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Mineral Biofortification Strategies for Food Staples: The Example of Common Bean

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ABSTRACT: Common bean is the most important directly consumed legume, especially in the least developed countries of Africa (e.g., Burundi, Democratic Republic of Congo, Rwanda, and Uganda) and Latin America (e.g., Guatemala, Nicaragua, and El Salvador). Biofortification is the process of improving staple crops for mineral or vitamin content as a way to address malnutrition in developing countries. The main goals of mineral biofortification have been to increase the concentration of iron or zinc in certain major cereals and legumes. In humans, iron is essential for preventing anemia and for the proper functioning of many metabolic processes, whereas zinc is essential for adequate growth and for resistance to gastroenteric and respiratory infections, especially in children. This paper outlines the advantages and needs of mineral biofortification in common bean, starting with the steps of breeding for the trait such as germplasm screening, inheritance, physiological, or bioavailability studies and finishing with product development in the form of new biofortified varieties.

KEYWORDS: *grain legumes, Phaseolus vulgaris, cereal, nutritional improvement, molecular breeding*

INTRODUCTION

Advantages of Common Bean for Mineral Biofortification. Common bean (*Phaseolus vulgaris* L.) is one of five cultivated species from the genus *Phaseolus* and is a major grain legume crop, third in importance after soybean and peanut, but first in direct human consumption.[1] Common beans originated in Latin America and have two primary centers of origin in the Mesoamerican and Andean regions that are easily distinguished by molecular means including phaseolin seed protein markers using SDS-PAGE and microsatellite fingerprinting using simple sequence repeat based DNA markers that are detected with silver stain gels or fluorescently.[1−4] Major producing countries for national consumption are Brazil and Mexico, whereas the United States, Canada, Argentina, and China are all exporting countries; beans are consumed around the world. The crop is also important in a range of developing countries of Central America, of the Andean region of South America, and of eastern and southern Africa.[3] Common beans are especially important in the countries of Burundi, Rwanda, and Uganda in Central Africa.[4] In these regions, beans are grown both for subsistence agriculture and for regional markets, where they play an important role in food security and income generation. Overall, common beans are important for nutritional well-being as well as poverty alleviation among consumers and farmers with few other food or crop options. Much of the world's bean production is on small farms ranging from 1 to 10 ha in size. Multiple commercial seed types or horticultural classes exist based on seed color, with white, yellow, cream, brown, pink, red, purple, black and mottled, pinto, stippled, or striped seed types popular in different regions of the world and with different cultures.[5,6]

Per capita consumption varies with each producing and consuming country and also among regions within a country depending on consumer preferences but can be as high as 66 kg/capita/year in Rwanda and parts of western Kenya.[1] Averages in the Americas are from 4 to 5 kg/capita/year in the United States to >10 kg/capita/year in Brazil to as much as 35 kg/capita/year in Nicaragua. These quantities of beans can provide substantial amounts of both protein and calories in the diet. In nutritional terms, beans are often called the "poor man's meat" for their inexpensive price as a protein source and their rich content of minerals (especially iron and zinc) and vitamins.[7]

In production terms, two general types of common beans are grown: bush beans, as a short-season crop, and climbing beans, as a long-season crop.[6] Bush beans produce a crop in as little as 65 days and may yield up to 2.5 t/ha per season (although average yields in Latin America are between 600 and 800 kg/ha, and yields in eastern and southern Africa are lower still). Climbing beans, on the other hand, have a slightly longer growing season (100−120 days; some even up to 240 days) and have a yield potential of 4.5 t/ha.[1] One advantage of bush beans over climbing beans is that in tropical regions two seasons might be grown; however, early-maturing climbing beans with adaptation to lower elevations have the potential to be grown in two seasons as well.[4,5] Bush and climbing beans in small farmer fields are often intercropped or used as a relay crop and planted at the end of the season to take advantage of residual moisture in the soil and are often not captured by official statistics.[1] One advantage of climbing beans over bush beans is that they fix larger amounts of nitrogen.[6]

As mentioned earlier, common bean is a valuable source of protein, minerals, and vitamins. In terms of biofortification, improvement of mineral content is advantageous precisely

