From harvest to health: Challenges for developing biofortified staple foods and determining their impact on micronutrient status

Christine Hotz and Bonnie McClafferty

Abstract

Background. The use of conventional breeding techniques and biotechnology to improve the micronutrient quality of staple crops is a new strategy to address micronutrient deficiencies in developing countries. This strategy, referred to as “biofortification,” is being developed and implemented through the international alliance of HarvestPlus to improve iron, zinc, and vitamin A status in low-income populations.

Objective. The objective of this paper is to review the challenges faced by nutritionists to determine and demonstrate the ability of biofortified crops to have an impact on the nutritional and health status of target populations.

Methods. We reviewed available published and unpublished information that is needed to design and evaluate this strategy, including issues related to micronutrient retention in staple foods, micronutrient bioavailability from plant foods, and evidence for the efficacy of high-micronutrient-content staple foods to improve micronutrient status.

Results. Further information is needed on the retention of micronutrients in staple foods, in particular of provitamin A carotenoids, when stored and prepared under different conditions. The low bioavailability of iron from staple foods and the ability to demonstrate an impact on zinc status are specific challenges that need to be addressed. In target countries, infections and other micronutrient deficiencies may confound the ability to affect micronutrient status, and this must be taken into account in community-based studies.

Conclusions. Information to date suggests that biofortification has the potential to contribute to increased micronutrient intakes and improved micronutrient status. The success of this strategy will require the collaboration between health and agriculture sectors.

Key words: Agriculture, bioavailability, biofortification, diet, iron, micronutrients, provitamin A, zinc

Background

Micronutrient deficiencies are among the major causes of poor health and development in the developing world. The prospects of using conventional breeding techniques, biotechnology, and micronutrient-containing fertilizers to improve the micronutrient quality of staple crops and the micronutrient status of the poor have been put forth in recent years [1]; this new strategy is referred to as “biofortification.” Biofortification proposes to use agricultural modifications as a public health intervention and, as a result, has the potential to reach more effectively the rural poor—those who are often at highest risk for micronutrient deficiencies.

HarvestPlus is a Challenge Program of the Consultative Group on International Agricultural Research (CGIAR) that undertakes to design, produce, test, and disseminate staple food crops that are biofortified with iron, zinc, and provitamin A. As with any intervention of this type, many challenges are faced in relation to the appropriate design, implementation, and evaluation. This article focuses on the particular challenges faced in the area of nutrition.

Under the agricultural component of HarvestPlus, progress in breeding for biofortified crops has been steady, with promising levels of zinc and provitamin A carotenoids and, to a lesser extent, iron being achieved in several staple food crops, including maize, rice, wheat, pearl millet, potatoes, and bananas or plantains. However, a challenge still faced by nutritionists is to determine and demonstrate the ability of biofortified crops to have an impact on the nutritional and health status of target populations.
Objective

The objective of this paper is to review the challenges and additional information needed to determine and demonstrate the ability of biofortified crops to have an impact on the nutritional and health status of target populations. To design biofortified staple food crops with an increased micronutrient content that is biologically important, nutritionists and breeders must consider the following: breeding targets for micronutrient content are set to provide adequate amounts of micronutrients in the crop food in the form in which it is consumed, after accounting for losses in micronutrients that occur during storage, milling, processing, and cooking; any limitations in the bioavailability of the micronutrients are overcome; and improvement in micronutrient status can be achieved and demonstrated in target populations. These are the topics of this review. A summary of the research stages and associated challenges is presented in figure 1.

Methods

We reviewed information from published studies as well as preliminary information from unpublished reports derived from HarvestPlus research. Although this was not a systematic review, sufficient information was compiled to enable us to outline research and information gaps and to identify challenges and precautions for the design of the biofortification strategy.

