Biofortification: Introduction, Approaches, Limitations, and Challenges

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Abstract

Micronutrient malnutrition is known to affect more than half of the world's population and considered to be among the most serious global challenges to humankind. Modern plant breeding has been historically oriented toward achieving high agronomic yields rather than nutritional quality, and other efforts related to alleviating the problem have been primarily through industrial fortification or pharmaceutical supplementation. Micronutrient malnutrition or the hidden hunger is very common among women and preschool children caused mainly by low dietary intake of micronutrients, especially Zn and Fe. Biofortification, the process of increasing the bioavailable concentrations of essential elements in edible portions of crop plants through agronomic intervention or genetic selection, may be the solution to malnutrition or hidden hunger mitigation. The Consultative Group on International Agricultural Research has been investigating the genetic potential to increase bioavailable Fe and Zn in staple food crops such as rice, wheat, maize, common beans, and cassava.

Keywords

Biofortification • Breeding • Genetic engineering • Limitations • Strategy

1.1 Introduction

Biofortification, the process of breeding nutrients into food crops, provides a comparatively cost-effective, sustainable, and long-term means of delivering more micronutrients. This approach not only will lower the number of severely malnourished people who require treatment by complementary interventions but also will help them maintain improved nutritional status. Moreover,

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biofortification provides a feasible means of reaching malnourished rural populations who may have limited access to commercially marketed fortified foods and supplements.

The biofortification strategy seeks to put the micronutrient-dense trait in those varieties that already have preferred agronomic and consumption traits, such as high yield. Marketed surpluses of these crops may make their way into retail outlets, reaching consumers in first rural and then urban areas, in contrast to complementary interventions, such as fortification and supplementation, that begin in urban centers. Biofortified staple foods cannot deliver as high a level of minerals and vitamins per day as supplements or industrially fortified foods, but they can help by increasing the daily adequacy of micronutrient intakes among individuals throughout the life cycle (Bouis et al. 2011).

1.2 Minerals and Vitamins

Minerals, in the context of the human diet, are inorganic chemical elements (or more properly their dissociated ions) that are required for biological or biochemical processes including the accumulation of electrolytes. Carbon, hydrogen, nitrogen, and oxygen are excluded from the list as these are found in common organic molecules. There are 16 essential minerals, but 11 of them are required in such small amounts and/or are so abundant in food and drinking water that deficiency arises only in very unusual circumstances. The remaining five are present in limiting amounts in many foods, so a monotonous diet can easily result in deficiency. These minerals are iodine (I), iron (Fe), zinc (Zn), calcium (Ca), and selenium (Se). Deficiency diseases arise when diets are based predominantly on staple foods, such as milled cereals, which have a low bioavailable mineral content (Christou and Twyman 2004). Mineral deficiency is therefore most prevalent in developing countries, where there is poor access to fresh foods, although Ca deficiency is also widespread in the industrialized world (Galera et al. 2010).

1.2.1 Iodine

Iodine is an essential component of the thyroid hormones thyroxine and triiodothyronine, which regulate growth and development and maintain the basal metabolic rate. However, only 30 % of the body's iodine is stored in the thyroid gland, and the precise role of the 70 % distributed in other tissues is unknown. It may overlap with the function of other minerals such as Se or Fe and Zn (Lyons et al. 2004). Goiter is another important symptom of iodine deficiency and results from the lack of thyroxine inducing the production of thyroid-stimulating hormone, which in turn causes the thyroid gland to swell (Dunn 2003). India is one of the worst affected countries in the world, with more than 50 million cases of goiter and more than two million of cretinism.

1.2.2 Vitamin A

Deficiency associated with blindness and increased risk of disease and death for small children and pregnant women can be addressed through supplements, which are now estimated to reach children at least once a year in 40 countries. The UN Standing Committee on Nutrition (UN/SCN) estimates that 140 million children and 7 million pregnant women are VA deficient, primarily in Africa and South/Southeast Asia. In 1998, WHO, UNICEF, Canadian International Development Agency, USAID, and the Micronutrient Initiative launched the VA Global Initiative. This provides support to countries in delivering VA supplements.

1.2.3 Iron

Iron has numerous important functions in the human body, reflecting its ability to act as both an electron donor and acceptor. In this role, it forms the functional core of the heme complex, which is found in the oxygen-binding molecules hemoglobin and myoglobin, and the catalytic center of cytochromes, which carry out redox reactions. Iron is therefore required for oxygen transport in the body and for energy metabolism, also contributing to the catalytic activity of a range of nonheme enzymes such as ribonuclease reductase (WHO/FAO 1998). The immediate outcome of Fe deficiency is iron deficiency anemia (IDA), which is thought to affect at least two billion people worldwide. More than half of these cases could be addressed by increasing the amount of Fe in the diet, but as for iodine this is difficult in developing countries where the population relies on staples, because cereal grains contain very low levels of Fe and also contain antinutritional compounds such as phytate that inhibit Fe uptake (Zimmermann et al. 2004).

1.2.4 Zinc

Zinc is an essential functional component of thousands of proteins. Many contain zinc prosthetic groups (e.g., zinc finger, zinc twist) and approximately 100 enzymes require Zn as a cofactor. Some olfactory receptors cannot function without Zn. Many cells in the body also secrete Zn as a signaling molecule, including cells in the immune and nervous systems (WHO/FAO 1998). Nearly two billion people are at risk of Zn deficiency, predominantly children and pregnant women. Signs of severe zinc deficiency include hair loss, skin lesions, wasting, and persistent diarrhea. The mineral appears to be particularly important during periods of rapid growth, and insufficient intake during childhood and adolescence can delay growth, sexual development, and psychomotor development (WHO/FAO 1998).

