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REVIEW ARTICLE

Biofortification of Cereals for Enhanced Nutrition: Strategies, Status and Future Directions

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ABSTRACT
Biofortification is the idea of breeding crops to increase their nutritional value which is more beneficial to human race. This can be done either through traditional breeding or through innovative breeding techniques. Great achievements have been made from the last decade with respect to the application of biotechnology to generate nutritionally improved food crops. Biofortified staple crops such as rice, maize and wheat harboring essential micronutrients to benefit the world's poor are under development as well as new varieties of crops which have the ability to fight with the chronic diseases of human race. The implementation of agronomic biofortification of cereal crops with Fe, Zn, and Se appears to be a rapid and simple solution to the deficiency of these elements in soils and plants. These deficiencies are a reason for serious public health concerns. Low levels of Fe, Zn, and Se are important soil constraints to crop production, especially in the developing world. Iron and zinc are 2 important nutrients in the human diet. Their deficiencies in humans lead to a variety of health-related problems. Iron and zinc biofortification of cereals is considered a cost-effective solution to overcome the malnutrition of these minerals. in cereal grains. However, the genes underlying this variation have rarely been identified and never used in breeding programs. Genetically modified cereals developed by modulation of genes involved in iron and zinc homeostasis, or genes influencing bioavailability, have shown promising results. However, iron and zinc concentration were quantified in the whole grains during most of the studies. Food and nutrition security are intimately interconnected, since only a food based approach can help in overcoming malnutrition in an economically and socially sustainable manner. Food production provides the base for food security as it is a key determinant of food availability. By mainstreaming ecological considerations in technology development and dissemination, we can enter an era of evergreen revolution and sustainable food and nutrition security. Public policy support is crucial for enabling this.
***Key words**: Biofortification, cereal crops, zinc, iron, malnutrition, breeding approaches*

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INTRODUCTION

Biofortification is the idea of breeding crops to increase their nutritional value. This can be done either through conventional selective breeding, or through genetic engineering. Biofortification differs from ordinary fortification because it focuses on making plant foods more nutritious as the plants are growing, rather than having nutrients added to the foods when they are being processed. This is an improvement on ordinary fortification when it comes to providing nutrients for the rural poor, who rarely have access to commercially fortified foods. As such, biofortification is seen as an upcoming strategy for dealing with deficiencies of micronutrients in the developing world. In the case of iron, WHO estimated that biofortification could help curing the 2 billion people suffering from iron deficiency-induced anemia.