Results

Filling the gap: Setting appropriate target levels for the micronutrient content of biofortified staple foods to achieve adequate intakes

One of the first questions asked of nutritionists by breeders in the development of the HarvestPlus program strategy was “By how much do we need to increase...
the micronutrient content of our crops to improve the micronutrient status of their consumers?" As a food-based strategy, the additional micronutrient intake resulting from biofortification would ideally be enough to fill the gap between current intakes and the amount that would result in the majority of the population having intakes above the theoretical mean dietary requirement level (the estimated average requirement, or EAR). However, with the use of this approach, there is not one unique answer that fits all possible target populations. Further, quantitative information on micronutrient intakes for most potential target populations does not exist or exists in very limited form. There are also differences in processing practices and inclusion of other foods that can result in large differences in the micronutrient content and bioavailability in the staple food.

Because this was a new strategy, breeders needed to get started in judging progress and possibilities for producing biofortified crops, and therefore multiple target levels for micronutrient content were not initially needed, or necessarily desirable. Therefore, for practical reasons, preliminary or “generic” target levels for micronutrient content were set using gross assumptions about staple food intake (grams/day); bioavailability (percent nutrient absorbed) or, in the case of vitamin A, the retinol equivalency of provitamin A carotenoids; losses of the target nutrient with milling, processing, storage, and cooking; and the proportion of the daily nutrient requirement that should be achieved from the additional amount of micronutrient in the staple food. Examples of the types of data used to estimate target levels for micronutrient contents of biofortified crops are presented in Table 1. As information of this type becomes available for specific populations, target levels for micronutrient contents in different staple food crops can be refined and adjusted. It must be recognized, however, that if preliminary target levels are not judged to be adequate for a specific population, the breeding process will continue until the necessary micronutrient content is achieved.

As with universal fortification of staple foods, biofortification will lead to some degree of increased micronutrient intakes among individuals in all life stages. A possible exception is breastfed children who have not yet been weaned onto staple foods, but even in this case,

<table>
<thead>
<tr>
<th>Information</th>
<th>Iron</th>
<th>Zinc</th>
<th>Cassava</th>
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<tbody>
<tr>
<td>Age/physiological status group</td>
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<tr>
<td>Non-pregnant, nonlactating, pre-menopausal women</td>
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<td>Children 4–6 yr of age</td>
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<td>Percent of daily micronutrient requirement to achieve&lt;sup&gt;a&lt;/sup&gt;</td>
<td>~30%–50%</td>
<td>~40%–50%</td>
<td>~30%–50%</td>
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<tr>
<td>Estimated average requirement (µg/day)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1,460</td>
<td>1,860</td>
<td>500</td>
</tr>
<tr>
<td>Intake (g/day)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>400</td>
<td>400</td>
<td>500</td>
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<tr>
<td>Baseline micronutrient content (µg/g)</td>
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<td>16</td>
<td>0.5</td>
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<tr>
<td>Food retention of micronutrient following cooking</td>
<td>90%</td>
<td>90%</td>
<td>50%</td>
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<td>Bioavailability&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10%</td>
<td>25%</td>
<td>12:1</td>
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<tr>
<td>Additional content required (µg/g)</td>
<td>11</td>
<td>8</td>
<td>15</td>
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<tr>
<td>Total content to achieve (µg/g)</td>
<td>14.5</td>
<td>24</td>
<td>15.5</td>
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<sup>a</sup> For iron and zinc, level is expressed as a percentage of the physiological requirement for absorbed iron [2] and zinc [3]. For provitamin A carotenoids, level is expressed as a percentage of the estimated average requirement for retinol activity equivalents [4].

<sup>b</sup> Expressed as dry weight equivalents for rice and fresh weight for cassava. Estimates for staple food intakes were derived from a composite of household food expenditure data from various countries, as reported by Meenakshi and colleagues (unpublished data, 2006). Intakes for adults and children were estimated on the basis of consumer equivalence.

<sup>c</sup> For iron and zinc, bioavailability is expressed as the percentage of total micronutrient intake that would be absorbed by the body; for provitamin A, bioavailability is expressed as the retinol equivalency.
increased intakes of provitamin A by lactating women may result in increased content in the breastmilk and hence transfer to the breastfed infant. Although all age groups could be affected, young children and women of reproductive age typically suffer the greatest consequences of micronutrient deficiencies because of their increased requirements for growth and for pregnancy and lactation, and hence they may be considered the primary targets for this strategy. Biofortification as the sole micronutrient strategy may not be sufficient to cover the deficit in micronutrient intakes by young children (e.g., under 2 years of age), who have particularly high micronutrient needs and relatively low staple food intakes. Therefore, the estimation of appropriate target levels for the micronutrients of biofortified foods were initially set considering the potential impact in children approximately 4 to 6 years of age and in adult women (table 1).

