Review

Benefits and Limitations of Non-Transgenic Micronutrient Biofortification Approaches

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Abstract: Increasing the amount of micronutrients in diets across the world is crucial to improving world health. Numerous methods can accomplish this such as the biofortification of food through biotechnology, conventional breeding, and agronomic approaches. Of these, biofortification methods, conventional breeding, and agronomic approaches are currently globally accepted and, therefore, should be the primary focus of research efforts. This review synthesizes the current literature regarding the state of biofortified foods through conventional breeding and agronomic approaches for crops. Additionally, the benefits and limitations for all described approaches are discussed, allowing us to identify key areas of research that are still required to increase the efficacy of these methods. The information provided here should provide a basal knowledge for global efforts that are combating micronutrient deficiencies.

Keywords: conventional breeding; agronomic; fertilizers; biofertilizers; hydroponic; microorganisms; nutri-priming

1. Introduction

Micronutrient deficiencies, also known as hidden hunger, are a global health issue that affects approximately two billion people, with the majority of cases found in Asia, Africa, and Latin America [1–3]. The most common micronutrient deficiencies concern iron, iodine, zinc, and vitamin A [4]. In general, micronutrient deficiencies can have severe health ramifications such as impaired cognitive development, stunted growth, perinatal complications, and even premature death [5]. For instance, vitamin A deficiency is the leading cause of childhood blindness (i.e., xerophthalmia), and it has been estimated that over 190 million children are affected by this deficiency worldwide [4].

Numerous strategies and methods have been recommended to combat micronutrient deficiencies, one of which is biofortification, or the process of increasing essential nutrients in food through biotechnology, conventional breeding, and agronomic practices [2,6] (Figure 1). Importantly, biofortification is generally considered a sustainable, cost-effective, and efficient method to increase micronutrients in the diets of underserved populations [6–8]. Additionally, biofortified food has become a niche commodity in developed countries in the form of functional foods, which are foods with a potentially positive effect on human health in addition to basic nutrition [9]. As some consumers have become more aware of particular nutrients' positive health effects, their food preference has shifted to foods enriched with those nutrients [10–13]. For example, NuLin® is a flax (*Linum usitatissimum* L.) variety developed to have a high alpha-linolenic acid content [14,15]. As a result, it is utilized to increase omega-3 concentrations in food and animal feed. As of 2019, the global functional food market size was estimated to be $173.26 billion and is projected to reach $309 billion by 2027 [16].
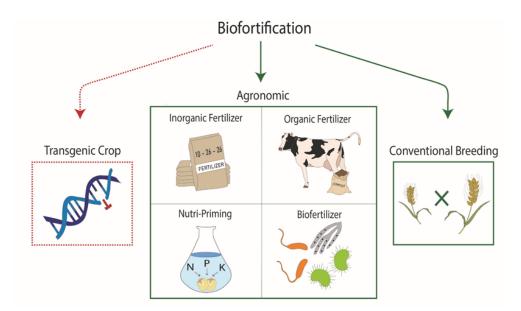
Figure 1. The three primary biofortification approaches for crops are transgenic, agronomic, and conventional breeding. Green solid arrows signify globally accepted approaches, while the red dashed arrows signify non-globally accepted approaches. Illustrated by Jorge Ruiz-Arocho.

Despite the benefits of biofortification to combat nutrient deficiencies, some biofortification methods have drawn major criticism from the public, primarily biofortification of food through transgenic biotechnological processes, or the process of introducing a gene from one organism into the genome of another organism [17–20]. Even though limited scientific evidence suggests foods developed by transgenic biotechnology have detrimental effects on human health, the majority of criticism focuses on its safety and ethical concerns [17–20]. In a recent survey conducted by the Pew Research Center with 1480 correspondents, 39% of Americans considered genetically modified foods harmful to human health, while only 10% believed they were beneficial [21]. The negative perception of transgenic crops is even higher in Europe and also occurs in regions where malnutrition is prevalent such as Africa and Asia [18,22]. Due to the strong negative public perception of these foods, many consumers refuse to purchase or consume transgenic foods. Therefore, for biofortification to have an actual impact on health around the globe, for now, biofortification research may need to shift to other non-transgenic processes that have greater public acceptance. This review will identify and describe non-transgenic biofortification processes for plant-derived products and discuss the benefits and limitations of each (Table 1). The overarching goal of this review is to explore various strategies to biofortify foods with broad global appeal.

Table 1. Benefits and limitations of conventional breeding and agronomic biofortification approaches.

