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Biofortification of Staple Crops to Alleviate Human Malnutrition: Contributions and Potential in Developing Countries

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Abstract: Micronutrient malnutrition is a global health challenge affecting almost half of the global population, causing poor physical and mental development of children and a wide range of illnesses. It is most prevalent in young girls, women, and pre-school children who are suffering particularly from the low consumption of vitamins and micronutrients. Given this global challenge, biofortification has proven to be a promising and economical approach to increase the concentration of essential micronutrients in edible portions of staple crops. Produce quality and micronutrient content can be further enhanced with the use of micronutrient fertilizers. Especially developing countries with a high percentage of malnourished populations are attracted to this integrated biofortification, combining modern agronomic interventions and genetic improvement of food crops. Consequently, maize, rice, wheat, beans, pearl millet, sweet potato, and cassava have all been biofortified with increased concentrations of Fe, Zn, or provitamin A in various developing countries. Today, there are several large-scale success stories in Africa and Asia that support the research and development of biofortified crops. In this review, we summarized what has been achieved to date and how edible crops can be further improved by integrating agronomic and genetic strategies to upgrade the nutritional status of children and adults around the world.

Keywords: micronutrients; developing countries; agronomic biofortification; genetic biofortification; malnutrition; hidden hunger

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1. Introduction

Mineral micronutrient deficiencies in humans are widespread globally, especially among women and children. Micronutrients are needed in the human diet in minute quantities, yet it is estimated that over 3 billion people suffer from micronutrient deficiencies, resulting in deleterious impacts on human health [1–5]. The prevalence and devastating impacts of micronutrient deficiencies are exacerbated in areas where cereal grains constitute a large portion of the diet, dietary diversity is low, and/or supplementation/fortification programs are lacking [1,2]. Micronutrient deficiencies impair growth, cognitive development, and immune function, with often lifelong consequences [6]. Therefore, this 'hidden hunger' poses many burdens on human health, economic growth, and constrains efforts to alleviate poverty. With a continuously growing population and changing diets, food demand is increasing drastically but our space for agriculture is limited leading to intensive use of natural resources and often poor-quality agricultural produce. Agricultural interventions to enhance the nutritional value of edible and forage crops feeding livestock could help to improve human nutrition, especially in developing countries.

One option to reduce micronutrient deficiencies is biofortification, defined as the process of improving the nutritional value of edible crops through selective conventional breeding, mineral fertilization, or advanced transgenic approaches. Additionally, the quality of the produce, post-harvest losses, food taste, cooking time, yield, and pest resistance can also be improved along with the biofortification process. Biofortification targets concentrate mostly on people living in rural areas depending mostly on their staple crops to fulfil their food requirements. Biofortified crops may make their way into local retail outlets and as a result, particularly people in rural areas could be reached comfortably. Their diets consist mostly of staple foods, and they are hard to reach through other strategies of nutritional improvement (e.g., fortification of processed food or supplements). Therefore, the lack of nutrients in the food they consume daily can at least be minimized if not eliminated entirely [7]. Although populations in urban areas may have some access to commercially fortified and processed foods, biofortified staples may still be more beneficial to them, especially for poor people who have limited access to expensive fortified and diversified food.

Biofortification offers many advantages for developing countries by using various strategies including engineering of staple crops [8]. As the World Health Organization considers iron (Fe), zinc (Zn), and vitamin A as the most limited micronutrients, research and programs such as HarvestPlus are focusing on these micronutrients to improve the most common crops such as corn, wheat, and rice [6]. These common crops are available for everyone globally and do not need special management, because the enrichment of produce can be achieved without affecting the crop's productivity. As most of the target minerals are also important for the plant's own nutritional requirements and may contribute to environmental stress tolerance, it can even result in better growth and higher yields. This is particularly important when the environmental conditions for farming are inferior, as they often are in developing countries, and the new varieties have an advantage over conventional varieties [8].

It is the general target in all breeding efforts for biofortification to reach or even improve the yield level of non-fortified varieties. Despite the complex breeding process, most biofortified crops have been developed and mapped using conventional breeding, and transgenic approaches were used in a few cases only [6]. For example, conventional breeding led to the development of rice with enhanced Zn and Se content [9], wheat with higher grain Fe and Zn [10], or maize enriched with provitamin A [11]. A common example of a genetically biofortified crop is golden rice, which consequently has faced a lot of criticism due to various social and political concerns. The issue of genetically modified crops is still under debate in most developed countries, nevertheless, developing countries discuss the potential of genetically modified nutrient-enriched crops because of the large number of poor people prone to micronutrient malnutrition.