Progress in breeding to achieve target levels of nutrient contents is very promising for several crops. For example, within the next four years, meeting theoretical target levels for zinc will be achievable for rice, wheat, maize, and pearl millet; meeting target levels for iron will be achievable for beans and potatoes; and meeting target levels for provitamin A carotenoids may be achieved in bananas and plantains. HarvestPlus research has determined that several crops simply do not contain enough natural genetic variation in provitamin A carotenoids content to expect reasonable increases in content through conventional breeding; this is the case for rice, wheat, pearl millet, beans, cowpeas, and lentils, and therefore the use of transgenic approaches can be justified. Good potential for achieving important levels of provitamin A carotenoids in maize exists, and there is some potential for cassava. The target level for iron will be difficult to achieve in most crops. This is largely because of the expected low bioavailability of iron from most staple foods in unrefined form; very large increases in iron intake would be needed to result in biologically important increases in the amount of absorbed iron. This justifies the importance of identifying and testing various plant compounds that can substantially improve the bioavailability of iron, and of determining the impact and agronomic feasibility of reducing the content of known inhibitors of iron bioavailability, mainly phytate.

Retention of micronutrients in biofortified staple foods: Preserving the nutritional value

Provitamin A carotenoids

In humans, retinol is the main biologically active form of vitamin A. Plant foods do not contain vitamin A in the form of retinol, but some are rich sources of provitamin A carotenoids that can be converted to vitamin A in the body. Losses and degradation of provitamin A carotenoids with storage, processing, and cooking are potentially important and need to be quantified in biofortified crops. There have been few data of this type generated so far, with carotenoid-rich grains (e.g., maize). The US Department of Agriculture [5] reports that approximately 80% of vitamin A precursors are retained after boiling of cereals. However, another study (N. Palacios, unpublished data) showed that 64% of provitamin A carotenoids were retained after tortillas were prepared from maize that had been nixtamalized, a soaking process used in Central America to prepare maize dough.

Orange-fleshed sweet potato is a very rich source of the major provitamin carotenoid, β-carotene. The majority of β-carotene is maintained after sweet potatoes are boiled. For example, the true food retention of β-carotene of the orange-fleshed sweet potato variety Resisto ranged from 83% to 92%, depending on the exact boiling procedure [6]. In contrast, processing of sweet potatoes to make flour, a practice used in some areas to preserve sweet potatoes for use outside the growing season, can result in anywhere from approximately 60% to less than 15% food retention of β-carotene, depending on the variety (S. Thilsted, unpublished data). For cassava, preliminary data suggest that the mean food retention of carotenoids after boiling is about 50%, but it ranges widely from 27% to 83%, depending on the specific genotype [7]. Once they are verified, factors responsible for higher food retention of β-carotene may become other traits for which cassava is selectively bred. The highly variable and potentially low food retention of provitamin A carotenoids in different staple foods highlights the need to accumulate further data on food retention and to take them into account when setting appropriate target levels for provitamin A carotenoid content in biofortified foods.

Iron and zinc

In grains, micronutrients are most concentrated in the germ and/or aleurone layer, with lesser amounts in the endosperm; unfortunately, as grains are milled and refined, these fractions are lost and the micronutrient content decreases substantially. For example, only about two-thirds of iron may be retained after rice is polished [8]. The food retention of iron and zinc after milling can range from 20% to 60% of the whole grain content, depending on the grain and the extraction.
rate [9]. For populations that typically consume whole grains, achieving the target increments for micronutrient contents may be relatively easy, since the greatest potential for increase will be exploited by including the aleurone and germ. However, to affect the substantially large populations in developing countries that consume refined grains, it will be more challenging to reach target micronutrient increments through breeding. For minerals, the physiology of loading iron and zinc into the endosperm and the genes involved in regulating that process need to be better understood so that selective breeding for higher endosperm content can be achieved.