1.2.5 Calcium

Calcium is the most abundant mineral in the human body, accounting for 1–2 % of an adult's body mass. Over 99 % of Ca is stored in the teeth and bones, where it plays an important structural role (WHO/FAO 1998). However, Ca, like Zn, is also an enzyme cofactor and an important signaling molecule (a secondary messenger). It plays a pivotal role in the blood clotting cascade. Calcium deficiency has a profound impact on bone health, resulting in rickets if deficiency occurs in the young and osteoporosis if it persists into old age.

1.2.6 Selenium

Selenium is found in two unusual amino acids—selenocysteine and selenomethionine—which are the principal functional components of selenoenzymes. It is an essential cofactor in approximately 50 enzymes, including those whose function is to reduce antioxidant enzymes (such as glutathione peroxidase) and those whose function is to remove mineral ions from other proteins (such as thyroid hormone deiodinases) (Lyons et al. 2004). Se is an antioxidant with health benefits including the prevention of cancer and heart disease (WHO/FAO 1998).

1.2.7 Folate

Deficiency associated with increased risk of maternal death and complications in birth and also associated with neural tube defects in infants and with an estimated 200,000 severe birth defects every year can be addressed through fortification of wheat products (Haddad et al. 2004).

1.3 Alleviating Hidden Hunger: Interventions

The term "hidden hunger" has been used to describe the micronutrient malnutrition inherent in human diets that are adequate in calories but lack vitamins and/or mineral elements. The diets of a large proportion of the world's population are deficient in Fe, Zn, Ca, Mg, Cu, Se, or I, which affects human health and longevity and therefore national economies. Mineral malnutrition can be addressed by increasing the amount of fish and animal products in diets, mineral supplementation, and food fortification and/or increasing the bioavailability of mineral elements in edible crops. However, strategies to increase dietary diversification, mineral supplementation, and food fortification have not always proved successful. For this reason, the biofortification of crops through the application of mineral fertilizers, combined with breeding varieties with an increased ability to acquire mineral elements, has been advocated (White and Broadley 2009).

Food fortification and supplementation are currently the most cost-effective strategies to address global mineral malnutrition. The most successful strategy has been salt iodization (fortification with iodine) which has reduced the incidence of goiter and other IDD symptoms markedly where the scheme has been introduced (Galera et al. 2010). Most strategies to improve mineral nutrition have been less successful because of political, socioeconomic, infrastructure-related, and technical constraints that are apparent in most developing countries.

1.3.1 Food Fortification

Food fortification is one of the most cost-effective long-term strategies for mineral nutrition (Horton 2006). Fortification of dairy products such as bread and milk with different minerals (and vitamins) has been successful in industrialized countries However, this strategy is difficult to implement in developing countries because it relies on a strong food processing and distribution infrastructure. Fortification takes place during food processing and increases the product price. These factors make fortified products unaffordable to the most impoverished people living in remote rural areas. Since many parts of the world suffer from multiple deficiencies, strategies must also be developed to fortify foods simultaneously with several micronutrients without adverse interactions among them (Zimmermann et al. 2004). The addition of a single micronutrient would have more or less the same cost implications as the addition of several (Alavi et al. 2008).

Zinc fortification has been implemented in the industrial world but rarely in developing countries. One exception is Zn-fortified wheat and maize flours in Mexico, which are used to make bread and tortillas, the two principal staples (IZINCG 2007). Organizations such as the Zinc Task Force (ZTF) and the International Zinc Nutrition Consultative Group (IZiNCG) are fighting Zn malnutrition by promoting diverse strategies to eliminate it. As Zn and Fe deficiencies tend to go hand in hand, it has been suggested that double fortification would be effective with little additional cost, particularly if Fe fortification were already in place.

1.3.2 Industrial Fortification

The marketed supply of a widely consumed staple food can be fortified by adding micronutrients at the processing stage, and historically this is how micronutrient deficiencies have been addressed in the developed world. Concentration in the food industry also tends to strengthen compliance and quality assurance. Consumption of wheat flour products is growing around the world, even where wheat is not a traditional food staple, opening new fortification opportunities at the milling stage. Public support for traditional fortification has recently been enhanced by new promotion and coordination efforts: Micronutrient Initiative (based in Canada), Flour Fortification Initiative (based in Emory University), Mid Day Meal Scheme (India), and the Global Alliance for Improved Nutrition (GAIN, based in Geneva). Other important global actors include the Network for Sustained Elimination of Iodine Deficiency and the International Zinc Nutrition Consultative Group. In addition, efforts are underway to set regional standards for fortification. The Flour Fortification Initiative provides an assessment of global progress, and they report that 26 % of the global wheat market is fortified, benefiting 1.8 billion people. Most wheat fortification efforts in the developing world are still preliminary or on a pilot scale; they are primarily in the Western Hemisphere, with little sustained activity in Asia and Africa, where most of the micronutrient deficient populations live. Certain kinds of fortification may be impractical for some important food staples (e.g., VA fortification of milled rice) or may introduce off colors or flavors (e.g., VA fortification of white maize).