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because the baseline grain iron content is high at 55 ppm (ppm) and variability for the trait is great, ranging up to 110 ppm, allowing initial breeding attempts to be much more successful than in the cereals in overall iron and zinc content increases.[8,9]

Estimates for the Harvest Plus challenge program on biofortification are that an addition of approximately 40 ppm to baseline iron levels in common bean can meet a large proportion of the recommended daily intake of iron.[10,11] This target level takes into account the amount of beans consumed by the undernourished, any loses during storage, cooking, and processing, and the extent to which humans will take up and absorb the extra iron. Given a diet-based approach of combining several biofortified foods, for example, high-iron beans and high-iron rice or maize, this level could be even more rapidly enhanced. The target areas for biofortified beans are in iron deficiency anemia prone areas of Latin America and eastern and southern Africa, where the crop is important and consumption rates are high, such as Central America, northeastern Brazil, and the Great Lakes region of Africa.[1,4]

Principals of Mineral Biofortification for Legumes. Biofortification is the process of breeding for improved nutrient content in a crop and is considered a sustainable and cost-effective strategy to address malnutrition in developing countries because it targets staple foods that are consumed daily.[12]

Two types of biofortification processes are being pursued, one to improve mineral micronutrient content and one to improve vitamin amounts.[13,14] Given the focus of this review on common bean biofortification, only the first type of breeding will be discussed because beans are a major source of minerals but not vitamins. The major staples that have been targeted for mineral biofortification breeding at the international scale include mainly the seed crops of rice, wheat, maize, and common bean along with related cereals and legumes in certain more intensive national research programs that are part of the overall Harvest Plus biofortification program.[15] Most mineral biofortification work has been through conventional breeding with some attempts at transgenic technology, but the first delivery products are from breeding pipelines using standard varieties and non-GMO methods. Rice is one exception, where mineral biofortification by transgenesis may soon be a reality with high potential for delivery (G. Berry, personal communication).

Mineral deficiencies in human populations are one of the greatest health concerns given that half the current population of the world is affected by some sort of mineral deficiency.[15] Iron-deficiency anemia is especially prevalent, affecting over 3 billion human beings, and zinc deficiency is thought to also affect a similar number of people.[12] Vulnerable groups such as pregnant women, young children, and those affected by illness are especially affected by mineral deficiencies. Mineral concentration in major staples has in many cases decreased with "green revolution" varieties, where higher productivity has been suggested to have diluted some mineral constituents to a certain extent.[10,13−15] Therefore, a challenge will be to breed for higher mineral concentration combined with higher productivity.

The effects of climate change present a challenge as well to mineral biofortification as higher temperatures may reduce protein levels in crops that are needed for essential minerals or may slow the uptake of these minerals.[12] Certainly, soil fertility decline will have unwanted interactions on mineral accumulation in the major cereals.

The focus of the rest of this review will be targeted to two types of mineral biofortification: one for iron and one for zinc. The justification for this is that iron-deficiency anemia (IDA) affects >50% of women and preschool children in developing countries, is responsible for 20% of deaths among women during childbirth, and impairs physical and mental development in childhood and adolescence.[15]

Zinc deficiency (ZD) is probably as widespread globally as IDA but is not as well documented due to less testing for this nutrient.[12] ZD is possibly the leading cause of child and infant stunting, impairs immunity, vitamin A use, and vitamin D function, and leads to decreased health, higher mortality, and greater prevalence of some parasitic diseases.[13] Therefore, there is an imperative to work on iron and zinc concentrations and bioavailability in grain crops and especially in the legumes where their concentrations are higher than in the cereals.[14] For example, common bean has naturally 4−10 times the amount of iron as milled rice and 2−3 times the zinc.[15] A major need is also promotion of the legumes and economic policies that favor legume production.[13] This is because legumes often cost more than cereals, and therefore their overall consumption is more limited compared to the consumption of starchy staples.