Losses of iron and zinc with typical household cooking procedures are not typically very high. For example, in cooked beans, 75% to 90% of iron and 85% to 90% of zinc is retained, depending on the boiling time and whether water is drained or not [5]. Food retention of up to 90% to 95% of iron and 95% to 100% of zinc content with boiling is reported for rice, with the lower food retention occurring when cooking water is drained [5]. Boiled and drained potatoes also retain 95% of their iron and zinc content [5]. Therefore, losses of these minerals during cooking will probably not be as important a factor in achieving target levels in biofortified crops as losses due to milling of grains.

**Bioavailability: Can plants provide a good source of bioavailable micronutrients?**

**Provitamin A carotenoids**

Provitamin A carotenoids must be released from the plant matrix and incorporated into micelles, absorbed, and then cleaved to form retinol. The conversion of provitamin A carotenoids is not 100% efficient, thus making plant-based diets poorer sources of vitamin A activity. The efficiency with which provitamin A carotenoids are converted to retinol in humans is often expressed as the retinol activity or equivalency. It has generally been assumed that 12 µg of all-trans β-carotene is required to produce 1 µg of retinol [4, 10], although this equivalency rate depends on factors such as the food matrix and the presence of dietary fat, which improves carotenoid bioavailability.

The retinol equivalency of provitamin A carotenoids from specific foods has largely been determined from the change in serum retinol concentration after consumption for a specified period of time, although this is not the most reliable way of determining retinol equivalency. Stable isotope tracer techniques have been developed and, when combined with long-term consumption, can be used to quantify changes in retinol stores [11]. Studies of this type have not yet been conducted for a large number of foods and have focused more on vegetables than on staple foods. In vitamin A–deficient men, canned, puréed orange-fleshed sweet potato sautéed in oil was determined to have a retinol equivalency of 13:1, close to the standard value of 12:1 [12]; it is possible that whole boiled sweet potato prepared without oil will have a somewhat less favorable retinol equivalency, and this is currently under study. Interestingly, recent evidence from a study in Mongolian gerbils indicated that the retinol equivalency of β-carotene from maize was equivalent to that of β-carotene in oil [13]. In this animal study, the retinol equivalency of β-carotene from both sources was determined to be 3:1; the retinol equivalency of β-carotene in oil in humans has been established to be about 6:1. This study suggests that the bioavailability of β-carotene from maize is favorable, and this needs to be confirmed in human studies.

**Iron and zinc**

There are several common plant food components that interact with iron and zinc during digestion to alter their bioavailability. For iron, staple food components that enhance the bioavailability of nonheme iron include ascorbic acid, and those that inhibit iron bioavailability include phytate and some polyphenolic compounds. The main staple food component that inhibits the bioavailability of zinc is phytate.

The proportion of zinc that is absorbed from typical diets appears to range from about 18% to 34%, where lower bioavailability is associated with a higher molar ratio of phytate:zinc in the diet [3]. This range applies to diets for a whole day that would supply just enough absorbed zinc to meet the physiological zinc requirement; for higher levels of daily zinc intake, the percentage of zinc absorbed would be lower. Because the phytate content of whole grains and legumes tends to be very high, it is expected that breeding for lower phytate content of staple foods would lead to improved zinc bioavailability and hence increase dietary zinc adequacy. Collectively, data from clinical studies measuring zinc absorption from a range of typical, whole diets [3] and some cross-sectional studies relating dietary phytate:zinc molar ratios and indicators of zinc status [14, 15] support this approach. More data from long-term intervention studies with low-phytate staples would be useful to quantify the impact of low-phytate staple foods in comparison with the impact achieved by simply increasing the zinc content.

The proportion of dietary iron that is absorbed is substantially lower than that of zinc; iron bioavailability typically ranges from 5% to 15% of total iron intake [2]. Direct measurement of iron absorption from meals representing different types of largely plant-based diets suggests that the bioavailability of iron can be even less than 5% [2]. This presents a particular challenge for the biofortification strategy, because a very large amount of additional iron would be needed to compensate for its very low bioavailability.