Iron and zinc deficiencies are widespread health problems. Iron deficiency is the most common nutritional disorder in the world, and almost 1.6 billion people are suffering from iron deficiency[1] Iron deficiency anemia is by far the most widespread micronutrient deficiency, and it results in impaired physical growth, mental development, and learning capacity [2] . Zinc deficiency is equally serious and is ranked as the 5th leading risk factor for diseases in the developing world [3]. Numerous health problems link zinc deficiency to retarded growth, skeletal abnormalities, delayed wound healing, increased abortion risk, and diarrhea [4]. Approximately one-third of the world's population is suffering from zinc deficiency. The situation is even more adverse in developing countries where more than half of the children and pregnant women are suffering from iron and zinc deficiencies [5]. This situation is largely attributed to the high consumption of cereal based foods, rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), and maize (*Zea mays* L.), in these countries [6]. Edible parts (endosperms) of modern cereal cultivars are inherently poor in iron and zinc. Iron and zinc concentration in whole grain of wheat are in the range of 29 to 73 mg/kg and 7 to 85 mg/kg, respectively. However, more than 75% of these nutrients is located in the seedy parts other than endosperm which is lost during milling [7]. The concentration of iron in the brown rice ranges from 6.3 to 24.4 mg/kg and zinc concentration ranges from 15.3 to 58.4 mg/kg [8]. However, polished rice, the principal form of rice consumed, on an average contains only 2 mg/kg iron and 12 mg/kg zinc. Apart from the cereals inherent inability to accumulate high iron and zinc, one major reason for their low accumulation in edible parts is the cultivation of cereals on zinc-deficient soils, particularly in developing countries like Pakistan, China, India, Iran, and Turkey [9]. For instance, in Turkey, zinc concentration in wheat grains grown on zinc-sufficient soils ranged between 20 and 30 mg/kg, whereas on the zinc-deficient soils this range fell enormously to 5 to 12 mg/kg [10].The Green Revolution is also considered to have contributed to the prevalence of these micronutrient deficiencies in soils because it promoted the use of high-yielding varieties, large-scale irrigation, and macronutrient fertilizers (nitrogen, phosphorus, potassium; [11]. It is considered that high yielding varieties led to the dilution effect of micronutrients due to increased 1000 kernel weights. The existence of a negative relationship between irrigation and iron and zinc uptake [12] and a similar negative relationship between phosphorus and iron and zinc uptake [13] also lead to lower the accumulation of these micronutrients in the cereal grains. Since the edible parts of the cereals are poor in iron and zinc, thus heavy dependence of people from developing countries on these foods results in the development of large-scale iron and zinc malnutrition. To alleviate iron and zinc deficiency, it is required to increase iron and zinc concentration in the endosperm to 8 and 30 mg/kg, respectively (www.harvestplus.org). Currently, there is growing concern to address micronutrient malnutrition through different interventions. Typically, these interventions are categorized into 4 major groups: pharmaceutical supplementation, industrial fortification, dietary diversification, and [14]. Iron and zinc pharmaceutical supplementation and industrial fortification are not considered cost-effective and only very few governments have the resources to finance such kinds of expensive interventions [14]. Moreover, several other issues such as negative interaction between iron and zinc bioavailability [15], frequency of supplementation, selection of food products to be fortified, impact of fortificants on taste, texture, and appearance of food, and availability of fortificant [16] need to be carefully addressed before undertaking these interventions. Dietary diversification offers greater prospects to overcome micronutrient malnutrition [17]. For instance, in India, Bangladesh, and Tanzania, small scale kitchen-gardening projects have shown promising results by diversifying cultivation and consumption of high β–carotene fruits and vegetables [18]. However, to achieve success on a large scale, poverty alleviation and change in food habits of high-risk groups, the rural poor, children under 5, and child bearing women, are prerequisites. [19] based on surveys in Bangladesh and the Philippines, showed that rich families already consume a diversified diet, whereas poor families do not have enough resources to purchase a diversified diet. Thus, considering the limitations linked to the above-mentioned 3 types of interventions to alleviate iron and zinc deficiencies, the biofortification of staple food crops is considered the most viable option to help alleviate iron and zinc malnutrition. We will discuss the opportunities and challenges associated with iron and zinc biofortification of cereals in this review. Iron and zinc biofortification of cereals aims at either increasing the accumulation of these minerals in edible parts or increasing their bioavailability. Currently, 2 research strategies to achieve this are mineral fertilization and crop biofortification. Mineral fertilization can mainly increase the accumulation of iron and zinc in edible parts, whereas crop biofortification has the potential to fulfil both aims. Both these strategies have their own advantages and limitations that will be discussed below.

MINERALS FERTILIZATION

Iron fertilization

Soil iron fertilization is believed to have little or no effect on iron concentration of grains [20] Thus much of the work has been focused to identify the effects of foliar application on iron accumulation in grains. As shown in Table 1 foliar application is reported to increase iron concentration by 20% to 70% in the grains of bread wheat [21] Recently, effects of foliar application of different forms of iron fertilizer at different plant developmental stages were studied in rice and it was shown that application of the DTPA-Fe form at the anthesis stage resulted in about 20% increase in iron content of polished rice grains [22] In addition to grain iron concentration, iron fertilization positively influences the grain zinc concentration in rice and wheat [23] . Zinc fertilization It is generally found that zinc deficiency in human beings is associated with zinc-deficient soils[22]. This led to numerous studies to identify the effect of soil or foliar zinc fertilization on grain zinc concentration under varied agro-ecological conditions. Soil application of zinc resulted in 20% to 90% and 60% to 250% increase in grain zinc concentration in bread wheat (*Triticum aestivum* L.) and durum wheat (*Triticum durum* L.), respectively [24]. Foliar application of zinc resulted in even a higher increase in grain zinc concentration than soil application in both bread and durum wheat [25, 26]. Studies of natural variation revealed the existence of notable differences for zinc accumulation in wheat grains between different wheat cultivars in response to soil and foliar application of zinc [26]. Moreover, the timing of foliar application of zinc was also found to be crucial in determining the wheat grain zinc contents. Foliar application of zinc around flowering time was shown to produce the highest increase in zinc contents in the endosperm of wheat grains [27] These findings suggest that both the zinc uptake and the remobilization are important factors that determine the zinc concentration in wheat grains, and natural variation for response to zinc fertilization is present in this species. Phloem is the only vascular tissue to reach the developing wheat grains, therefore zinc has to leave the xylem at some stage and become actively loaded into the phloem to reach the grains [28] The genetic and molecular mechanisms controlling this re-translocation from leaves to grains are still unknown, now considered a bottleneck to explain the natural variation of response to zinc fertilization between wheat cultivars, and to successfully exploit the potential of this mechanism to zinc biofortify wheat. In rice, the impact of soil or foliar application of zinc on increasing grain zinc concentration is not as strong as in wheat; generally, an increase in the range of 10% to 60% is found in grain zinc concentration in response to zinc fertilization [29]. Similarly, very low increase in grain zinc concentration (up to 40%) was observed after soil application of zinc fertilizer in maize [30]. Foliar application of zinc, besides influencing grain zinc concentration, could also increase iron concentration in wheat, rice, and maize grains [31], and reduce cadmium toxicity and accumulation in cereals grown on cadmium-contaminated sites. These findings strongly support the utilization of zinc fertilizer in cereal cultivation to produce zinc/iron-enriched cereals, but the success may depend upon the cultivar being used and agro-ecological conditions.