Industrial fortification will only apply to marketed supplies and therefore may not reach those among the poor who obtain food outside of commercialized channels. Given these limitations, it is clear that industrial fortification of food cannot provide a complete solution to the problem of micronutrient deficiencies in the medium term. It is in this context that a role emerges for biofortification as a complementary strategy.

1.3.3 Promotion of Dietary Diversification

Education is an important element in ensuring that improvements in income result in better maternal and child health. However, dietary diversification is constrained by resource availability for poor households and seasonal availability of fruits and vegetables. Promotion of home gardens is often touted, but the poor have a high opportunity cost for their labor and often limited land. Increased production of fruits and vegetables for household use reduces resources available for other income-earning or food production activities. This type of effort is also relatively expensive and difficult to sustain on any large scale.

1.3.4 Food Supplementation

Supplementation is the best short-term intervention to improve nutritional health, involving the distribution of pills or mineral solutions for immediate consumption. This helps to alleviate acute mineral shortages but is unsustainable for large populations and should be replaced with fortification at the earliest opportunity (Shrimpton and Schultink 2002). In industrialized countries, with few mineral malnutrition problems, supplementation is focused on a small subset of the population with specific deficiencies resulting from medical conditions. In developing countries, where acute and chronic deficiencies are common, supplementation is highly recommended to complement the diet (fortified or otherwise) of the entire population (Nantel and Tontisirin 2002).

Periodic provision of supplements (often in the form of tablets) can address deficiencies of micronutrients that are stored in the body, such as vitamin A and iron. Supplementation can be cheap compared to the large public health benefit. The total annual cost of iron tablet supplementation in India to reach 27 million women and 128 million children at risk is only $5.2 million. Yet even small budgets can be difficult to sustain year after year when they are dedicated to the welfare of politically weak or socially marginal beneficiaries. Furthermore, while certain populations are easy to reach through existing institutions (e.g., schoolchildren through schools), it is often difficult to accomplish full coverage of those most at risk—poor women and very young children. Thus, supplementation has often been most effective when delivered together with other maternal and child health interventions.

The distribution of vitamin A supplements has been one of the most cost effective and successful acute intervention programs in the developing world (Shrimpton and Schultink 2002), but this is a rarity. Like fortification, successful supplementation strategies require a robust infrastructure and a government determined to improve the nutritional health of its population (Shrimpton and Schultink 2002). Even more than fortification, supplementation requires compliance monitoring because more people will neglect to take regular supplements at prescribed intervals than fortified staple foods. Mineral supplements are prescribed for acute deficiency diseases in industrialized countries as well as serving a niche health market. Zn supplements rank very highly according to the Copenhagen Consensus report cost–benefit analysis.

1.3.5 Biofortification

Conventional interventions have a limited impact, so biofortification has been proposed as an alternative long-term approach for improving mineral nutrition (Zhu et al. 2007). Biofortification focuses on enhancing the mineral nutritional qualities of crops at source, which encompasses processes that increase both mineral levels and their bioavailability in the edible part of staple crops. The former can be achieved by agronomic intervention, plant breeding, or genetic engineering, whereas only plant breeding and genetic engineering can influence mineral bioavailability. Plant breeding and genetic engineering are often compared because, in contrast to agronomic interventions, both involve changing the genotype of a target crop. The two processes are similar in aim, albeit different in scope. Both attempt to create plant lines carrying genes that favor the most efficient accumulation of bioavailable minerals—plant breeding achieves this by crossing the best performing plants and selecting those with favorable traits over many generations—whereas genetic engineering accesses genes from any source and introduces them directly into the crop. Plant breeding is limited to genes that can be sourced from sexually compatible plants, whereas genetic engineering has no taxonomic constraints and even artificial genes can be used.

The main advantage of genetic engineering and plant breeding approaches for mineral enhancement is that investment is only required at the research and development stage, and thereafter the nutritionally enhanced crops are entirely sustainable. Furthermore, as stated above, mineral-rich plants tend to be more vigorous and more tolerant of biotic stress, which means yields are likely to improve in line with mineral content (Frossard et al. 2000; Nestel et al. 2006). Unlike conventional intervention strategies, genetic engineering and plant breeding are both economically and environmentally sustainable (Stein et al. 2008). Although there are no commercial nutritionally enhanced plants derived from either method at the current time, this approach has the greatest long-term cost-effectiveness overall and is likely to have an important impact over the next few decades. Biofortification is also likely to be more accessible than conventional interventions in the long term because it removes hurdles such as the reliance on infrastructure and compliance. Moreover, plants assimilate minerals into organic forms that are naturally bioavailable and contribute to the natural taste and texture of the food. Economic studies have shown many potential health benefits of biofortification strategies, especially in combination with conventional strategies (Buois 2002; Stein et al. 2008).

1.3.5.1 Plant Breeding

Plant breeding programs focus on improving the level and bioavailability of minerals in staple crops using their natural genetic variation (Welch and Graham 2005). Breeding approaches include the discovery of genetic variation affecting heritable mineral traits, checking their stability under different conditions, and the feasibility of breeding for increasing mineral content in edible tissues without affecting yields or other quality traits. Breeding for increased mineral levels has several advantages over conventional interventions (e.g., sustainability); no high-mineral varieties produced by this method have been introduced onto the market thus far. This reflects long development times, particularly if the mineral trait needs to be introgressed from a wild relative. Breeders utilize molecular biology techniques such as quantitative trait locus (QTL) maps and marker-assisted selection (MAS) to accelerate the identification of high-mineral varieties, but they have to take into account differences in soil properties (e.g., pH, organic composition) that may interfere with mineral uptake and accumulation. For example, the mineral pool available to plant roots may be extremely low in dry, alkaline soils with a low organic content (Cakmak 2008).