Overall, the goals of mineral biofortification are to reduce the prevalence of IDA and ZD in a large part of the human population.[12,13] Within any given population group, certain individuals will be more affected by the deficiency and will need supplementation methods, whereas others will be near the limits of deficiency and can be positively affected by the biofortification strategy.[14] In this sense biofortification is like flour fortification; however, it is targeted to unmilled grains and to rural populations that are distant from formal fortification strategies.[12] Biofortification is complementary to other interventions that promote the consumption of mineral-rich vegetables or vitamin-rich fruits.[13]

One issue to consider with all food-based approaches is bioavailability of the nutrients involved.[12] Bioavailability is defined as the proportion of a consumed nutrient that is digested, absorbed, and utilized by a human being. This is in turn determined by food composition and the nutrient status of the consumer. In the case of iron and zinc uptake, there are promoter and inhibitory substances that work in various ways to affect human use of the minerals.

Promoters include sulfur amino acids such as methionine and cysteine, vitamin A, vitamin C, and certain lipids but have not been combined with mineral biofortification through breeding. Antinutritional factors for iron and zinc absorption include phytates, polyphenolics and tannins, calcium and manganese ions, lectins, and some fibers, and all of these could be reduced by breeding efforts at least in the legumes. However, many of these substances are nutrients or health-promoting factors in their own right, so many breeders have preferred not to modify antinutrient content over total mineral accumulation.

Another consideration for biofortification strategies is an agronomic one. This is because crop vigor can change with increases in iron, zinc, or phytate content, which are all related to seedling vigor and establishment. Efficient plant nutrient scavenging and uptake mechanisms are useful agronomically for the enhancement of crop health. This review will not touch on the agronomic benefits of biofortification breeding as this has been less well studied in common bean than in wheat, for example. The review will, however, touch on advances in

breeding and genetic analysis for iron and zinc concentration achieved in the Harvest Plus Challenge Program, which has been a major 10-year program to improve nutritional quality in basic staples aimed at producing international public goods in terms of new knowledge and germplasm. The steps in biofortification with the example of common beans are outlined in Figure 1 and as separate sections below.

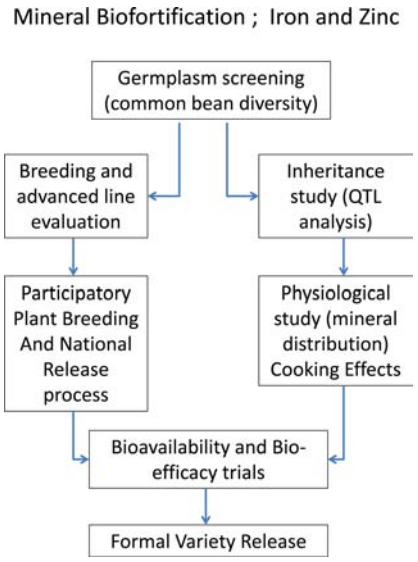

Figure 1. Steps in the nutritional breeding pathway, including germplasm screening (common bean diversity), inheritance study (QTL analysis for iron and zinc), physiological study (seed iron and zinc distribution) and cooking effects, breeding and advanced line evaluation, participatory plant breeding and multilocational testing, bioavailability and bioefficacy tests, and variety release.

METHODOLOGIES OF BEAN BIOFORTIFICATION

Germplasm Screening. In any breeding program, germplasm screening for a trait of interest is an important first step to genetic improvement. In the case of biofortification, nutritional breeding also starts with assembly of parental germplasm for crosses based on the evaluation of a large amount of genetic material. For beans, a core collection of 1400 genotypes was the starting point for screening of mineral traits.[16] Ranges of 30−110 ppm iron and 25−60 ppm zinc were found in the germplasm analyzed, and the high-iron genotypes, G14519 and G21242 from the FAO germplasm treaty collection held at the International Center for Tropical Agriculture, were selected to initiate crosses.[8]

This variability for iron or zinc content is slightly larger than what was found in the analysis of a more limited range of genotypes.[17−20] In addition, screening of advanced lines within each of the gene pools has been important for identifying potential commercial type parents, as many of the core collection high-iron or high-zinc lines were of noncommercial seed types. The range of mineral accumulation in the two gene pools of common bean (Andean and Mesoamerican) is similar, although many Andean beans or inter-gene-pool hybrids have higher iron concentration than Mesoamerican beans.[4,16] Therefore, some breeders choose a control genotype for each genepool that is a standard variety or breeding line that can be multiplied to a large quantity, ground, and used for standardizing measurements across sites and screenings. CAL96 (Andean breeding line) has been used for this standardization in some cases.[8] DOR390 (Mesoamerican black-seeded breeding line) is an alternative for the other genepool as it is a variety in some parts of Central America.