Breeding Approaches to Increase Prebiotics

Until now, very few studies have been performed to explore the natural variation for the accumulation of fructans in cereal grains and thereafter to dissect the genetic architecture of this trait. Wheat and rye are known to be better sources of fructans and contain 0.7% to 2.9% and 3.6% to 6.4% in grain (dry weight), respectively [32]. Further analyses revealed that inulin concentration of wheat grain varies from 0.4 to 14.6 mg/g dry weight [33]. Significant genetic variation in inulin concentration has also been reported in rice and maize, but their grain inulin concentration is far lower than those found in wheat and rye [32]. The highest rice variety contains approximately 7 times less inulin than the lowest wheat variety, and the highest maize variety contains 2 times less inulin than the lowest wheat variety. Genetic mapping studies were performed in wheat to identify the QTLs controlling the variation in grain fructan concentration [32] and specifically inulin concentration [33]. In total, 5 QTLs were found to be controlling the variation in grain fructan concentration in a doubled-haploid population of a cross between Berkut and Krichauff, two being the most important ones, located on chromosomes 6D and 7A, which explained 17% and 27%, respectively, of the total phenotypic variance [32]. A genetic mapping study performed by [33] using a doubled-haploid population generated from a cross between AC Reed and Grandin showed that the 2 QTLs controlling grain inulin concentration in wheat are located on chromosomes 2B and 5B and explained approximately 20% and 15%, respectively, of the total phenotypic variance. The presence of approximately 4-fold variation in the grain inulin concentration and the modest effect of QTLs controlling this variation indicate that inulin concentration can be significantly ameliorated in wheat grains through molecular breeding. Yet, numerous studies using different genetic backgrounds of wheat need to be performed under variable environmental conditions to identify robust QTLs that can be of interest to a wider scientific community. In the case of rice, an ideal starting genetic material could be a large set of rice accessions designed by [24] for which genotypic data are already available. These accessions can be phenotyped under variable environmental conditions to identify genomic regions controlling variation of grain inulin concentration through association mapping. The availability of Nested Assn. Mapping population in the case of maize [34] is considered highly valuable material to identify genetic determinants of inulin accumulation in maize grains. The way forward would be to identify the markers that can help molecular breeding programs aiming at increasing grain inulin concentration.

Genetic Engineering Approaches to Increase Prebiotics

As mentioned earlier, rice and maize varieties analyzed to date contain lower grain fructan, but it is believed that exploring new materials like landraces, wild relatives, and so on may help identify the sources that can be used in breeding programs to increase grain fructan content. As for now, rice and maize transgenics have been developed that can accumulate high amounts of fructan in grains. Fructans are produced by the combined action of various fructosyltransferases (FTs) [35]. The enzyme 1-SST catalyzes the initial fructosyl transfer between 2 sucrose molecules. Then FTs (1-FFT, 6G-FFT, 6-SFT, and so on) catalyze chain elongation adding β-(2→1)- or β-(2→6)-linked fructose units. Inulin-type fructan biosynthesis in plants is generally believed to occur through the collective action of 2 vacuolar enzymes, 1-SST and 1-FFT. The expression of these enzymes, 1-SST and 1-FFT, from Jerusalem artichoke (a high-inulin-accumulating plant) in high-sucrose maize under the control of an endosperm-specific promoter increased the fructan content by 2-fold [36]. Moreover, kernel development and seed germination of these transgenic maize plants were not hampered. The overexpression of 1-SST enzyme from Jerusalem artichoke as well as Yacon (another high-inulin-accumulating plant) in rice under the control of a constitutive promoter significantly enhanced the production of fructan in plant tissues of transgenic rice [35]. Constitutively, overexpression of 1-SST enzyme of wheat in rice could increase seed fructan of rice transgenics, with a slight decrease in seed weight [37]. It is likely that increasing fructan content could have a positive effect on iron and zinc absorption. Beside the nutritional aspects, agronomic performance of the plants should also be considered while developing high-fructan-accumulating cereals.