1.3.5.2 Conventional Plant Breeding

This allows crop scientists to make significant improvement in the nutritional, eating quality, and agronomic traits of major subsistence food crops. Conventional breeding is limited, however, because it can only use the genetic variability already available and observable in the crop being improved or occasionally in the wild varieties that can cross with the crop. Furthermore, conventional breeders usually have to trade away yield and sometimes grain quality to obtain higher levels of nutrition. One example is quality protein maize (QPM), which has taken decades of conventional plant breeding work to develop into varieties acceptable to farmers. However, multiple gains are at times possible, as with iron and zinc in rice and wheat, where the characteristics that lead to more iron and zinc in the plant can also lead, by some accounts, to higher yield. Other biofortified crops, such as the orange-fleshed sweet potatoes (OFSP) promoted through the HarvestPlus program in Africa, have been successfully selected and developed for both nutrient and (at least rainy season) yield traits (Unnevehr et al. 2007).

1.3.5.3 Mutation Breeding

Mutation breeding has been used extensively in developed and developing countries to develop grain varieties with improved grain quality and in some cases higher yield and other traits. This technique makes use of the greater genetic variability that can be created by inducing mutations with chemical treatments or irradiation. The FAO/International Atomic Energy Agency (IAEA) website contains more than 2500 varieties that have been developed through mutation breeding (Mutant varieties database). Of these, 1568 are in Asia, 695 in Europe, and 165 in the United States. Most of the European and US mutants are flowers, but most in Asia are basic food crops such as wheat, rice, maize, and soybeans. According to their website, FAO/IAEA include biofortification as one of the objectives of their mutagenesis program, but there do not seem to be any applicable results yet. Varieties produced using mutagenesis can be grown and certified as organic crops in the United States, whereas transgenic crops developed using recombinant DNA (rDNA) technology cannot.

1.3.5.4 Molecular Breeding

Also called marker-assisted breeding, this is a powerful tool of modern biotechnology that encounters little cultural or regulatory resistance and has been embraced so far even by organic growers because it relies on biological breeding processes rather than engineered gene insertions to change the DNA of plants. This technique is expanding rapidly with the development of genomics, which is the study of the location and function of genes, and with the rapid decline in costs of screening plant tissue. Once scientists have identified the location of a gene for a desirable trait, they build a probe that attaches itself only to a DNA fragment, a so-called marker, unique to that gene. They then can use this marker as a way to monitor and speed up their efforts to move this trait into relatives of the plant using conventional breeding. For example, since the marker can be detected in the tissue of new seedlings, the presence or absence of the desired trait can be determined without having to wait for a plant to mature, often reducing by years the length of a typical crop development process. If molecular breeding reduces the number of generations required to develop a pure line variety by three generations, this can save 3 years of research time. The use of molecular breeding has increased dramatically both by private seed companies and government plant breeders in developed countries, and it is gradually spreading to developing countries (Pray 2006). Using this technique, plant breeders also can stack into one variety several different genes that code for different traits, for example, QPM, disease resistance, and drought tolerance in maize (Pray 2006). This technique has also been used to find recessive traits in plants that cannot be located by conventional breeding or other techniques.

1.3.5.5 Genetic Engineering

Genetic engineering is the latest weapon in the armory against mineral deficiency and uses advanced biotechnology techniques to introduce genes directly into breeding varieties. The genes can come from any source (including animals and microbes) and are designed to achieve one or more of the following goals (Zhu et al. 2007): (a) improve the efficiency with which minerals are mobilized in the soil, (b) reduce the level of antinutritional compounds, and (c) increase the level of nutritional enhancer compounds such as inulin. Genetic engineering, or rDNA, is a technique that offers still greater speed and reach because it moves specific genes with desired traits from a source organism—one which does not have to be a related organism—directly into the living DNA of a target organism. The *transgenic* trait is added without normal biological reproduction, but once in the plant it becomes inheritable through normal reproduction. Scientists first developed this technique in the laboratory in 1973 and have been using it to transform agricultural crop plants since the 1980s. Once a useful gene has been identified (which can require a major research project and many years), it is attached to both marker and promoter genes and then inserted into a plant, usually using a nonviable virus called *Agrobacterium* as a carrier. GE produces plants that are known as transgenics or less precisely as GMOs. GE has great reach because it can add valuable characteristics that are not currently found in the seeds of individual plant species. GE was necessary for the development of golden rice, which contains the precursor to VA from a daffodil plant. This was a trait missing from rice plants, and it could not be introduced conventionally since daffodils cannot be crossed with rice plants. In addition, GE can take much less time to incorporate desired traits into a crop plant than either traditional or molecular breeding. The choice of which technology to use when biofortifying crops comes down to a calculation by breeders of how to get the best results most quickly, given their budget constraint. Conventional plant breeding requires less investment in labs or highly trained human resources (molecular biologists) than either marker-assisted selection or genetic engineering, and it faces lower and less costly regulatory hurdles. However, if there are no genes for the VA precursors in the genome of a crop (as one example), no amount of conventional plant breeding can put them there, and scientists must turn to GE. Molecular breeding and GE also have advantages over traditional breeding because they make it easier to *develop* crops with multiple desired nutritional traits, maintain agronomic viability of biofortified crops, adapt agriculture improvements arising in the United States for obscure crops in developing countries, etc.