A big challenge of biofortified food crops is acceptance by the consumers, which determines the demand for the crop to be cultivated by farmers [11]. For example, the color difference between the ordinary and a biofortified crop with higher concentration of β-carotene demands considerable efforts to generate its market. In Africa, where common corn for human consumption is white and the yellow one is for animals, consumers need to be convinced to buy yellow corn for human use [8]. If the crops have the same color, consumers must be able to distinguish the ordinary crop and the one enriched with nutrients. Both problems must be solved with information and communication between scientists, farmers, and consumers until the biofortified food is accepted and well known [8]. However, even then, the transport to remote markets may be difficult and indigenous markets need to be developed [8].

Nonetheless, biofortified food crops have great potential for many people in developing countries because their diet is mainly based on local staple crops, often causing an unbalanced nutrition. Enrichment of nutrients in these crops can prevent or reduce malnutrition because they are consumed in large quantities on a daily basis. Furthermore, biofortification has low costs, spreads itself through seed sharing, may improve plant growth, and has other positive health effects. In the case of conventionally bred crops, there is no further need for regulatory management of biofortified crops, enhancing its potential for developing and poor countries further. The objective of this review is to give an insight into biofortification of staple crops to address human malnutrition in developing countries, keeping in view recent contributions and future perspectives.

2. Existing Micronutrient Deficiencies in People of Developing Countries

Balanced daily nutrition should contain macronutrients, including carbohydrates, proteins and fat, as well as micronutrients such as electrolytes, minerals and vitamins. Macronutrients cover the need for energy throughout the body through large intake quantities, whereas micronutrients are involved in the essential functions of the body such as metabolism, cell growth, and immune reactions, and are required in minimal amounts. Therefore, the generic term "malnutrition" includes protein/energy undernutrition and micronutrient deficiencies, representing a major public health problem particularly in southern Asia and sub-Saharan Africa [4]. Undernutrition concerns around 821 million people and ~2 billion people in the world are affected by one or more forms of micronutrient deficiency [12]. Micronutrient deficiency is also called "hidden hunger" or "silent epidemic" because it can exist even where the food supply is adequate and those affected at first glance seem well fed [13]. There are specific diseases caused by micronutrient deficiencies, but they often act as exacerbating factors in infectious and chronic diseases, greatly impacting mortality and quality of life [4]. Additionally, diseases consume micronutrients through the body's immune system, leading to a vicious cycle of malnutrition and infection [14,15]. Micronutrient deficiencies affect people all over the world, but the nutrition of the underprivileged population in developing countries is particularly low in micronutrients (Figure 1), because they use most of their low income for staple food [13].

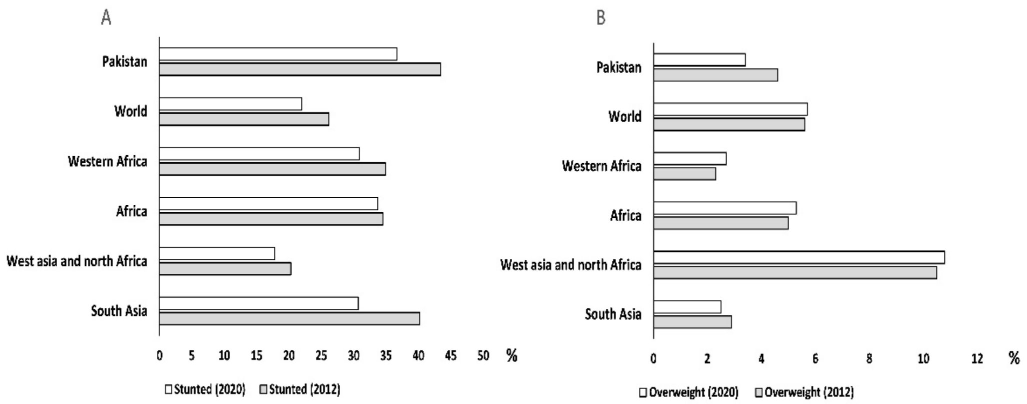

Figure 1. Prevalence of undernutrition (**A**) and obesity (**B**) in children under five years of age in Pakistan and other global regions during 2012–2020, data extracted from FAO 2021 [13].