Approach	Benefits				Limitations					
	Cost-Effective	Simple	Eco-Friendly	Timely	High-Cost	Labor Intensive	Unsustainable	Time Consuming	Reliant on Variation [1]	Environment-Dependent [2]
Conventional Breeding	✓		✓		✓	✓		✓	✓	✓
Agronomic Inorganic Fertilizers		✓		✓	✓	✓	✓		✓	✓
Organic Fertilizers		✓		✓		✓				✓
Biofertilizers		✓	✓	✓	✓			✓	✓	✓
Nutri-Priming	✓	✓	✓	✓					✓	✓

[1] Relies on organismal variation, or genotype or phenotype, for successful biofortification. [2] Environmental factors such as soil type, soil pH, temperature, etc., may affect this approach's efficacy for biofortifying plant products.

2. Conventional Breeding

Conventional plant breeding increases the essential nutrients of foods through the improvement of cultivars by the conservative manipulation of plant genomes within their natural genetic boundaries [23]. While conventional breeding has received less relative research attention than transgenic approaches to biofortify crops, as of 2018, it still has a higher success rate, with more released biofortified cultivars on a percentage basis [7]. Of those released cultivars, 58.1% have been cereals, followed by vegetables (19.8%), legumes (13.2%), and fruits (9%) [7]. There are many methods for breeding biofortified crops without transgenic technologies such as single-seed descent, marker-selected-breeding, and genomic selection [23–25]. For instance, zinc biofortification of rice (*Oryza sativa* L.) has been facilitated through the identification of gene-specific markers and quantitative trait loci associated with increased grain zinc content [26,27].

Major research programs worldwide such as the European Union's Health Grain Project, the global HarvestPlus program, and the BioCassava Plus program have utilized conventional breeding to generate some of the most successful biofortified cultivars [7]. For instance, the biofortified, orange-fleshed sweet potato (*Ipomoea batatas* L.) cultivars were developed to comprise a greater content of β-carotene to help combat vitamin A deficiency in developing countries [28,29]. Nutritional analyses demonstrate that orange-fleshed sweet potato varieties contain up to 21-fold more β-carotene than other sweet potato varieties [30]. Research has shown that the consumption of orange-fleshed sweet potato varieties alleviated vitamin A deficiency in children in Mozambique, Uganda, and South Africa [31–33]. Another prominent example is the iron-biofortified rice variety IR68144–2B-2–2-3, developed by the International Rice Research Institute in conjunction with the HarvestPlus program [34]. This rice variety contains four- to five-fold more iron after processing and cooking than other commercial rice varieties [34,35]. In a controlled diet study, this variety increased ferritin and whole-body iron by 20% in Filipino women [35]. For more examples of biofortified crops, please refer to the cited reviews [3,6,7].

2.1. Benefits of Conventional Breeding

One of the major advantages of utilizing conventional breeding practices to biofortify foods is that the public widely accepts this process. Humans have been consciously and unconsciously altering domesticated plants for thousands of years, making this process a societal norm [36,37]. Therefore, biofortified foods developed by this process will be more likely accepted by a large portion of the world's population than food biofortified through transgenic approaches [6,20]. In addition to having a global appeal, this method is cost-effective for growers [6,38]. As mentioned above, micronutrient deficiencies occur mainly in vulnerable populations in developing regions such as Africa, Asia, and Latin America [1,2]. Most of the growers in these areas are smallholder farmers that generally live in poverty [39]. Unlike other biofortification processes, the biofortification of food through conventional breeding practices adds minimal additional costs to growers [40]. The majority of the costs associated with this practice would be incurred by the breeding institute through research and development of the biofortified cultivars [40].

Conventional breeding is also a more sustainable option for food biofortification than agronomic approaches [6,8,41]. As more research highlights the inadvertent negative effects of agricultural practices on the environment, the need to sustainably produce food has become more important than ever [42–45]. Specifically, the overuse of fertilizers in agroecosystems has been linked to algal blooms, reduced biodiversity, and polluted air and waterways [42–45]. In contrast, conventional breeding is considered a sustainable biofortification process, as it does not depend on the additional use of synthetic inputs in agroecosystems like some agronomic approaches.