1.3.5.6 Tissue Cultures

Modern tissue culture techniques can allow scientists to reproduce plants from a single cell. These techniques are now used extensively to produce disease-free planting material of clonally propagated crops such as bananas. When tissue culture is combined with embryo rescue techniques, plant breeders can use the genes from wild and weedy relatives of a crop, which would normally not cross with the cultivated crop. This allows breeders to increase genetic variability of the cultivated crop and then bring in valuable traits of the wild and weedy relatives. These techniques have allowed scientists to cross Asian and African rice varieties and develop Nerica rice varieties with agronomic traits, such as higher yield and resistance to water stresses, that have met with growing success in Africa. Tissue culture is an important tool for propagation of roots and tubers, such as potatoes and cassava, and both of these crops are part of current biofortification research.

1.3.6 Microbiological Interventions

1.3.6.1 *Plant Growth Promoting Rhizobacteria* (PGPR)

These include beneficial bacteria that colonize plant roots and enhance plant growth by a wide variety of mechanisms. The use of PGPR is steadily increasing in agriculture, as it offers an attractive way to reduce the use of chemical fertilizers, pesticides, and related agrochemicals (Rana et al. 2012). Interventions using PGPR or other biological agents are limited. Secretion of phytosiderophores by microorganisms and plants in restricted spatial and temporal windows represents an efficient strategy for uptake of iron and other micronutrients by plants from the rhizosphere. Analysis of the complex interactions between soils, plants, and microbes in relation with micronutrient dynamics represents a unique opportunity to enhance our knowledge of the rhizosphere ecology. Such progress can provide information and tools enabling us to develop strategies to improve plant nutrition and health with decrease in the application of chemical inputs. Microorganisms are known to differ significantly in competing with higher plants for micronutrients (Steven 1991). Among bacteria, a lot of attention has been dedicated to the siderophore-mediated iron uptake by fluorescent pseudomonads. A number of other mechanisms are also involved in the sequestration and transformation by microorganisms in soil such as production of acids, alkalis, etc. PGPR constitute a significant part of the protective flora that benefit plants by enhancing root function, suppressing disease, and accelerating growth and development (Glick 1995). Microorganisms differ in competing with higher plants for micronutrients. Species of *Azotobacter* differed in their competitiveness with wheat plants in extracting Fe and Zn (Shivay et al. 2010). Biofortification of crops through application of PGPR can be therefore considered as a possible supplementary measure, which along with breeding varieties can lead to increased micronutrient concentrations in wheat crop, besides improving yield and soil fertility.

1.3.6.2 AM Fungi

Most plants, including all major grain crops and almost all vegetables and fruits, are associated with mycorrhizal fungi that improve the uptake of essential mineral elements from soils and, therefore, enhance plant growth and productivity. These symbiotic fungi, therefore, change, directly or indirectly, the mineral nutrition of plant products that are also essential for humans. However, the role of mycorrhizas on element biofortification may be piloted through agricultural practices. Mycorrhizas can potentially offer a more effective and sustainable element biofortification to curb global human malnutrition. Approximately 90 % of land plants form mycorrhizas (literally “fungus roots”): they exist everywhere, from tiny home gardens to large ecosystems (Smith and Read 1997). Six types of mycorrhizas (arbuscular, arbutoid, ecto-, ericoid, monotropoid, and orchid) are categorized by their distinct morphological characteristics (Wang and Qiu 2006). Of them, arbuscular mycorrhiza (AM) is the most common and predominant type. Arbuscules, specific “little-tree-shaped” fungal structures inside root cortical cells, serve as the main sites of nutrient exchange between the plant and the fungus. AM also has external hyphae that provide an extensive surface area or network for nutrient uptake from soils. Thus, AM is the most important mycorrhiza in agriculture and closely relates to human nutrition. Obligately depending on plant photosynthates as energy sources, the extensive AM mycelial systems (the vegetative parts of the fungus) effectively explore soil substrates and acquire soil inorganic nutrients, including major macronutrients N, P, and K and micronutrients Cu, Fe, and Zn (Caris et al. 1998), with some capacity for acquiring organic N and P (Koide and Kabir 2000). These soil-derived nutrients are not only essential for AM development but are also partly transferred to the host plant. It is believed that many plants that usually form this symbiotic relationship would be unable to survive without the mycorrhiza. Mycorrhizal mycelia and their exudates also constitute a large carbon source (20–30 % total soil microbial biomass) for the functioning of other belowground microorganisms that ameliorate soil nutrient availability through decomposition of organic compounds and weathering of inorganic materials. So, not only do the activities of AM fungi have multiple functions that enhance plant performance but they also play crucial roles in the development of soil properties and the health of the entire ecosystem.

1.3.7 Agronomic Intervention

Farmers have applied mineral fertilizers to soil for hundreds of years in order to improve the health of their plants, but within certain limits the same strategy can also be used to increase mineral accumulation within cereal grains for nutritional purposes (Rengel et al. 1999). This strategy only works if the mineral deficiency in the grain reflects the absence of that mineral in the soil and if the mineral fertilizer contains minerals that are rapidly and easily mobilizable. Also, even if plants can absorb minerals efficiently from the soil, they may store the mineral in leaves but not fruits or seeds, or they may accumulate the mineral in a form that is not bioavailable, thus having no impact on nutrition (Frossard et al. 2000). Like supplements and fortification, agronomic intervention is probably best applied in niche situations or in combination with other strategies (Cakmak 2008). One drawback of agronomic intervention is the cost and impact of the fertilizers. Fertilizer use is likely to increase the cost of food, thus reducing its availability to the most impoverished people. The expensive fertilizers must be applied regularly, with no direct yield incentive to farmers in developing countries, so the intervention would likely be omitted to save costs even though seeds produced under rich mineral conditions germinate more vigorously than those in poor soils (Cakmak 2008). There is also concern about the impact of increased fertilizer use on the environment (Graham 2003).

