and M.T. Ruel. 2006. Understanding the links between agriculture and health. p. 1–32. *In* C. Hawkes and M.T. Ruel (ed.) Int. Food Policy Res. Inst., Washington, DC.
Horton, S., and J. Ross. 2003. The economics of iron deficiency. Food Policy 28:51–75.
Hotz, C., B. McClafferty, C. Hawkes, M. Ruel, and S. Babu. 2007. From harvest to health: Challenges for developing biofortified staple foods and determining their impact on micronutrient status. Food Nutr. Bull. 28:S271–S279.
House, W.A. 1999. Trace element bioavailability as exemplified by iron and zinc. Field Crops Res. 60:115–141.
House, W.A., and R.M. Welch. 1989. Bioavailability of and interactions between zinc and selenium in rats fed wheat grain intrinsically labeled with ^{65}Zn and ^{75}Se. J. Nutr. 119:916–921.
Kemna, E., H. Tjalsma, C. Laarakkers, E. Nemeth, H. Willems, and D. Swinkels. 2005. Novel urine hepcidin assay by mass spectrometry. Blood 106:3268–3270.
Kennedy, G., G. Nantel, and P. Shetty. 2003. The scourge of "hidden hunger": Global dimensions of micronnutrient deficiencies. Food Nutr. Agric. 32:8–16.
King, J.C. 2002. Biotechnology: A solution for improving nutrient bioavailability. Int. J. Vit. Nutr. Res. 72:7–12.
Lee, M., N. Sharopova, W.D. Beavis, D. Grant, M. Katt, D. Blair, and A. Hallauer. 2002. Expanding the genetic map of maize with the intermated B73 × Mo17 (IBM) population. Plant Mol. Biol. 48:453–461.
Lonnerdal, B. 2009. Soybean ferritin: Implications for iron status of vegetarians. Am. J. Clin. Nutr.
Low, J.W., M. Arimond, N. Osman, B. Cunguara, F. Zano, and D. Tschirley. 2007. A food-based approach introducing orange-fleshed sweet potatoes increased Vitamin A intake and serum retinol concentrations in young children in rural Mozambique. J. Nutr. 137:1320–1327.
Lucca, P., R. Hurrell, and I. Potrykus. 2001. Genetic engineering approaches to improve the bioavailability and the level of iron in rice grains. Theor. Appl. Genet. 102:392–397.
Lucca, P., S. Poletti, and C. Sautter. 2006. Genetic engineering approaches to enrich rice with iron and vitamin A. Physiol. Plant. 126:291–303.
Mäkelä, A.-L., V. Näntö, P. Mäkelä, and W. Wang. 1993. The effect of nationwide selenium enrichment of fertilizers on selenium status of healthy Finnish medical students living in south western Finland. Biol. Trace Elem. Res. 36:151–157.
Manning, T.S., and G.R. Gibson. 2004. Prebiotics. Best Pract. Res. Clin. Gastroenterol. 18:287–298.
Moraghan, J.T. 1980. Effects of soil temperature on response of flax to phosphorus and zinc fertilizers. Soil Sci. 129:290–296.
Nestel, P., H.E. Bouis, J.V. Meenakshi, and W. Pfeiffer. 2006. Biofortification of staple food crops. J. Nutr. 136:1064–1067.
Ortiz-Monasterio, J.I., N. Palacios-Rojas, E. Meng, K. Pixley, R. Trethowan, and R.J. Pena. 2007. Enhancing the mineral and vitamin content of wheat and maize through plant breeding. J. Cereal Sci. 46:293–307.
Peck, N., D.L. Grunes, R.M. Welch, and G.E. MacDonald. 1980. Nutritional quality of vegetable crops as affected by phosphorus and zinc fertilizers. Agron. J. 72:528–534.
Pfeiffer, W.H., and B. McClafferty. 2007. HarvestPlus: Breeding crops for better nutrition. Crop Sci. 47:S88–S105.
Qaim, M., A.J. Stein, and J.V. Meenakshi. 2007. Economics of biofortification. Agric. Econ. 37:119–133.
Ren, Q., F. Fan, Z. Zhang, X. Zheng, and G.R. DeLong. 2008. An environmental approach to correcting iodine deficiency: Supplementing iodine in soil by iodination of irrigation water in remote areas. J. Trace Elem. Med. Biol. 22:1–8.
Rouse, T.I., and D.P. Davis. 2004. Exploring a vision: Integrating knowledge for food and health. A workshop summary. Board on Agric. and Nat. Resources, Division on Earth and Life Stud., Natl. Res. Counc. of the Natl. Acad. of Sci. Natl. Acad. Press, Washington, DC.
Saied, H.T., and A.M. Shamsuddin. 1998. Up-regulation of the tumor suppressor gene p53 and WAF1 gene expression by IP6 in HT-29 human colon carcinoma cell line. Anticancer Res. 18:1479–1484.