Nineteen vitamins and minerals are considered essential for human health, of which the deficiencies of iodine (I), Fe, Zn, and vitamin A are the most widespread forms of micronutrient malnutrition [16]. Especially women in the reproductive age and young children under the age of 5 years are affected by the lack of micronutrients due to an increased need during the developmental phases such as pregnancy and early childhood [17,18]. Another problem is Folate deficiency, causing problems especially in pregnant women, and developing countries with poor quality food are more prone to this deficiency [19]. An overview of the deficiencies of Fe, I, vitamin A, and Zn is given in Table 1 mentioning possible causes, symptoms, and possible solutions.

Table 1. Causes, manifestations, management, and prevention of the major micronutrient deficiencies based on [16].

Nutrient	Specific Function	Causes of Deficiency	Manifestation of Isolated Deficiency	Management and Prevention
Iron	Hemoglobin, various enzymes, myoglobin	Poor diet and elevated need (e.g., while pregnant, in early childhood); chronic loss from parasite infections (e.g., hookworms, schistosomiasis, whipworm)	Anemia and fatigue impaired cognitive development, reduced growth and physical strength	Food richer in iron and with fewer absorption inhibitors, iron-fortified weaning foods, low-dose supplements in childhood and pregnancy, cooking in iron pots
Iodine	Thyroid hormone	Except where seafood or salt fortified with iodine is readily available, most diets worldwide are deficient	Goitre, hypothyroidism, constipation, growth retardation, endemic cretinism	Iodine supplement, fortified salt, seafood
Vitamin A	Eyes, immune system	Diet poor in vegetables and animal products	Night blindness, xerophthalmia, immune deficiency, increased childhood illness, early death, contributes to development of anemia	More dark green leafy vegetables, animal products, fortification of oils and fats, regular supplementation
Zinc	Many enzymes, immune system	Diets poor in animal products, diets based on refined cereals (e.g., white bread, pasta, polished rice)	Immune deficiency, acrodermatitis, increased childhood illness, early death, complication in pregnancy, childbirth	Zinc treatment for diarrhea and severe malnutrition, improved diet

Iron as the central ion of hemoglobin is essential for the oxygen transport of the erythrocytes. It is also a component of various enzymes and the muscle protein myoglobin. Iron deficiency leads to anemia (a decrease in the hemoglobin concentration in the blood), affects cognitive development, growth, and physical fitness. Anemia affects 43% of children under the age of 5 and 38% of pregnant women worldwide. Furthermore, hemoglobin deficiency increases maternal mortality and the risk of low birth weight. This effect is reinforced by genetic dispositions, blood loss due to menstruation, and infections such as malaria and other parasites [16].

Vitamin A is essential for eyesight and a healthy immune system. Its deficiency increases the risk of blindness, contributes to the development of anemia, and is associated with an increased infection rate and child mortality. Vitamin A deficiency affects around 190 million preschoolers worldwide [13,16].

Iodine is required to produce thyroid hormones and there is an increased need especially during pregnancy and the physical and cognitive development of children. Around 1.8 billion people worldwide are insufficiently supplied with I through their diets. Except in countries where food is artificially enriched with I, its deficiency is widespread worldwide [13,16].

Zinc is an essential component in over 300 enzymes that the body needs for metabolic processes, RNA and DNA synthesis, and the immune system. More than 17.3% of the global population is at great risk of Zn deficiency. Zinc deficiency deteriorates the immune system, increases the risk of infectious diseases, and can have a negative impact on child development during and after pregnancy [13,16]. In recent studies, the significance of Zn has been further revealed in context with its role in developing immunity against COVID-19 [20].

In order to counteract the shortage of micronutrients, experts present four different integrated solutions (Figure 2): dietary diversification, fortification, biofortification, and supplementation [13]. Supplementation is the direct supply of nutrients in the form of syrup or pills and is used as a short-term solution in emergency situations. A more sustainable method is fortification, in which micronutrients are added to staple foods. The most common form is iodized salt, but some wheat and milk products are enriched with various micronutrients too [17]. In biofortification, the nutritional properties of plant varieties are increased through classic breeding and sometimes through genetic engineering (genetic biofortification), and/or fertilizer use (agronomic biofortification). The most sustainable way to prevent micronutrient deficiencies is a dietary diversification, meaning basic staples are adequately supplemented with fruits, vegetables, and animal products year-round. This can be achieved by changing the nutritional culture of communities, through e.g., small-scale community gardens, small livestock, and techniques of preserving [13].