Agronomic strategies to increase the concentrations of mineral elements in edible tissues generally rely on the application of mineral fertilizers and/or improvement of the solubilization and mobilization of mineral elements in the soil (White and Broadley 2009). When crops are grown where mineral elements become immediately unavailable in the soil, targeted application of soluble inorganic fertilizers to roots or to leaves is practiced. In situations where mineral elements are not readily translocated to edible tissues, foliar applications of soluble inorganic fertilizers are made. It has been observed that the human population of the world has exceeded the carrying capacity of low-input agriculture, and modern inorganic fertilizers are necessary to obtain the crop yields required to prevent starvation (Graham et al. 2007). Essential plant nutrients are mainly applied to soil and plant foliage for achieving maximum economic yields. Soil application method is more common and most effective for nutrients, which are required in higher amounts. However, under certain circumstances, foliar fertilization is more economic and effective. Foliar symptoms, soil and plant tissue tests, and crop growth responses are principal nutrient disorder diagnostic techniques. Soil applications of fertilizers are mainly done on the basis of soil tests, whereas foliar nutrient applications are mainly done on the basis of visual foliar symptoms or plant tissue tests. Hence, correct diagnosis of nutrient deficiency is fundamental for successful foliar fertilization. In addition, there are some more requirements for successful foliar fertilization. Foliar fertilization requires higher leaf area index for absorbing applied nutrient solution in sufficient amount; it may be necessary to have more than one application depending on severity of nutrient deficiency.

1.3.7.1 Parboiled Rice

Fe fortification in parboiled rice is a rapid and cost-effective solution to Fe deficiency anemia in economically disadvantaged populations with rice as the major staple food and poor access to animal proteins. It focused initially on the feasibility of this innovative approach, by examining the effectiveness of Fe fortification and retention, the solubility of Fe in the grain in response to fortification treatments, and the likely pathway of Fe movement into the endosperm. NaFeEDTA has been recently approved as an ingredient to be used in supervised food fortification programs (Hurrell 2003) and is the most promising Fe fortification compound for food additives and is used intensively to prevent oxidation and color changes in food and promote its bioavailability in the human diet. The effectiveness of enhancing Fe density in white rice through the Fe-fortified parboiling process is far greater than that achieved from conventional and transgenic rice breeding. For example, Fe concentration in milled rice of IR68144-2B-2-2-3, an improved rice cultivar by conventional breeding, is 7–13 mg Fe kg^{-1} (Graham et al. 1999) and 37 mg Fe kg^{-1} in rice containing transferred soybean ferritin gene (Vasconcelos et al. 2003). In contrast, Fe concentration in the milled rice grains Fe fortified in the parboiling had 70–144 and 30–110 mg Fe kg^{-1} in 60 and 120 s milled grains, respectively.

Comparatively, a substantial loss of Fe sprayed on raw rice surface occurs if the rice is rinsed before cooking. Although a polymer coating technique has been advocated for painting Fe on the rice grain surface to minimize Fe loss from washing and/or cooking, these techniques are expensive and not practical in rice mills of developing countries. In comparison, parboiled rice is a rapid and cost-effective vehicle to deliver Fe nutrition benefits through the already established parboiling infrastructure, market network, and consumers' acceptance in Asia (particularly in the subcontinent) and Africa, where the high risk of Fe malnutrition-induced anemia is present (Graham et al. 1999). Most of the fortified Fe in the milled rice grains remained in the dilute acid-soluble pool, which is considered as potentially bioavailable in the human diet in all cultivars.

One of the significant advantages of parboiled rice is that parboiling resulted in a significant inward movement of the fortified Fe into the endosperm, countering milling-induced Fe loss in raw rice grains due to restricted distribution of Fe in the aleurone and embryo (the bran fraction) of brown rice (Bhattacharya 2004). Parboiling itself may cause inward migration of some mineral nutrients present in the surface layers of rice grain, resulting in a higher retention rate of these nutrients when being milled to produce white rice (Ali and Bhattacharya 1980; Palipane and Swarnasiri 1985), such as P, Ca, Fe, Mn, Mo, and Cr in milled parboiled rice.

1.3.8 Biofortification of Feed for Livestock

For many decades plant breeding primarily focused on yield, and little attention was given to the nutritional value of cereal residues (bran and straw) that were not used for human consumption. However, bran and straw are among the most important feed for ruminant livestock in many parts of the world, where sorghum and millet are important staple cereals in addition to rice and wheat. In sorghum and pearl millet, various crop management interventions (Reddy et al. 2003) and plant breeding (Zerbini and Thomas 2003) strategies were shown to influence yield and improve quality of the straw for animal feed. These efforts are now being complemented with the introduction of molecular markers (QTL mapping and MAS) as tools to increase breeding efficiency (Hash et al. 2003). Progress has also been reported for a completely different approach to improving cereal nutrient availability. In transgenic animals like the Enviropig, phytase is produced in the salivary glands and the active enzyme is secreted into the saliva (Forsberg et al. 2003). These pigs show improved phosphorus uptake. Such a transgenic pig might also show improved iron uptake in the intestine, owing to lower content of the antinutritional phytate.