2.2. Limitations of Conventional Breeding

Despite the numerous advantages of utilizing conventional breeding to biofortify foods, there are several limitations, the first of which is the reliance of this method on

the standing diversity in the targeted crop gene pools. Biofortification can only occur if there is trait diversity present in the primary, secondary, or tertiary gene pool of the targeted crop [7,40]. If no diversity is present, then the targeted crop cannot be biofortified through conventional breeding. For example, oilseed biofortification has only occurred through transgenic processes due to its limited genetic diversity, low heritability, and linkage drag [7]. Additionally, ancillary gene pools that have been underutilized in crop improvement efforts may provide the greatest opportunity to identify lost beneficial nutritional traits [46–49]. For example, the stay-green phenotype in chickpeas (*Cicer arietinum* L.), a potentially lost trait as a result of domestication and breeding processes, was seen to be associated with greater levels of lutein and β-carotene when compared to conventional chickpea varieties [50]. Moreover, nutritional analyses of modern crops and their wild relatives revealed that some modern crops are of lower nutritional value than their wild counterparts [51–54]. Consequently, the wild relative gene pool may have potentially more beneficial phenotypes for biofortification than the domesticated gene pool [48,49]. However, the utilization of wild relatives could be a hurdle for several target crops due to pre- and post-zygotic reproductive barriers [55–58], the potential for unfavorable traits to be tightly linked to favorable traits [59,60], and wild relatives being severely underrepresented in world gene banks [61]. The limited availability of wild accessions results from inadequate systematic collection of wild relatives in their native range. Furthermore, the current collection of wild relatives has become increasingly difficult due to the limited in situ conservation efforts placed on wild relatives. For example, 2 of 24 sites containing wild chickpea populations in southeastern Anatolia have been permanently lost due to rapid human development [47].

Another limitation of conventional breeding is that the process is rather time-consuming [40,62]. It can take several years before a cultivar is deemed releasable by the breeding institute. This is because introgressing a trait into an elite cultivar requires extensive selection, at least until the sixth generation [62]. However, there are strategies such as high-throughput phenotyping platforms, seed chipping technology, molecular markers, genomic selecting breeding approaches, and the manipulation of day length that can expedite the release of a cultivar [62–64]. However, several of these methods have a higher upfront cost than traditional breeding methods. Thus, they are not widely utilized in some public breeding institutes even though they are more efficient on a per-dollar basis [64–67]. Moreover, before a cultivar is released, it needs to be tested in diverse growth environments, since genetic-by-environment interactions can substantially alter a crop's phenotype and potentially the nutritional components of the cultivar [62,68,69]. Therefore, due to genetic-by-environment interactions, a biofortified crop may lose its enhanced nutritional trait [68].

3. Agronomic Biofortification Approaches

Agronomic biofortification approaches are globally utilized because they are straightforward and timely. These approaches are defined as pre-harvest agronomic practices that enhance the nutritional content of food [70]. A caveat of these approaches is that they must occur pre-harvest for the food to be considered biofortified. If the approaches occur post-harvest, then the food is categorized as fortified [70]. Some agronomic biofortification practices are the application of soil or foliar inorganic fertilizers, organic fertilizers, and biofertilizer as well as nutri-priming.

The most prominent agronomic practices used to biofortify food crops are the soil or foliar application of inorganic fertilizers. The use of these fertilizers has resulted in enhanced micronutrient content for a variety of crops in various agroecosystems [7,71,72]. For instance, soil- or foliar-applied zinc fertilizers led to increased zinc content in corn (*Zea mays* L.) [73–77], wheat (*Triticum aestivum* L.) [72,76–79], peas (*Pisum sativum* L.) [80], chickpeas [81], potatoes (*Solanum tuberosum* L.) [82–84], and rice [85–89]. For more examples of crops biofortified through agronomic approaches, please refer to the cited reviews [7,40,71,89,90].

A similar method to applying fertilizers and foliar sprays with enhanced micronutrient content is the supplementation of micronutrient media to soilless cultivated food [91]. In soilless cultivation, plant productivity is optimized due to strictly regulated environmental conditions such as temperature, light, and nutrient solution [92,93] and by maximizing root contact with the nutrient supply [94]. The constant root contact with the nutrient solution maximizes the uptake, translocation, and accumulation of micronutrients and allows for consistent crop nutritional quality [91,95]. In addition to these benefits, soilless cultivation extends the cultivation cycle and allows for year-round production due to, in part, the avoidance of soil limitations such as soil pollution, soil fertility reduction, and soil-borne diseases [91,96]. Furthermore, soilless cultivation has been described as having low labor requirements due to the absence of weeds, straightforward harvesting and processing, and the utilization of automated systems for plant care [96].