Increasing Grain Iron and Zinc Concentration

In order to increase the accumulation of iron and zinc in cereal edible parts, we need to have good understanding of physiological, genetic, and molecular mechanisms involved in iron and zinc homeostasis in plants. Numerous reviews have been published regarding iron and/or zinc homeostasis in plants [38]. Here, instead of discussing in detail the iron and zinc homeostasis networks in plants, attention will be focused on the strategies that can be chosen to develop cereal crops with a high accumulation of iron and zinc in their edible parts. Moreover, as there exists crosstalk between different minerals in crops which could be synergistic or antagonistic depending upon the minerals under consideration, we thus advocate looking into the opportunities and challenges related to accumulation of iron and zinc, and also other heavy metals such as cadmium and arsenic at the same time. The accumulation of arsenic is of particular concern in rice because this crop is mainly cultivated under anaerobic conditions where arsinite (As(III)) is more available [39].

Breeding Approaches to Increase Iron and Zinc Accumulation

Natural variation for accumulation of iron and zinc in grains of cereals has been explored to a great extent, and significant differences for iron and zinc concentration in grains of cereals have been observed. The grain iron, as well as zinc concentration, varies 2-fold in cultivated wheat species (*Triticum aestivum*, *Triticum turgidum* spp. *durum*) as well as in their wild relatives [40]. Compared to cultivated wheat, wild relatives belonging to the genus *Aegilops* can accumulate significantly higher iron and zinc in their grains [41]. Thus, it could be hypothesized that synthetic hexaploid wheat, developed by crossing tetraploid wheat cultivars with diploid wild relatives, would contain higher iron and zinc in the grains. Synthetic hexaploids developed by crossing *Triticum turgidum spp. durum* with *Ae. tauschii* indeed accumulated about 30% higher iron and zinc in the grains [42]. Unfortunately, grain yield of these synthetics was reduced by about 25%, which is by no means desirable for farmers. Such negative correlations between iron and zinc concentration and grain yield have been reported in numerous studies, although the strength was greatly influenced by the environment [43]. Several QTLs controlling iron and zinc concentration in wheat grains have been mapped using RIL or DH population issued from crosses of different parents [44]. Remarkably, 3 of these QTL mapping studies, performed over different years and under various agro-ecological conditions, revealed a common QTL on chromosome 7A around 70 centimorgan to be contributing about 10% of the phenotypic variance to the grain iron and zinc concentration. Further, positional cloning of Gpc-B1, a wheat quantitative trait locus associated with increased iron and zinc concentration, identified a no-apical meristem (NAM) transcription factor to be responsible for this variation [45]. QTL mapping studies have also been performed in rice using RIL populations [46]. QTLs affecting both iron and zinc concentration in grains are more often co-localized on chromosome 7 and chromosome 12 in rice. The genes underlying these QTLs have not yet been identified. Co-localization of QTLs affecting grain iron and zinc concentration has also been found in maize mapping populations [47]. These are very encouraging findings because it suggests that iron and zinc concentration in cereal grains can be increased simultaneously by exploiting the same chromosomal regions in MAS. However, QTL mapping studies performed until now have only addressed variation for accumulation of iron and zinc in whole-grains, and none of the QTL mapping studies has been undertaken to identify the QTLs controlling variation for the accumulation of these minerals in the endosperm of cereals. It is important to note here that more than 75% of minerals accumulated in whole grain are lost during milling [48]. Moreover, the accumulation of these minerals in grains of cereals is significantly influenced by the environment [47]. Thus, efforts are required to map QTLs controlling iron and zinc accumulation in endosperm of cereal grains, and to identify genetic determinants and developing markers that would be of use in various genetic backgrounds and under variable environmental conditions. An important mechanism that scientists may focus to exploit is transgression, because positive transgressive segregants that could accumulate about 2-fold higher iron and zinc in the grains than the highest parent were consistently found in almost all of the above-mentioned QTL mapping studies, which indicates that it is possible to increase the concentration of these minerals beyond what is found in the parents. In addition, characterizing cereal germplasms, including landraces and wild relatives, can also help finding genotypes with higher iron and zinc concentration that can be used in breeding programs.