1.4 Hindrances or Limiting Factors

1.4.1 Antinutrients

Phytate and tannins are the limiting factors in the absorption of Fe, Zn, and Ca by the gut (Mendoza 2002). Phytate occurs widely in plant tissues but is concentrated in seeds or grain. There is considerable intraspecific variation in phytate concentration in edible portions (Glahn et al. 2002; Coelho et al. 2005) that is independent of variation in Fe and Zn concentrations. In addition, several low phytic acid (lpa) mutants have been produced by non-transgenic techniques in rice, maize, wheat, barley, and soybean (Banziger and Long 2000). Fortuitously, plants with lpa mutations often show raised levels of seed Fe, Zn, and Mg (or similar levels to those found in wild type), although they do have reduced concentrations of seed Ca. Tannin concentration in edible tissues also varies greatly between varieties (Lin et al. 2005). Hence, breeding for reduced concentrations of these antinutrients appears feasible.

Phytic acid, as well as other metabolites produced by plants, such as PP, is considered an "antinutrient" because by chelating iron, it can reduce its absorption in the human gut (Jin et al. 2009). In plants, however, phytic acid fulfills essential biological functions (Murgia et al. 2012). Phytate, a mixed cation salt of D-myo-inositol, hexakisphosphate, commonly known as PA, InsP6, or IP6, constitutes up to 1–8 % of mature seed dry weight and accounts for up to 90 % of phosphorus content in cereal grains, legumes, nuts, and oil seeds. Phytate represents an important metal cation reserve (magnesium, potassium, calcium, manganese, barium, and iron) in seeds, either in the aleurone cell layer or in the seed embryo, depending on the plant species. Degradation of phytate occurs during seed germination, by means of phytases, a class of phosphatases capable of releasing at least one phosphate from phytic acid (IP6) (Bohn et al. 2008). The consequent release of phosphorus and mineral nutrients supports growth and development of the seedling. Besides its well-known role in mineral storage in seeds, IP6 also acts in the leaves in the signaling cascade triggered by drought/osmotic stress leading to stomatal closure. In guard cells ABA produces rapid changes in IP6 which trigger release of Ca2+ from endomembrane stores and inhibition of K+ inward rectifying channels. Some low IP6 cereals are less tolerant to stress and possess undesirable agronomical traits, such as reduced seed yield and lower seed viability, as observed in bread wheat (*Triticum aestivum*), rice (*Oryza sativa*), and barley (*Hordeum vulgare*) (Zhao et al. 2008), suggesting that IP6 is indeed involved in essential biological functions in the whole plant. More recently, a key role for IP6 in the maintenance of basal resistance against a wide range of pathogens has been demonstrated transgenic potatoes with compromised synthesis of IP6 (through IPS antisense RNA) are less resistant to virus infection. Disruption of IP6 biosynthesis in *Arabidopsis* is also associated with increased susceptibility to viruses and to bacterial and fungal pathogens (Murphy et al. 2008).

1.4.2 Promoters

Some organic compounds stimulate absorption of essential mineral elements by humans (Table 1.1). These include ascorbate (vitamin C), b-carotene (provitamin A), protein cysteine, and various organic and amino acids. There is considerable intraspecific variation in both ascorbate and b-carotene concentrations in fruit and vegetables (Frossard et al. 2000). For example, ascorbate concentration in cassava varied by 250-fold in leaves and 40-fold in roots among the 530 accessions of the CIAT core collection, whereas b-carotene concentration varied by 3.7-fold in leaves and 10-fold in roots. Similarly, ascorbate concentration varied almost 20-fold among *Dioscorea alata* accessions. There is also appreciable intraspecific variation in amino acid concentrations in edible tissues (Guzman-Maldonado et al. 2000). However, the complement of amino acids present in different foodstuffs is constrained by evolutionary heritage such that cereal and vegetable crops contribute complimentary amino acids to the diet (White and Broadley 2005).

Table 1.1 Mineral nutritional enhancers and antinutrients

Nutritional enhancers	Antinutrients
b-Carotene (provitamin A)	Oxalic acid (oxalate)
Inulin	Phytic acid (phytate)
Long-chain fatty acids	Polyphenols
Certain amino acids (cysteine, lysine, etc.)	Tannins
Certain organic acids (ascorbic acid, citrate, etc.)	Others
Vitamin D	Others

Adapted from White and Broadley (2005); Welch and Graham (2005)

1.5 Assessing Iron Bioavailability from Biofortified Foods

Iron bioavailability from biofortified food can be assessed through four different approaches: (i) algorithmic approximations, (ii) in vitro digestion and Caco-2 intestinal cell uptake assay, (iii) animal studies, or (iv) human studies (Cockell 2007; Fairweather-Tait 2001). The algorithmic method is the least suited to predict the effects of new circumstances, such as the nutritional impact of a new biofortified crop. By contrast, the choice between the remaining methods should take into account experimental costs, short- and long-term responses, and differences in iron absorption between laboratory animals and humans (Cockell 2007; Walter et al. 2003). It is however important to note that at least two types of human bioassays for bioavailability have qualified as necessary: efficacy and effectiveness trials. Efficacy trials assess the beneficial effect of iron biofortification under ideal conditions and thus depend only on biological factors (such as in clinical trials); effectiveness trials take into account behavioral factors, because trials are performed under "real-life situations" (Davidsson and Nestel 2004).