For breeding, it has also been important to evaluate locally available germplasm to have a baseline of information on the nutritional traits. Screening of local germplasm of the countries involved in biofortification has included a range of Andean varieties,[21,22] a regional collection of eastern and southern African released varieties (unpublished data, this laboratory), and a large collection (over 350 entries) of Rwandan genotypes.[4] Additional diversity for mineral concentration has been found in wild or weedy germplasm[19] (unpublished data, this laboratory). Finally, screening of related species such as *Phaseolus coccineus* or *Phaseolus dumosus* and *Phaseolus acutifolius* has been used to identify high iron content genotypes in the secondary and tertiary gene pools, respectively.

One characteristic of this stage of nutritional breeding is that it has allowed the development of methodologies for nutritional analysis, which was very important to avoid mineral contamination in iron analysis, which is a common problem. Although initial sample preparation was done with regularly harvested seed and aluminum grinding equipment in a modified Retsch mill,[21] currently a more careful method for sampling

Table 1. Types of QTL Mapping Populations and Studies Used for Biofortification Breeding of Mineral (Iron and Zinc), Phytate, or Tannin Concentrations in Common Bean Based on Gene Pool and Growth Habit Assignment

trait	type of genetic cross	type of morphological cross	genepool 1 (Andean)	genepool 2 (Mesoamerican)
minerals (Fe/Zn)	intergene pool	bush × bush	Mesoamerican × Andean; Blair et al. (2009)[21]	
	intergene pool	wild climbing × bush	Andean × Mesoamerican; Guzman-Maldonado et al. (2003),[27] Blair and Izquierdo (2012)[9]	
	intragene pool	climbing × climbing	Andean × Andean; Blair et al. (2011)[25]	Mesoamerican × Mesoamerican (tbd)
	intragene pool	climbing × bush	Andean × Andean (tbd)	Mesoamerican × Mesoamerican; Blair et al. (2010)[24]
	intragene pool	bush × bush	Andean × Andean; Cichy et al. (2009)[26]	Mesoamerican × Mesoamerican; Gelin et al. (2007)[29]
phytates	intergene pool	climbing × bush	Mesoamerican × Andean; Blair et al. (2009)[41]	
	intergene pool	bush × bush	Mesoamerican × Andean; Blair et al. (2012)[40]	
	intragene pool	bush × bush	Andean × Andean; Cichy et al. (2009)[26]	Mesoamerican × Mesoamerican (tbd)
tannins	intergene pool	bush × bush	Mesoamerican × Andean; Díaz et al. (2010)[37]	

and analysis is used that involves hand harvesting and threshing of grain followed by seed milling in Teflon chambers with zirconium grinding balls.[22−25] In studies by Blair et al.[4,24,25] additional goals were (a) to determine the validity and precision of various mineral analysis methods such as atomic absorption spectrophotometry versus inductively coupled plasma−optical emission spectrometry and (b) to calibrate near-infrared reflectance spectroscopy for iron.

As part of germplasm screening and evaluation, the stability of the genotypes for a given trait is usually evaluated. In the case of iron and zinc accumulation, G×E was best evaluated with the most promising local germplasm and potential parents to determine if mineral accumulation was stable across sites. As part of the Harvest Plus challenge program, G×E has been analyzed for a high-mineral nursery of advanced lines tested across sites in Central and South America and appears to be moderate.[8]