Salminen, S., C. Bouley, M.-C. Boutron-Ruault, J.H. Cummings, A. Franck, G.R. Gibson, E. Isolauri, M.-C. Moreau, M.B. Roberfroid, and I. Rowland. 1998. Functional food science and gastrointestinal physiology and function. Br. J. Nutr. 80:S147–S171.
Schiffrin, E.J., and S. Blum. 2002. Interactions between the microbiota and the intestinal mucosa. Eur. J. Clin. Nutr. 56:S60–S64.
Shamsuddin, A.M. 1999. Metabolism and cellular functions of IP6: A review. Anticancer Res. 19:3733–3736.
Sharopova, N., M.D. McMullen, L. Schultz, S. Schroeder, H. Sanchez-Villeda, J. Gradiner, K. Edwards, T. Ruff, J.C. Register, C. Brouwer, R. Thompson, R. Velasco, E. Chin, M. Lee, W. Woodman-Clikeman, M.J. Long, E. Liscum, K. Cone, G. Davis, and E.H. Coe, Jr. 2002. Development and mapping of

SSR markers for maize. Plant Mol. Biol. 48:463–481.
Sobal, J., L.K. Khan, and C. Bisogni. 1998. A conceptual model of the food and nutrition system. Soc. Sci. Med. 47:853–863.
Steer, T., H. Carpenter, K. Tuohy, and G.R. Gibson. 2000. Perspectives on the role of the human gut microbiota and its modulation by pro- and prebiotics. Nutr. Res. Rev. 13:229–254.
Teitelbaum, J.E., and W.A. Walker. 2002. Nutritional impact of pre- and probiotics as protective gastrointestinal organisms. Annu. Rev. Nutr. 22:107–138.
Theil, E.C., J.W. Burton, and J.L. Beard. 1997. A sustainable solution for dietary iron deficiency through plant biotechnology and breeding to increase seed ferritin control. Eur. J. Biochem. 51:S28–S31.
Van Campen, D.R., and R.P. Glahn. 1999. Micronutrient bioavailability techniques: Accuracy, problems and limitations. Field Crops Res. 60:93–113.
Wang, Z.M., G.M. Xiao, Y.M. Yao, S.M. Guo, K.M. Lu, and Z.M. Sheng. 2006. The role of bifidobacteria in gut barrier function after thermal injury in rats. J. Trauma-Injury Infect. Crit. Care 61:650–657.
Welch, R.M. 1986. Effects of nutrient deficiencies on seed production and quality. Adv. Plant Nutr. 2:205–247.
Welch, R.M. 1995. Micronutrient nutrition of plants. Crit. Rev. Plant Sci. 14:49–82.
Welch, R.M. 1999. Importance of seed mineral nutrient reserves in crop growth and development. p. 205–226. *In* Z. Rengel (ed.) Mineral nutrition of crops. Fundamental mechanisms and implications. Food Products Press, New York.
Welch, R.M. 2001. Micronutrients, agriculture and nutrition; linkages for improved health and well being. p. 247–289. *In* K. Singh et al. (ed.) Perspectives on the micronutrient nutrition of crops. Sci. Publ., Jodhpur, India.
Welch, R.M. 2002. Breeding strategies for biofortified staple plant foods to reduce micronutrient malnutrition globally. J. Nutr. 132:495S–499S.
Welch, R.M., and R.D. Graham. 1999. A new paradigm for world agriculture: Meeting human needs—Productive, sustainable, nutritious. Field Crops Res. 60:1–10.
Welch, R.M., and R.D. Graham. 2004. Breeding for micronutrients in staple food crops from a human nutrition perspective. J. Exp. Bot. 55:353–364.
Welch, R.M., and W.A. House. 1984. Factors affecting the bioavailability of mineral nutrients in plant foods. p. 37–54. *In* R.M. Welch and W.H. Gabelman (ed.) Crops as sources of nutrients for humans. ASA, Madison, WI.
Welch, R.M., W.A. House, and W.H. Allaway. 1974. Availability of zinc from pea seeds to rats. J. Nutr. 104:733–740.
White, P.J., and M.R. Broadley. 2009. Biofortification of crops with seven mineral elements often lacking in human diets—Iron, zinc, copper, calcium, magnesium, selenium and iodine. New Phytol. 182:49–84.
WHO and FAO. 2003. Joint WHO/FAO expert consultation on diet, nutrition and the prevention of chronic diseases. World Health Organ., Geneva, Switzerland.
World Bank. 2007. From agriculture to nutrition: Pathways, synergies and outcomes. Rep. 40196-GLB. Int. Bank for Reconstruction and Dev./The World Bank, Washington, DC.
Zarocostas, J. 2009. Malnutrition and hunger rise as economic crisis hits world's poorest people. BMJ 338:B1651.
Zhou, J.R., and J.W. Erdman, Jr. 1995. Phytic acid in health and disease. Crit. Rev. Food Sci. Nutr. 35:495–508.