Research has shown that the supplementation of specific micronutrient media in soilless cultivation results in greater contents of folate in spinach (*Spinacia oleracea* L.) [97], silicon in green bean (*Phaseolus vulgaris* L.) [98], zinc and selenium in lettuce (*Lactuca sativa* L.) [99], and zinc in white cabbage (*Brassica oleracea* L.) [100]. Moreover, the role of microgreens as a soilless biofortified food product to combat micronutrient deficiencies, or as functional food, has become promising in recent years [101,102]. This is because microgreens have a high phytonutrient content, flavor-enhancing properties, a short cultivation cycle (harvested 7-21 days after germination), and can be biofortified through soilless cultivation [101,102]. For instance, the microgreens basil (*Ocimum basilicum* L.), coriander (*Coriandrum sativum* L.), and tatsoi (Brassica rapa *subsp. narinosa* (L.H. Bailey)) were biofortified with selenium [103,104], Brassicaceae microgreens with zinc and iron [105], and buckwheat microgreens (*Fagopyrum esculentum* (Moench)) with selenium and iodine [106]. Additionally, selenium biofortification of wheat microgreens has also led to increased levels of carotenoids or other bioactive compounds (phenolics, flavonoids, vitamin C, anthocyanin) [107]. More examples of biofortified food through soilless cultivation are specified in Rouphael et al. [91].

3.1. Limitations of Inorganic Fertilizers to Biofortify Crops

Although the application of inorganic fertilizers is an effective, relatively simple, and quick agronomic approach to biofortify crops in soil and soilless systems, there are several limitations to this approach. The primary limitations are the detrimental environmental effects that result from the overuse of inorganic fertilizers. As previously mentioned, the overuse of fertilizers in soil systems has led to water pollution, algal blooms, and a reduction in biodiversity in natural systems [52–55]. Moreover, inorganic fertilizers are expensive and labor-intensive to apply, which would add further financial strain to impoverished smallholder farmers, who make up a large portion of farmers in food-insecure areas [39]. Additionally, identifying when to apply fertilizers to achieve the maximum increase in nutrient content is another challenge [68,108,109]. Phattarakul et al. [101] found that foliar zinc application to rice after flowering, during the early milk plus dough stages, increased grain zinc content more than earlier applications [108]. However, Rodrigo et al. [102] found that foliar selenium application to wheat pre-flowering and in between the booting and heading stages increased selenium grain content the most [109].

Furthermore, similar to soil cultivation practices, the timing, chemical form, and amount of fertilizers in soilless cultivation are critical for effective biofortification [91]. For instance, when comparing carrots (*Daucus carota* subsp. *sativus* (Hoffm.)) biofortified hydroponically to field foliar fertilizer applications, the same rate of iodine fertilization resulted in cumulative toxic levels of iodine in the hydroponically biofortified carrots [110]. Moreover, the biofortification of multiple micronutrients at once may be problematic in soilless cultivation due to the potential antagonistic effects micronutrients have on crop accumulation. Germ et al. [96] found that selenium content was greatest in buckwheat when fertilized in combination with selenium and iodine; however, iodine content was greatest when fertilized with iodine alone [106]. Therefore, for fertilization to be an effective

and efficient soil and soilless biofortification approach, the proper timing, amount, and chemical form of the application are critical.

Furthermore, crop phenotype, genotype, and soil conditions are additional factors that influence the effectiveness of fertilizers to biofortify crops [111,112]. It has been documented that phenotypic differences in nutrient uptake, translocation, and accumulation result from plant genotype [112–114]. For example, rice [68,115] and corn [74] genotypes differed in grain zinc content when applied with zinc fertilizers. Additionally, soil factors can exacerbate nutrient accumulation differences between genotypes. Mabesa et al. [68] showed that only one out of the eight tested rice biofortification breeding lines reached the targeted zinc concentration when grown in a severe zinc-deficient site with fertilization [68]. Likewise, iron biofortification via iron fertilization of calcareous soil with high pH is ineffective due to the reduced mobility of iron and the rapid conversion of iron into unavailable forms [116]. Therefore, crop genotype and the growth environment can severely hinder the biofortification of crops with fertilizers. Detailed mechanisms of how plants uptake, translocate, and accumulate micronutrients can be found in White and Broadley [112]. This limitation is less pronounced in soilless systems, as environmental conditions in these systems can be tailored to any crop genotype. However, soilless biofortification has several unique limitations: It is considered expensive due to the necessary equipment and energy costs [117], is not applicable to all crop types, is considered unsustainable in some regions [117], and is limited in capacity due to the physical constraints of the soilless system being used (e.g., size of the greenhouse, pots, etc.).