REVIEW AND DISCUSSION OF BEAN BIOFORTIFICATION

Inheritance of Seed Mineral Accumulation. One step in a biofortification program that is important in assessing the feasibility of improving common beans for micronutrient quality following germplasm screening and in conjunction with the initiation of crosses is to study the inheritance and physiology of the accumulation of seed iron and zinc. Most studies have indicated multigenic inheritance of micronutrient traits[9,21,24−27] even while a few initial reports suggested that the inheritance of zinc concentration in common beans might be by a few genes.[28] Germplasm evaluation shows a normal distribution for iron and zinc concentrations.[16]

Specific quantitative trait loci (QTL) studies for iron accumulation have been conducted on intergene pool populations[21] intragene pool populations[24−26] and on a wild × cultivated population[9,27] as summarized in Table 1. The intergene pool study based on DOR364 × G19833 used a genetic map that covered the full common bean genome and found a large range of iron and zinc values among the recombinant inbred lines and 13 QTL for iron content, of which 5 were clustered on linkage group b11.[21] Other QTL were identified on linkage groups b03, b06, b07, and b09 for zinc and b04, b06, b07, and b08 for iron of a total of 26 QTL detected for both minerals.[21] Previously, some zinc QTL studies on a limited genetic map postulated a limited number of QTL[29] but this seems to be erroneous. This may be explained by the growth of populations on highly zinc-deficient soils in cases where simpler inheritance has been suggested.

In intragene pool crosses additional QTL have been found to be important. For example, in cross G14519 × G4825, the most important QTL were found overlapping for iron and zinc on linkage group b06.[24] Other QTL for mineral concentration or content were found on linkage groups b02, b03, b04, b07, b08, and b11 and, together with the b06 cluster, were mostly novel compared to loci found in previous studies of the intergene pool crosses or Andean gene pool.[21,25] In the Andean cross G21242 × G21078 there were nine seed mineral QTL on five linkage groups, with the most important being new loci on b02 but with some overlapping QTL from the intergene pool cross on b06, b08, and b07 near phaseolin.[25] An interesting feature of the studies of Blair et al.[21,24,25] was that several QTL for iron and zinc colocalized or overlapped, suggesting possibly pleiotropic loci and common physiology for mineral uptake or loading, although this was not the case in the study of small white navy beans,[29] perhaps due to differences in genetic map coverage. If shown to be the case in further studies, we may postulate that QTL for the accumulation of both minerals may be genetically linked or pleiotropic, controlling both traits at once.

The mechanism for this may have to do with protein balance in the seed as some association of iron and zinc content with the phaseolin seed storage locus on linkage group b07 was found by Blair et al.[21] Certainly ferritin as a storage protein for iron must play a role in total accumulation of iron, and the gene for ferritin has been associated with a QTL on linkage group b08.[9,21] An alternative is an association of mineral accumulation and seed size, although most QTL for seed minerals have been independent of seed size QTL. It is notable that for some studies and in certain genetic backgrounds, the efficiency of accumulation of seed zinc may relate to adaptation mechanisms for plant growth on zinc-deficient soils, which seems to be especially the case for small white navy beans derived from a bush bean breeding and mutagenesis program[30] as mentioned for the genetic study in this type of Mesoamerican bean.[29]

Iron accumulation may also be dependent on supply of iron taken up by the roots, especially under low-iron soils and iron-deficient conditions.[31] In this case the role of iron reductase as the major mechanism for uptake of iron by strategy I plants may be paramount. In this case iron reduction in common bean roots is required to convert ferric iron to ferrous iron that is able to be taken up by the plants.

One conclusion of the genetic analyses conducted to date has been that greater transgressive segregation for seed mineral content has been observed in wide crosses such as between gene pools compared to narrow crosses within a gene pool or a given commercial class.[21,24−29] Additional studies in the populations G21242 × G21078 derived from an Andean × Andean cross and G14519 × G4825 derived from a Mesoamerican × Mesoamerican cross have shown that some genes for zinc or iron content detected in an intergene pool cross[21] are also found in intragene pool crosses.[25,26]

A followup to these studies will be the application of marker-assisted selection (MAS) for the genes and QTL that have been identified so far in an attempt to move the loci from one genetic background to another. In this regard, initial marker validation for a QTL from G14519 appears promising in a red mottled Andean grain background. In terms of further MAS research, the colocalization of QTL for seed iron and zinc would be promising for plant breeding of higher micronutrient concentration given that if the same QTL contribute jointly to both minerals, it may be easy to select for these traits simultaneously, phenotypically and through MAS.