Advantages of Biofortification

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 fades. Biofortification provides a truly feasible means of reaching malnourished populations in relatively remote rural areas, delivering naturally fortified foods to people with limited access 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 spinoff effects for increasing farm productivity in developing countries in an environmentally-beneficial way. Mineral-packed seeds sell themselves to farmers because, as recent research has shown, these trace minerals are essential in helping plants resist disease and other environmental stresses. More seedlings survive and initial growth is more rapid. Ultimately, yields are higher, particularly in trace mineral "deficient" soils in arid regions.

Extent and Underlying Cause of Micronutrient Malnutrition

Billions of people in developing countries suffer from an insidious form of hunger known as micronutrient malnutrition. Even mild levels of micronutrient malnutrition may damage cognitive development, lower disease resistance in children, and reduce the likelihood that mothers survive childbirth. The costs of these deficiencies in terms of lives lost and poor quality of life are staggering.

It is important to identify who the malnourished are, where they are located, and what they eat in order to develop an effective strategy to reduce micronutrient malnutrition. We know, for example, that they are mostly women and children whose nutritional requirements are highest due to reproduction and rapid growth, who reside in developing countries where dietary quality is often poor.

Extent of Micronutrient Malnutrition

Iron deficiency anemia is by far the most common micronutrient deficiency in the world. Iron deficiency during childhood and adolescence impairs physical growth, mental development, and learning capacity. In adults, iron deficiency anemia reduces the capacity to do physical labor. Iron deficiency increases the risk of women dying during delivery or in the postpartum period. Estimates of prevalence of anemia by region for 1980. Though these figures also represent anemia brought on by infections, illness, and genetic factors, iron deficiency is by far the most important cause of anemia. Prevalence among children exceeds 50 percent in South and Southeast Asia, where 1.8 billion out of the approximately 4.5 billion people in developing countries live. Prevalence is equally high in Africa, although the number of persons affected is smaller. Prevalence is consistently highest for pregnant women and consistently lowest for adult males. Zinc deficiencies have equally serious consequences for health. For example, meta-analyses of recent randomized controlled trials show that zinc supplementation can reduce morbidity from a number of common childhood infections, especially diarrhea, pneumonia, and possibly malaria, by one-third [49]. In addition, zinc deficiency is an important cause of stunting.

Billions of people are also at risk for zinc deficiency. As for anemia, prevalence is highest for South and Southeast Asia and Africa. Because there is no widely accepted method for measuring of zinc deficiency, no estimates are available of numbers of people who are zinc deficient. Globally, approximately 3 million preschool-age children have visible eye damage owing to vitamin A deficiency. Annually, an estimated 250,000 to 500,000 preschool children go blind from this deficiency, and about two-thirds of these children die within months of going blind. Even more importantly, the last two decades have brought an awareness that vitamin A is essential for immune function. Estimates of the prevalence of subclinical vitamin A deficiency range between 100 million and 250 million for preschool children. People with subclinical vitamin A deficiency more often experience anemia, impaired linear growth [49] and morbidity from common childhood infections such as respiratory and diarrheal diseases and malaria. Most importantly, a number of randomized controlled trials in developing countries have shown that administration of vitamin A capsules among infants and preschool children helps reduce mortality rates from all causes by 23 percent [49], and that administration of capsules with vitamin A and beta-carotene among women during childbearing years can reduce maternal mortality related to pregnancy by 40 percent and 49 percent, respectively. Prevalence of vitamin A deficiencies by region is available only for preschool children. Again, similar to iron and zinc, prevalence is highest in South and Southeast Asia and Sub-Saharan Africa.

Cause of Micronutrient Malnutrition

The primary underlying cause of micronutrient malnutrition is poor quality diets, characterized by high intakes of food staples, but low consumption of animal and fish products, fruits, lentils, and vegetables, which are rich sources of bioavailable minerals and vitamins. As such, most of the malnourished are those who cannot afford to purchase high-quality, micronutrient-rich foods or who cannot obtain these foods from their own production. For rural Bangladesh are typical of diets and food expenditures for those who suffer from micronutrient malnutrition. Staple foods (overwhelmingly rice in this example) account for 80 percent of total per capita energy intakes. Animal and fish products are dense sources of bioavailable micronutrients that the poor wish to eat but cannot afford in large quantities. They account for 25 percent of total food expenditures but only 3 percent of total energy. Nonstaple plant foods such as fruits, vegetables, and lentils are also rich sources of vitamins and minerals. Together, nonstaple plant foods and animal and fish products account for only 20 percent of total energy intakes but 60 percent of food expenditures. What is perhaps most alarming, however, is the upward trend in nonstaple food prices. Cereal prices have fallen by 40 percent since the early 1970s. The Green Revolution can rightly take credit for its crucial contribution to this tremendous achievement. Falling cereal prices have not only led to increased food security in terms of energy, but also allowed greater purchases of nonstaples by freeing up cash. Unfortunately, however, the rate of production of nonstaple foods (e.g. fruits, vegetables, lentils) has not kept pace with demand, so that these micronutrient-rich food sources have become increasingly expensive for the poor.