In order to eliminate micronutrient deficiencies, the above-mentioned strategies must be combined along with other measures. At the political level, health care, education, sanitation, water supply, and housing must be further promoted, and at the same time education and awareness campaigns must be expanded. Through in-depth research in science and technology new helpful knowledge can be gained (20). Additional challenges such as the effects of population growth and climate change, will have to be overcome in the future [21]. Sustainable prevention of micronutrient deficiencies therefore requires a holistic approach that includes society, politics, and science [16].

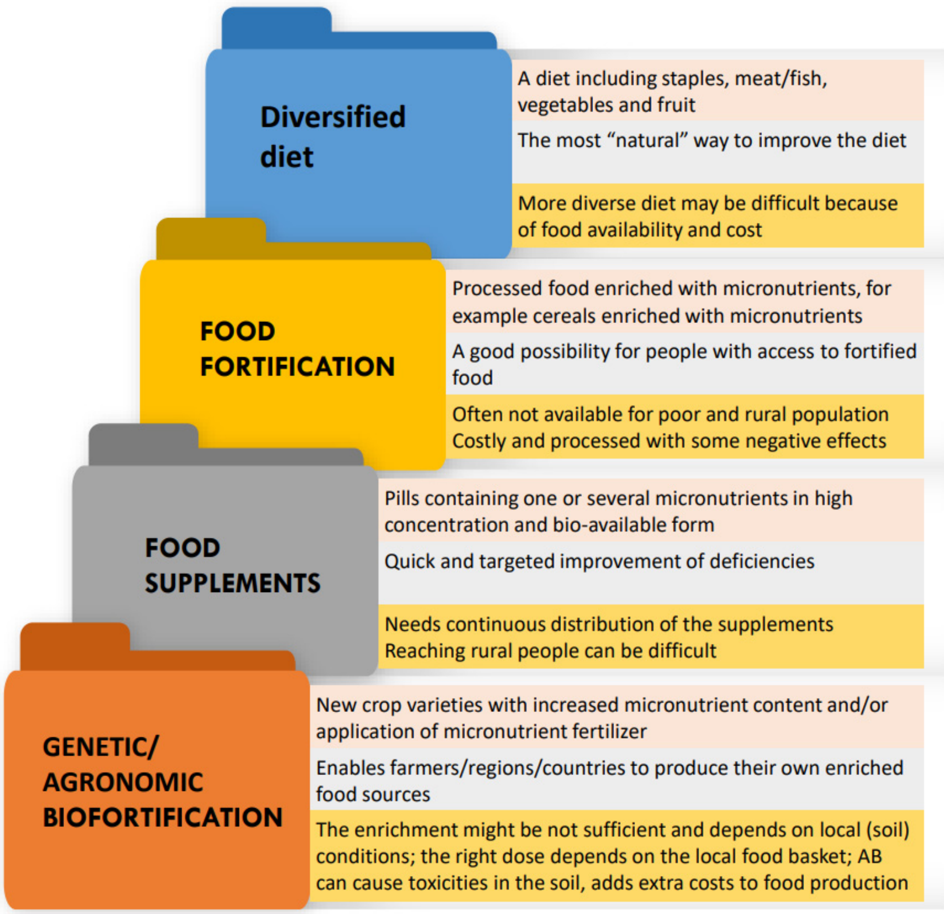

Figure 2. The principal options to address micronutrient deficiencies including examples, advantages, and disadvantages of each option. AB: agronomic biofortification.

3. Staple Food Contribution to "Hidden Hunger" in Developing Countries

Hidden hunger is among one of three types of hungers. There is acute, chronic, and hidden hunger. Acute hunger describes undernourishment over a specific period. It can occur after natural disasters, war, or other crises. In contrast to that, chronic hunger stands for long-term undernourishment with a consumption of an insufficient amount of food. Hidden hunger designates the lack of essential nutrients even if the number of calories consumed is sufficient. Among the essential minerals, especially I, Fe, Zn, and vitamin A occur in limited amounts in food, which can lead to deficiencies through unvarying nutrition. Furthermore, there are essential fatty acids and amino acids, which need to be provided through food [22]. The chronic lack of nutrients can lead to serious health issues, diseases, and diminished mental or physical development especially in children. It is estimated that two billion people suffer from nutrient deficiency worldwide, the majority in developing countries, without access to nutritious and safe food [23].