On the basis of quantitative inheritance and the number of QTL found, recurrent selection and/or advanced backcrossing could be predicted as options for developing high-mineral genotypes in common beans.[9] The discovery of QTL can specifically pave the way for molecularly supported breeding of new varieties of common beans with commercial seed types along with higher micronutrient concentration. The analysis of candidate genes for iron or zinc accumulation such as cation transporters or iron reductase will be useful for the development of more perfect markers, provided that these genes have an important phenotypic effect on mineral accumulation. Ferritin, as the major storage protein for iron in seeds and leaves, may also be an interesting candidate gene or molecular marker.[9,21] An advantage of the candidate gene work is that these genes could be used for creating high-iron transgenics;

however, the balancing activities of mineral homeostasis may restrict gene expression, and beans are still recalcitrant to transformation.[1]

Localization of Iron within the Seed and Bioavailability Tests. Three critical components of realizing the advantages of biofortification are to understand (1) the localization of minerals within the grain, (2) the effects of cooking on mineral concentration, and (3) the bioavailability of these components or of the overall food prepared from the grain. For bioavailability evaluations the most common test is one that mimics the human digestive system in vitro. The most widely used test for this involves Caco-2 cells, which are cultured human intestinal cells measured for uptake of specific nutrients across a filter membrane.[32,33] In vivo studies for iron absorption using laboratory animals such as rats have been questioned due to differences from human digestive systems, but recently a poultry model has been functional for common bean.[34] Caco-2 measurements of common bean indicated a significant correlation ($r = 0.383$, $P \leq 0.05$) between the iron to phytate concentration ratio and absorption in cooked cotyledons.[35]

The effect of cooking on iron concentration in the food has been a new area of research that brings together the plant breeders developing biofortified products and the food scientists evaluating these products.[36] In the case of beans, the effect of soaking was evaluated for iron loss from grain as this is a common practice before cooking beans with variability observed in both percentage (3−13%) and total amounts (1.9−6.5 ppm) of iron released.[35] In the same study, low variability in the weight gain from soaking (201−231%) was observed.[35] Release of tannins as an antinutrient that retains iron during digestion and prevents its absorption was another variable studied but not quantified beyond spectrophotometric methods.[35] The color of the water used to soak the beans varied with the anthocyanin content, and in turn there was variability among grains of different seed colors for types of tannins and tannin monomers.[37]

Localization of iron within the whole bean seed versus separated into the cotyledon, the embryo, and the seed coat is an important distinction that varies between genotypes of common bean.[35] The seed coat, in particular, can contain from 4 to 22% of total iron, having an important result on iron bioavailability because the seed coat is also the site of all the tannin binding of iron that occurs in the grain. The embryonic axis is rich in iron but is only a small percentage by weight of the seed and contains approximately 2−3% of the total iron. The other variable iron fraction is in the cotyledons, which also contains starch and protein reserves of the seed. Here in the cotyledonary tissue the percent of seed iron varies from 75 to 95%. This is important with respect to another antinutrient for iron, namely, phytates, which are found almost completely (95−97.5%) in the cotyledons. Phytates, especially phytic acid, bind iron, and this may affect the distribution of iron in the seed after the iron passes from the maternal seed coat tissue to the seed's cotyledonary tissue.[35]