PLANT BREEDING ACTIVITIES

Conventional

The target micronutrients to be improved are iron, zinc, and beta-carotene. Breeding activities include:

Micronutrient Bioavailability and Determination of Optimal Breeding Strategies

There is evidence that micronutrients may interact with each other and with other nutrients in their absorption and conversion to biologically active compounds in the human body. Such interaction may be either synergistic (for example, fat may possibly increase absorption of beta-carotene) or antagonistic (for example, iron from supplements appears less effectively absorbed when these supplements are concurrently taken with zinc supplements). The most convincing evidence for such interactions comes from physiological studies, but there is insufficient knowledge whether the mechanisms involved are important under real-life conditions. Most importantly, randomized controlled trials have conclusively shown that improved health can be achieved by increasing intake using single nutrients. Many other factors influence the degree to which ingested micronutrients are absorbed (bioavailability) and utilized (bioefficacy), including a person's micronutrient and health status. Given this complexity, three breeding sub-strategies may be applied individually or in various combinations. These are (1) increasing the mineral and vitamin content, (2) reducing the level of anti-nutrients in food staples that inhibit the bioavailability of minerals and vitamins, and (3) increasing the levels of nutrients and compounds that promote the bioavailability of minerals and vitamins. For example, phytates and tannins inhibit iron and zinc absorption. Proposed in vitro and animal studies and efficacy feeding trials will evaluate effects of these inhibiting compounds. With respect to compounds that promote bioavailability, certain amino acids (such as cysteine) enhance iron and/or zinc bioavailability [50]. These amino acids occur in many staple foods, but their concentrations are lower than those found in meat products. A modest increase in the concentrations of promoter amino acids in plant foods may have a positive effect on iron and zinc bioavailability in humans. Iron and zinc occur only in micromolar amounts in plant foods, so only micromolar increases in the amounts of these amino acids may be required to compensate for the negative effects of anti nutrients on iron and zinc bioavailability. These amino acids are essential nutrients for plants as well as for humans, so relatively small increases of their concentrations in plant tissues should not have adverse consequences on plant growth [50]. While the Biofortification Challenge Program pursues breeding for higher micronutrient content as its initial breeding objective, at the same time an effort will be made to investigate the possibly large nutritional impacts of the extended breeding strategies suggested above. This component of the project explores genetic variation in nutrient content in staple crop plants and the interactions among nutrients in animals and humans that can dictate improved strategies of plant breeding for nutritional quality. The idea is to exploit potential synergies in the human body to simplify the breeding objectives by decreasing the number of genes needed in a breeding program, thereby optimizing the cost-effectiveness of the strategy. The results of the research from this component of the project will inform breeding strategies for all crops.

- ✓ Additional germplasm evaluation of materials with potential for short-term impact;
- ✓ Implementation of molecular markers for large-scale selection programs;
- ✓ Genetic and QTL analyses to determine loci involved in micronutrient content; and
- ✓ Development of varieties with high micronutrient concentration and superior agronomic traits, in collaboration with NARES and farmers' groups.