Phytate is also a potent inhibitor of iron bioavailability. Whereas for zinc the dose-response inhibitory
effect of phytate is more gradual, for iron it is more acute, so that even relatively small amounts of phytate can have a substantial inhibitory effect on iron bioavailability [16]. Polyphenolic compounds are also strong inhibitors of iron bioavailability. Studies using in vitro models of iron bioavailability have shown that even after reduction of the phytate content of staple foods such as sorghum and millet, iron bioavailability remains low in the presence of polyphenolic compounds [17, 18]. Common beans (Phaseolus vulgaris), for example, typically have a high phytate content, and colored varieties (e.g., red, brown, black, mottled) have large amounts of polyphenolics. Even in the absence of polyphenols, as would be the case with white beans, it is not yet clear to what extent phytate content would need to be reduced so that beans might be considered a useful food to biofortify with iron. HarvestPlus is addressing these research questions.

However, there is some information suggesting that phytate and polyphenols may have protective effects against chronic diseases, as suggested by their effects on various biological indicators in vitro and in vivo [19–21]. Although the effects of long-term intake of different dietary levels of phytate and polyphenols on chronic disease are not known, it may be prudent to weigh the potential benefits of reducing phytate and polyphenol content for improved iron and zinc absorption against any potential risks of reducing the intake of these compounds. The risk-benefit ratio may be very different in the context of developing countries from that in affluent populations. From a breeder’s perspective, it may well be possible to alter the content of these plant components, although this adds an additional trait that would need to be combined with higher iron or zinc content. From an agronomist’s perspective, staple crop components such as phytates may have importance for crop yield; for example, low-phytate barley mutants were shown to have reduced yield [22]. Polyphenols may impart resistance to pests. The heritability of these traits and environmental determinants also needs to be assessed. Phytate content in grains and legumes appears to be greatly affected by soil conditions, in particular the phosphorus content [23, 24]. Therefore, overcoming the low bioavailability of minerals by reducing the content of absorption inhibitors presents a further challenge for the biofortification strategy, as the implications of such modifications may extend beyond bioavailability to broader issues of public health and agronomic feasibility. Further research is needed in this area.

Efficacy: Determining the biological impact of biofortified crops in controlled feeding studies

In order to be accepted as a viable, cost-effective strategy to improve the health and development of micronutrient-deficient populations, the biological impact of biofortification will need to be demonstrated. Studies to determine the efficacy of biofortified foods would measure the effect of consumption on biological and/or functional indicators of micronutrient status under controlled study conditions in comparison to a similar but nonbiofortified genotype of the same staple food. Some evidence for the potential of biofortified staple foods to improve micronutrient status already exists.

The efficacy of orange-fleshed sweet potato and of high-iron-content rice has been demonstrated [6, 25, 26]. One study among 3- to 6-year-old Indonesian children demonstrated that consuming β-carotene-rich sweet potato providing an additional 375 µg of retinol activity equivalents (RAE) per day, 6 days a week, for 3 weeks resulted in increased serum retinol concentrations [25]. Another study conducted among 5- to 10-year-old South African schoolchildren provided them with an additional 1,031 µg RAE/day from a 125-g portion of orange-fleshed sweet potato served for 53 days over a period of 10.6 weeks [27]. On the basis of the modified relative dose-response test that determines the adequacy of liver stores of vitamin A, the vitamin A status of these children was improved in comparison with that of a control group. In both of these studies, the sweet potato provided well over the mean daily requirement of vitamin A for these children due to its very high concentration of β-carotene (~100 µg/g in the South African study). Consumption of other vitamin A–rich plant foods has also been shown to be efficacious in improving the vitamin A status of night-blind pregnant women [28]. This provides greater confidence that attaining reasonable levels of provitamin A carotenoids in biofortified staple crops will indeed result in a measurable impact on vitamin A status.