When the iron distribution is investigated within the seed at a microscopic level using nondestructive microparticle induced X-ray emission (PIXE) analysis of element distribution, high concentrations of iron are found in the embryonic axis (hypocotyl, radicle, and leaf primordial) as would be expected but also, surprisingly, in the vascular bundles of the cotyledons.[38] The proximity of the provascular bundles was found to hold up to 500 ppm of iron, depending on the genotype, which is 10 times the average for the seed overall. Iron distribution can be confirmed in a less quantitative way with Prussian Blue staining for iron localization, which is also useful for confirming that iron is found in the cytoplasm of epidermal (seed coat) cells and cells near the epidermis.[38,39] Overall results, however, show that concentration of iron seems to be greatest near the bundle sheath cells containing vacuoles with amylose, suggesting an association of starch and iron loading. In contrast, the protein ferritin that has been suggested as the major iron storage protein in legumes was only highly expressed in the amyloplasts of the seed embryo, although lower amounts of the protein could also be present in bundle sheath cells.[38] The highest concentration of zinc, meanwhile, has been found in the embryonic axis and also in the provascular bundles of the cotyledons.[39] This would be good news depending on the distribution of phytates, so more detailed studies are needed in this area of microlocalization. The role of ferritin in accumulating iron and the bioavailability of ferritin-held iron is another area worth studying. Genetic associations show that ferritin should play a role in overall iron accumulation in common bean seeds.

Breeding of High Mineral Bean Varieties. An initial goal in the biofortification of common beans has been to produce varieties with 80% more iron content and 40% more zinc while maintaining or improving the properties that farmers and consumers require in a variety, such as adaptation to abiotic or biotic stresses and seed shape or color. Breeding is concentrating on both gene pools of common bean, the large-seeded Andeans and the small-seeded Mesoamericans, and in bush beans as well as climbing beans. Various breeding technique or strategies have been used for the current biofortification breeding effort, including backcrossing, recurrent selection, and various permutations of gamete and pedigree selection. Secondary characteristics of phytate and tannin content have for the most part been screened only on advanced lines due to their more expensive assays. Bioavailability tests and bioefficacy trials have been undertaken on the best bet varieties.[34]

The first strategy applied for biofortification breeding of Andean beans was backcrossing with gamete selection, where selection was applied in the BC_1F_1 and again in the BC_1F_3 (back cross 1, filial 1 and 3 generations, respectively), at which point mineral analysis was used to select the most promising lines in the following generation.[8] Two pedigrees were used in this stage: CAL 96 (CAL 96 × G14519) and CAL 143 (CAL 143 × G14519), based on the recurrent parents CAL96 and CAL143, which have been or are being considered as released varieties in several countries.[8] Both of these red mottled beans were considered valuable recurrent parents because of their wide adaptation in eastern and southern Africa and in the Andes of South America.

From these crosses, a number of BC_1F_4 nutritionally enhanced Andean (NUA) lines were selected and used in multiple-site, on-station, and on-farm testing in Colombia, Kenya, and Malawi. Initial releases of the best lines with an increase of up to 25 ppm seed iron stable and were multiplied for a germplasm release.[8] Participatory plant breeding (PPB) can be used in the process of nutritional improvement to define the best advanced lines and to simultaneously increase seed supply for variety promotion and nutritional testing.[4,5] In one unpublished study in Colombia, PPB identified a high-iron genotype that was preferred by farmers due to its earliness and high production potential.

Currently, backcrossing, multilocation, and multiseason testing along with PPB are underway or being planned for the improvement of climbing beans for nutritional quality on the basis of the previous success of the bush bean crosses and the value of the high-iron source, G14519, and interspecific crosses. Climbing beans were selected as a good delivery system for biofortified grain, because of their high potential impact and intrinsic advantages of high yield in small space, large grain, good nitrogen fixation and weed suppression, and flexibility for various cropping systems (e.g., maize × beans), which makes them a good alternative for small farms.

The recurrent parents being used for climbing bean nutritional improvement have been BCMNV resistant genotypes with midaltitude adaptation, which are expected to also improve the major productivity constraints of virus and heat stress susceptibility (unpublished data, this laboratory). One line, MBC46, was found to have high yields, good resistance to diseases, and high iron concentration of >85 ppm in multiple testing in Colombia and was a candidate for release before biofortification for this region was abandoned.

Within the Mesoamerican bean gene pool, breeding for nutritional value likewise focuses on combining high grain mineral content with preferred agronomic traits that will make new varieties attractive to farmers, so as to speed adoption and ultimate impact. Because the small- and medium-seeded Mesoamerican bush beans are often grown in more stressful environments,[1] a top priority has been to combine high iron and zinc with drought tolerance, as well as resistance to important diseases such as bean golden yellow mosaic virus and angular leaf spot. Priority grain classes for this effort should be small black, small red, and cream-striped grain types.