Breeding has been the strength of the CGIAR centers since their inception, as a logical extension of the gene banks that are held in the centers. For every crop listed in this project, significant success has been obtained in the areas of disease resistance, tolerance to abiotic stresses and/or yield potential. Productivity of several crops has been increased dramatically, and these objectives continue to be important as breeders look to the future. From this perspective, can breeders assume still another breeding objective? Breeders involved in this project do not feel that this is unrealistic. The experience in IRRI's rice program suggests that a moderate level of iron was achieved even without directed screening in a few modern varieties. This modest success can be greatly amplified by breeding consciously and systematically for higher micronutrients, thus exploiting opportunities that have been observed in the germplasm where much greater levels are to be found. "Conventional" breeding is made even more efficient by integrating a set of tools such as: markerassisted selection for key agronomic and nutritional traits; molecular biology for gene discovery of relevant traits; and transgenics to increase existing levels or provide opportunities that are lacking within the respective crop species.In the final analysis, the requirement is that 3-4 genes be added to the suite of genes that breeders successfully work with in their breeding programs. This is totally feasible with the tools described above, and breeders feel comfortable that they can increase levels of micronutrients while continuing to make gains in agronomic breeding objectives. Materials to be released will have superior agronomic traits to facilitate adoption by farmers. QPM maize is now fully competitive with standard maize varieties and often out yields these, making them attractive to producers. Biofortified maize will build on progress in QPM and expand these successes to higher levels of iron and zinc. Progress has been registered in beans for superior drought tolerance and this in turn will be the basis for new varieties with better nutritional quality. Results for several crops show that there is no penalty in yield or other traits from increasing micronutrient content. For most crops, selection of specific sub-regions for which initial nutritionally improved varieties will be developed and disseminated, is still to be refined based on levels of malnutrition, diets, prospects for successful breeding, and opportunities for establishing effective partnerships. These decisions will be taken after start-up of the Program in full consultation. Prebreeding studies will be undertaken over three to four years to determine the feasibility and desirability of pursuing full-scale breeding activities for these crops. Analyses of the mineral and vitamin content of crop samples will be processed at a central collaborating partner facility to ensure quality control and comparability of measurements among crops. Based on rigorous peer review of the results of the prebreeding studies, additional funding may be sought to pursue a full breeding program for those crops with demonstrated potential for significant impact. The University of Adelaide's Plant Nutrition Group members (Robin Graham and James Stangoulis) will provide micronutrient expertise to crop leaders, particularly those of the Tier 2 crops, through regular visits, newsletters, relevant literature, and a laboratory and fieldwork handbook for micronutrient research in plant breeding and agronomy. Additionally they will provide support in data evaluation and interpretation, as well as provide strategic research essential to strategy development. Waite Analytical Services (WAS) will provide, at cost, analysis of grain and other food crop products involved in the Challenge Program, maintaining quality assurance standards certified by ASPAC. These analyses include all essential minerals, and fat-soluble vitamins.

Nutritional Genomics Activities

Nutritional genomics is a new approach to the study of complex biochemical pathways in plants. It seeks to elucidate the basic, underlying mechanisms involved in the synthesis and accumulation of essential vitamins and minerals in plant tissues. Because of the metabolic unity among organisms through evolution, the knowledge obtained in one organism by using this approach can be readily applied to many organisms. As a component of the overall biofortification research program, nutritional genomics will leverage the massive knowledge base resulting from the enormous investment of the developed world in

DNA sequencing of entire organisms (plants, bacteria, fungi, and animals). This knowledge base will be used to increase specific micronutrient levels in crops that will be of most benefit to the needs of the developing world. Once the genes of interest are identified, they are moved into a crop species to demonstrate proof of concept to effect the desired change in nutritional content of the target tissues. This process can provide novel traits for breeding that are not available in the existing germplasm (betacarotene in rice endosperm, for example). The expanding ability to manipulate pro-vitamin A carotenoid synthesis (such as in Golden Rice), vitamin E synthesis, and mineral composition in plants can be directly traced to advancements in nutritional genomics and exemplifies the power and potential benefits of the approach.

Activities to be undertaken in this project area aim to:

- ✓ Leverage and integrate new methods in genomics, genetics, and molecular biology to identify and understand plant biosynthetic genes of nutritional importance, specifically those related to zinc, iron, and vitamin A;
- ✓ Demonstrate proof of concept nutritional enhancements by engineering genes involved in the biosynthesis of essential vitamins and accumulation of essential minerals;
- ✓ Analyze the consequences of proof of concept enhancements on nutrients and bioavailability;
- ✓ Transfer proven materials to partner breeding centers for implementation in Phase 1 and 2 crops;
- ✓ Assist with analysis of micronutrient composition and agronomic traits; and
- ✓ Aid breeders in identifying molecular markers to nutritionally important genes for incorporation into molecular breeding programs.

On-Farm Testing and Adaptation

The on-farm testing phase is crucial, especially in regions and for crops for which adoption rates of modern varieties have been low. Simply, if the varieties are not grown or appreciated by farmers (widely and by large numbers), the positive impacts will not be achieved. The on-farm testing phase will have three basic aims: to assess whether varieties grow under farmer conditions and management; to get precise feedback from the range of potential producers on the acceptability of materials; and to give farmers exposure to what, in some cases, may be quite novel materials (e.g., yellow varieties where white varieties are preferred).