Iron

The potential for iron–biofortified rice to improve iron status was demonstrated in a carefully controlled study conducted among iron-deficient, nonanemic Filipino women [26]. The biofortified rice provided on average an additional 1.4 mg/day of iron. Both the serum ferritin concentration and the estimated total body iron were significantly higher in the group that received the iron-biofortified rice for a period of 9 months than in the control. Although the additional iron provided by the biofortified rice produced a measurable impact on the iron status of the iron-deficient women, the increased iron content was still quite modest. On the basis of a 10% bioavailability of iron from the rice-based diet, as estimated from this study, an additional 0.14 mg/day of iron would have been absorbed. This represents only about 10% of the physiological requirement for absorbed iron among premenopausal, non-pregnant adult women [4]. The increased amount of iron in the biofortified rice used in this study was only about 2.6 mg/kg greater than that in the control rice.
Under HarvestPlus the goal is to achieve an increase of about 10 mg of iron per kilogram of rice so that a more meaningful impact can be achieved. Nonetheless, this study provided an important proof-of-concept for iron-biofortified foods.

Zinc

To date, no efficacy studies with zinc-biofortified staple foods have been conducted. Demonstrating efficacy of a food-based intervention to improve zinc intakes will probably pose an important challenge. Randomized controlled trials providing zinc supplements have often resulted in significantly increased serum zinc concentrations [29], but this has not always been observed in studies using zinc-fortified cereal foods [30–32]. This may be partly due to the poor sensitivity of serum zinc concentration in response to relatively low amounts of additional zinc intake. Novel biochemical or functional indicators of zinc status, such as metallothionein mRNA expression [33], markers of DNA damage [34], and biochemical indicators of bone metabolism [35] or growth [36], should also be developed and tested.

Another important challenge for demonstrating the efficacy of micronutrient interventions, including biofortification, is to understand the role of infection in micronutrient status. Intestinal parasitic infections, such as helminth infections, are an important determinant of iron status [37, 38]. Hookworm causes intestinal blood loss, thus increasing dietary iron requirements [39]. The proposed target levels for iron content of biofortified foods may not be adequate to have an impact on the iron status of infected individuals. It was also found that the impact of β-carotene–rich sweet potato on serum retinol concentration was greater when antihelmintic treatment was provided to children with Ascaris lumbricoides [25]. Impaired intestinal health, as determined by measures of intestinal permeability, is a common condition in tropical environments [40] and could be associated with impaired zinc absorption [41].

Effectiveness: Further challenges to achieving population-level impact

Even when they have been shown to be efficacious, the large-scale impact of biofortified crops on the micronutrient status of populations will depend on many factors unrelated to nutrition. To be accepted by farmers and consumers, the new biofortified crops will have to have equivalent yield, good resistance to environmental stresses and pests, and acceptable sensory and cooking qualities. Under HarvestPlus, the main strategy to deal with these critical issues is to backcross biofortified staple crops with locally adapted, agronomically superior varieties. The existence of an effective seed and rural extension system for multiplication and dissemination of new varieties will also be important for an effective biofortification program. Although increased iron and zinc contents of crops are very unlikely to cause changes in color or taste, increased content of provitamin A–rich biofortified foods will result in a noticeable color change and could potentially affect taste. Studies to assess consumer preferences and perceptions are needed. If the problem is not insurmountable, a strong demand creation component will be needed, including effective nutrition education and social marketing, to promote consumption of such products. Good success was achieved with the introduction of orange-fleshed sweet potato in Mozambique by using intensive demand creation. The acceptability of orange-colored provitamin A–rich maize requires study and is being considered by HarvestPlus.

Conclusions

The development of biofortification as a micronutrient intervention strategy faces many challenges. Understanding and quantifying the losses of micronutrients, in particular of provitamin A, with storage, processing, and cooking, quantifying the bioavailability of micronutrients in usual diets, and overcoming the low bioavailability of iron require a comprehensive research program. Demonstrating the biological efficacy of biofortified foods needs to be approached cautiously, particularly since the changes in micronutrient intakes will be relatively modest in most cases, and confounding factors such as infection are likely to be present in the target populations of interest. This review highlights the fact that there is a fairly limited body of information on food and nutrient intakes, micronutrient bioavailability, and the efficacy of food-based intervention strategies to improve micronutrient intakes in populations where deficiencies are prevalent. Even once biofortification has been shown to be efficacious, successful introduction of biofortified foods will still require knowledge of food production, seed and rural extension systems, and consumer preferences, highlighting the continued need for collaboration between the nutrition/health and agricultural sectors.

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