1.6 Biofortification: Strategic Advantages

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 the one-time investment is made to develop 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, the biofortified crop system is highly sustainable. Nutritionally improved varieties will continue to be grown and consumed year after year, even if government attention and international funding for micronutrient issues fade. Moreover, biofortification provides a truly 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. Breeding for higher trace mineral density in seeds will not incur a yield penalty. In fact, biofortification may have important spin-off effects for increasing farm productivity in developing countries in an environmentally beneficial way. Mineral-packed seeds sell themselves to farmers because, as recent research developments proved that seeds rich in trace elements are stronger to resist against biotic and abiotic stresses including diseases and environmental stresses (Bouis 2003). Further, fortified or enriched seeds also have more plant vigour, seedling survival, faster initial emergence and grain yield.

1.7 Future Challenges

- Produce crops for human nutrition with increased iron concentration. Biofortification strategies alternative to reduction in concentration of phytic acid or polyphenols should be explored further, in order to increase iron absorption without loss of their beneficial effects. When overexpressing ferritin, such crops should be tested for concentration of various heavy metals, in laboratory as in open-field trials, before releasing to the public. Detailed knowledge on mechanisms regulating iron compartmentalization in various plant organs will offer a major contribution for reaching such goal.
- Expand research on prebiotics and iron absorption. Crops biofortified with prebiotics have the potential to partially circumvent the "iron paradox" caused by host–pathogen competition for iron, by favoring amelioration of gut health and gut-associated immune defense.
- Promote initiatives supporting large-scale prospective studies on the effects of iron biofortified crops on effectiveness of the adopted biofortification strategy in relieving iron deficiency anemia and in improving general health.
- Improve the efficiency with which minerals are mobilized in the soil.
- Improve the efficiency with which minerals are taken up from the soil into the roots of the plant.
- Improve the transport of minerals from the roots to storage tissues, such as grain.
- Increase the capacity of storage tissues to accumulate minerals in a form that does not impair plant vegetative growth and development, but remains bioavailable for humans.
- Reduce the level of antinutritional compounds such as phytic acid, which inhibit the absorption of minerals in the gut.

Glossary

Anemia condition in which the number of red blood cells or their oxygen-carrying capacity is insufficient to meet physiological needs. The number of red blood cells is dependent on age, gender, and altitude and is altered by smoking, or during pregnancy the hemoglobin threshold used to define anemia is <11 g/dL

Antinutrient a substance that impairs the absorption of an essential element by the gut

Bioavailability measure of fractional utilization of orally ingested nutrient and also defined as the proportion of a particular nutrient that can be used by the body to provide its associated biological function. In simple words it is the amount of an element in a food constituent or a meal that can be absorbed and used by a person eating the meal. Bioavailability also refers to the portion of an ingested nutrient that can be absorbed in the human gut. The bioavailability of minerals can be reduced or enhanced by the consumption of food rich in antinutrients (inhibitors of absorption) or nutritional enhancers, respectively

Biofortification process for improving the nutritional value of the edible parts of the plants, through mineral fertilization, conventional breeding, or transgenic approaches. It can also be defined as the process of increasing the bioavailable concentrations of an element in edible portions of crop plants through agronomic intervention or genetic approaches

Disability-adjusted life year (DALY) a time-based parameter for assessing global burden of disease that combines years of life lost due to premature mortality (years of life lost, YLL) and years of life lost due to time lived in states of less than full health (years lived with disability, YLD): DALY = YLL + YLD

Hidden hunger the term "hidden hunger" has been used to describe the micronutrient malnutrition inherent in human diets that are adequate in calories but lack vitamins and/or mineral elements

Hunger the physical sensation of desiring food. When politicians, relief workers, and social scientists talk about people suffering from hunger, they usually refer to those who, for sustained periods, are unable to eat sufficient food to meet basic nutritional needs

Food fortification with iron a way to increase iron concentration in food by adding an iron compound (e.g., ferrous sulfate heptahydrate, ferrous gluconate, and sodium FeEDTA among others) to processed food (e.g., infant formula or cereals, wheat flour products, and corn meal among others)

Fortification the addition of an ingredient to food to increase the concentration of a particular element

Malnutrition the condition that results from eating a diet in which certain nutrients are lacking, in excess (too high in intake), or in the wrong proportions. The verb form is "malnourish"; "malnourishment" is sometimes used instead of "malnutrition." A number of different nutrition disorders may arise, depending on which nutrients are under- or overabundant in the diet. In most of the world, malnutrition is present in the form of undernutrition, which is caused by a diet lacking adequate calories and protein and not enough food, and of poor quality. Extreme undernourishment is starvation, and its symptoms and effects are inanition. While malnutrition is more common in less developed countries, it is also present in industrialized countries. In wealthier nations it is more likely to be caused by unhealthy diets with excess energy, fats, and refined carbohydrates (WHO 2001)

Promoter a substance that stimulates the absorption of an essential element by the gut

Reference nutrient intake (RNI) the amount of an element that is enough, or more than enough, for most people in a group (usually at least 97 %). If the average intake of a group is at the RNI level, then the risk of deficiency in the group is small

Regulators organic compounds, either natural or synthetic, are able to modify or control plant growth and/or development. Also, plant growth regulators (PGRs)

Supplementation the addition of an element to the diet to make up for an insufficiency

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