The Scope of Research on Transgenic Crops

Breeding, dissemination, and impact activities, outlined in the ten-year plan, are focused on development of conventionally-bred crops. No activities involving the release of nutritionally-improved transgenic crops to farmers and consumers are proposed here or are included in proposal budgets for the initial four years for which funding is being requested. Research and development activities with respect to transgenic crops are confined to agricultural research centers and research laboratories. Transgenic methods hold great promise for improving the nutrient content of staple foods and speeding up the breeding process over what can be achieved using conventional methods. High social benefit and lower risk applications, such as the incorporation of desirable traits from crop wild relatives, will be favored throughout the program whenever transgenic methods are considered. The biofortification challenge program will implement a wide range of technologies including germplasm characterization, breeding, molecular marker assisted breeding and genetic transformation technologies. The mix and extent to which each of these technologies is used will vary by crop (see crop appendixes for further information). The first phase of the project will mainly involve germplasm deployment of land races and breeding genotypes. Genetic transformation technologies will be used in the first phase for research purposes to a better understanding of the targeted nutritional pathways resulting in better screening and to develop efficient genomic tools for marker assisted selection and breeding; and in the second and third phases for potential seed deployment by increasing the level of a trait beyond what is available in the gene pool and by providing traits not present in the gene pool. The project participating institutions have established strict regulations to develop and deploy genetically modified organisms consistent with the CGIAR policy. All institutions involved have well-established guidelines for biosafety related issues and will pursue rigorous scientific processes to ensure the safe use of transgenic plants when there is a high social benefit. Components of the project that involve genetically transformed plants will strictly adhere to international safety standards and the national regulations of the partner countries. As part of their research agenda, several institutions involved in the project are conducting gene flow, risk assessment project and food safety studies. Results from such studies will be incorporated during the implementation of the program. The Centers will distribute with caution and consultation/approval of civil society transgenic materials for experimental purposes with advanced informed consent only to countries where national biosafety legislations are in place. Development of Golden Rice is much further ahead of any other nutritionally -

improved transgenic crop. Research activities related to Golden Rice will be undertaken under the purview of the Biofortification Challenge Program and will adhere to the guidelines set forth above. Research will be undertaken during Phase 1 at IRRI and other collaborating research institutions to evaluate the agronomic and nutritional properties of various existing lines, and to develop experimental lines with even higher levels of beta-carotene. Initial evaluations of the bioavailability of beta-carotene in Golden Rice using human subjects is not expected to be undertaken until 2004. Decisions concerning possible release to farmers and consumers, therefore, can only be taken well after the first four years for which funding is being sought, pending results of an array of agronomic, nutritional, and safety tests.
Rigorous designs for Participatory Plant Breeding (PPB) and Participatory Varietal Selection (PVS) are well known among the groups. These will be employed if deemed technically useful and of interest to the potential stakeholders involved. It may be that on-farm and extensive community-based collaboration could be beneficial quite early in the breeding process, with early stabilized or segregating materials. This will greatly depend on the local context, the challenges adaptation, the stringency of producer/consumer requirements, and the diversity of materials on offer. On-farm research and development (R&D) remains a program component of many NARS' partners in this proposal and is supported by their collaborating commodity networks (for example, the African bean, cassava, and sweet potato networks). However, staffing and operating costs constraints have been progressively reducing the scale of on-farm research, particularly in some of the key Latin American and African target countries of this biofortification work. While there is a growing body of experience to work with and through these resource constraints, we aim to stimulate further creative on-farm R& D relationships, starting from the strengths of NARS and moving to joint work with, inter alia, civil society groups, farmers' organizations, agricultural schools, and even churches (when technically appropriate and mutually beneficial).

CONCLUSION

Biofortification of cereals is the most promising intervention to overcome iron and zinc malnutrition due to its cost-effectiveness. Bioavailability of iron and zinc from cereal grains can be increased by genetic interventions by modulating accumulation of either anti-nutrient agents or prebiotics. Encouraging results have been obtained in this regard in model experimental subjects like Caco-2 cell lines, but studies involving human beings as subjects are largely lacking, thus making it difficult to realistically assess the success of this approach. Enhanced accumulation of iron and zinc in cereal grains can be achieved by fertilization or genetically manipulating iron and/or zinc homeostasis-related genes. However, it is not fully known whether it is feasible to increase the accumulation of these minerals up to desired levels in the edible parts of cereals by these strategies under field conditions. Moreover, it is not clearly identified how agronomic or genetic biofortification interventions affect the accumulation of toxic heavy metals such as cadmium and arsenic in edible portions of cereal grains. Future research should involve analyzing the accumulation of iron and zinc, as well as other heavy metals, in the edible parts of cereal grains rather than whole grains. It is also advised that the impact of genetic modifications on the agronomic performance of crops, including grain yield, drought tolerance, insect resistance, disease resistance, and so on should also be assessed. In addition, the focus should be on studies involving field crop trials and human beings as experimental subjects to analyze the effectiveness of agronomic or genetic biofortification.

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