Interspecific crosses to introgress high iron from related species appears to hold promise, especially to reach levels of iron of 90+ ppm. Interspecific crosses with high iron accessions of *Phaseolus dumosus* (*Phaseolus polyanthus*) and *Phaseolus coccineus* have expressed as high as 127 ppm iron in grain harvested under greenhouse conditions (although field-harvested grain is often lower in iron). Whereas the highest levels are normally found in materials of poor adaptation (late maturity, poor pod set), even lines of 80 ppm iron or 45 ppm zinc would represent an important gain in genetic potential. However, caution should be exercised as interspecific crosses with an Andean bean (CAL 96) did not show evidence of significant introgression of the high-iron trait. It is expected that individual lines from the interspecific crosses will have different patterns of introgression from the related species that will determine the degree of expression of the high-iron trait.

One must also consider antinutritional factors when breeding for iron biofortification. The inheritance of phytate accumulation in the seed appears to be complex, with various QTL involved; however, several of these loci align with enzymes involved in the biochemical pathway to phytate. In an initial study by Blair et al.[40] a total of six QTL were found for total or net seed P in the cross G2333 × G19839, whereas three QTL were found for percentage or net seed phytates. In the study of an intergenepool climbing bean population QTL for seed P and percent phytates were located independently on linkage groups b02 and b11 versus b06, respectively. Consistent QTL for phytate with previous studies located on linkage groups b02 and b11 were found previously.[26]

In a more extensive study of genes involved in phytate synthesis and QTL for phytate, Blair et al.[41] identified an association of phytate concentration QTL with one of two paralogs of the *myo*-inositol (3)P1 synthase gene family, located on linkage group b01 and expressed in common bean seed rather than in vegetative tissues. Other QTL for phytate concentration were found on linkage group b06 and for phytate content on linkage groups b03, b04, and b10. The phytate concentration QTL are likely to affect nutritional quality, whereas the phytate content QTL (phytate on a per seed basis) are likely only to affect agronomic traits; however, some mutants for phytate production have no detrimental agronomic affect. In other words, one must be careful to maintain seed phosphorus levels in the overall seed so the best is to breed for higher seed weight with lower phytate per seed. However, QTL for total seed P or phytate content were related to seed weight QTL on linkage groups b06, b07, and b10 with one additional net phytate QTL on b05. Seed weight is a consumer trait that must be taken into account when any variety of common beans is bred.

In conclusion, the steps typical of biofortification are those of any crop improvement program starting with germplasm screening, followed by inheritance studies and breeding, but with a focus on the nutritional traits of seed mineral content. Within the breeding step, one must emphasize the use of hybridization to create both wide crosses followed by narrower crosses. Simultaneous evaluation of both mineral and antinutrient concentrations is advisable. Bioavailability evaluation can be saved for the final products because for the most part an increase in bean mineral concentration will translate to a difference in dietary absorption. The strategies for selection are part of traditional breeding methods and the potential of molecular markers based on QTL loci or candidate genes that are part of a modern plant improvement.

I recommends that the Harvest Plus challenge program utilize modern scientific tools and long-term science funding rather than only empirical breeding to make faster progress in nutritional traits. Replicable strategies of plant improvement for nutritional quality require an understanding of the genes and mechanisms of mineral accumulation, where many unanswered questions remain at large. The job security of plant breeders should not be threatened by investments made in plant genetics, rather more breeding solutions will be available with a commitment to genetic analysis.

Although phenotyping for nutritional quality has improved, traditional plant breeding alone cannot be expected to solve the problem of selection for an invisible trait such as seed nutritional quality in a cost-effective manner without the help of genomic studies and marker-assisted selection. Therefore, it is advisable that Harvest Plus and their donors prioritize science-based breeding. Only the judicious use of all technologies available to breeders in the modern world will ensure that biofortification becomes a regular part of plant breeding in multiple crops, with common bean as an example of this need.

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