3.2. Benefits and Limitations of Other Agronomic Approaches

Other agronomic approaches to biofortify crops include the application of organic fertilizers. Organic fertilizers have been categorized as a cost-effective, eco-friendly alternative to inorganic synthetic fertilizers [118–122]. Organic fertilizers are obtained from animal or plant sources such as animal manure or green manure, respectively, and have been shown to increase the micronutrient content in various crops [121,123,124]. For example, vermicompost (earthworms converting organic waste into fertilizer) increased the content of zinc by 14% and iron by 7% in barley (*Hordeum vulgare* L.) [125], and poultry manure increased the iron content in wheat by 15% [126] and in rice by 10% [127]. However, if not properly treated, a major disadvantage of some organic fertilizers is that they may contain human pathogens such as *Escherichia coli*, antibiotics, and heavy metals [128,129]. Additionally, organic fertilizers are considered an imprecise method to provide nutrients to crops because the nutrient content in organic fertilizers is highly variable, and when applied, the nutrients are not immediately available to the crops [122,130]. The amount of nutrients and the element types in organic fertilizers are mainly dependent on the age, origin (animal or plant), and environmental conditions from which the organic fertilizers were derived [130].

Biofertilizers differ from organic and inorganic fertilizers as they are substances that contain microbial inoculants, consisting of microorganisms that promote the growth and productivity of the host plant [118,119]. These bacteria are commonly referred to as plant growth-promoting bacteria. In addition to enhanced growth, these bacteria have also been shown to augment the nutrient content in crops by increasing the supply or availability of nutrients [118,119]. Specific biofertilizers such as cyanobacteria (*Anabaena Azotobacter* sp. Biofilm and *Anabaena* sp.–viz. *Providencia* sp) and *Bacillus aryabhattai* facilitated the zinc biofortification of corn [131], wheat [132], and soybeans (*Glycine max* L.) [132], respectively. Despite this practical promise, biofertilizers have not been shown to offset the use of fertilizers in agroecosystems due to several obstacles [133], one of which is the identification of the proper plant growth-promoting bacterium for each host crop, particularly one that can withstand packaging, storage, and application on a global scale [133]. Furthermore, the beneficial effects that biofertilizers may provide may be short-lived due to the agroecosystem's environment. This is because environmental factors such as soil pH have a more substantial influence on the presence of soil microorganisms

than the host plant [134,135]. Consequently, there is the potential that the supplied plant-growth-promoting bacteria may be maladapted to the environment, resulting in minimal crop biofortification.

Another biofortification approach is nutri-priming, which is the method of soaking seeds in solutions containing nutrients before planting [136–138]. Nutri-priming has been primarily used for yield improvement, germination, seedling establishment, and root system development in crops [136–138]. However, some studies have shown that this process improves grain nutrient content [138,139]. For instance, zinc nutri-priming increased grain zinc content by 29% in chickpeas [140] and from 12% to 15% in wheat [140,141]. Furthermore, nutri-priming flax and fenugreek with fish oil increased polyunsaturated essential fatty acids (i.e., docosahexaenoic acid and eicosapentaenoic acid) in sprouts [142]. An additional benefit of nutri-priming is that farmers can conduct this approach, as it is considered a low-cost and simple practice for nutrient enrichment [81,140]. However, the effectiveness of nutri-priming may be largely affected by crop type, genotype, time (duration of the priming process), osmotic potential of the priming solution, and environmental conditions (e.g., light and temperature) [136,137,143].

4. Conclusions

Biofortification of food through conventional breeding and agronomic approaches has been successful for various crops. As a result, biofortified food has enhanced nutrient concentration in diets, which benefits human health in food-insecure areas. However, there are several limitations to conventional breeding and agronomic approaches such as the detrimental effects of fertilizers on the environment, if misused, or the limited genetic variation present in some crops. These limitations dampen the efficacy and application of biofortification methods globally. Nonetheless, the first step in overcoming the described hurdles is identifying alternative practices, and critical research is required to broaden the applicability of biofortification approaches. However, if the efficacy and application of biofortification does increase, biofortification alone is not the sole answer to providing relief for the two billion people worldwide suffering from hidden hunger. Biofortification as a whole has limitations. In particular, the process does not address the high cost or accessibility of micronutrient-rich foods. Additionally, biofortified foods are generally limited in the amount and range of micronutrients [4]. Therefore, an assortment of intervention approaches, such as diversifying diets and the fortification of commercial food along with biofortification, will need to be utilized to help alleviate hidden hunger.

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