People in developing countries rely heavily on staple foods, such as cereals and beans. These foods are sufficient sources of macronutrients such as carbohydrates, proteins, fats, and calories, but they are inheritably deficient in micronutrients such as minerals and vitamins, especially staples grown in Zn-deficient soils [24,25]. Particularly children and pregnant women are at risk of becoming nutrient deficient. Children's health and their ability to absorb nutrients can also be harmed through poor water, sanitation, and hygiene conditions leading to a further reduced nutritional status [26,27]. Micronutrient deficiencies do not only harm the individual but also negatively impact the economy, as the people's ability to work is affected. Thereby, they are trapped in a vicious cycle which makes it difficult for them to escape poverty and get better nutrition.

Along with fortification of food during processing, increased diversity of food including staple grains, fruits, and vegetables can provide a balanced and appropriate combination of macronutrients, vitamins, and minerals. Different food groups, their suitability for fortification along with successful examples are listed in Table 2, however, poor people in developing countries often have financial or agricultural limitations to access them. Therefore, biofortification of staple crops with micronutrients is considered a promising way to combat malnutrition. It is a cost-effective and easily approachable strategy with minimal complexities of regulations. Nevertheless, nutritional education is needed to understand the different nutritional values of food to make it more successful [7].

Table 2. Examples of major food groups, their role in nutrition, and examples of fortification, based on [8].

Food Commodity	Disadvantages	Example
Cereals	Consumed in large quantities, throughout the year, and by all members of society, as part of typical diet but micronutrient concentrations usually low.	Wheat flour fortification with iron (Chile).
Fats, oils, and margarines	Intake generally not sufficient to supply 100% of recommended intake.	Margarine fortification with vitamins A and D (e.g., in Europe).
Dairy products	Favors mothers and children, but poor rural populations usually have limited access.	Fortification of milk with iron (Argentina).
Condiments	Sugar, spices, starches, sauces consumed regularly through the population, particularly SE Asia, but usually in small amounts.	Fish sauce fortification with iron (Thailand), sugar fortification with vit. A (Guatemala).
Value added products	May be consumed only sporadically by populations with deficiencies.	Water, bread, juices, bread fortified with Zn, Fe, and vitamins (e.g., in the United States).

However, not only the food people are consuming is lacking in nutrients but often also the soils where the crops are grown This can be due to geology and soil formation, but also to intense cropping in response to high population pressures, resulting in various nutrient element deficiencies essential for plant growth [28]. Furthermore, every soil naturally has a different composition and potential to grow crops. To rectify resulting deficiencies, fertilizers can be used both to improve the health of the plant and to increase the accumulation of minerals within cereal grains [29–31].

Furthermore, every soil naturally has a different composition and potential to grow crops. To rectify resulting deficiencies, fertilizers can be used both to improve the health of the plant and to increase the accumulation of minerals within cereal grains [29–31]. This is called agronomic biofortification, and fertilizers are applied either to the soil or to the leaves. While historically fertilizer was only used to improve yields, it is now another way to maintain and enrich nutrition. In a well-known example from Finland, wheat was fertilized with supplements of Zn and Fe through a foliar spray. This promoted root growth and resistance to pathogens, and later accumulated in the plant available for human consumption. Compared to breeding or biotechnological enhancement of plants, foliar spray is only a short-term solution and connected with high workload and costs, since the dose of fertilizers depends on cultivated species, application technique, and soil properties. In addition, some elements are difficult to deliver with foliar sprays and wrong amounts of fertilizers in foliar sprays can cause leaf damage and yield penalties. If applied to the soil, minerals provided as fertilizers need to be plant available which again might be affected by soil conditions (especially soil pH) or the growth stage of the plant. Additionally, even if the mineral uptake was successful, the plant might store it in the leaves instead of the fruits or seeds, or in a non-available form [32]. However, continued increased uptake of biofortified crops will eventually lead to low soil availability of micronutrients and some form of replenishment will be necessary in the long run anyway. A disadvantage specifically for poor people is an increasing food price caused by added fertilizer costs, and the risk to pollute the environment, groundwater, and whole ecosystems may not be ignored.

Besides the lack of nutrients, there are also "anti-nutrients" in many staple foods. These substances hinder or completely prevent the absorption of nutrients or in some cases are even toxic. They occur naturally in many plants and protect them from predation. Examples for anti-nutrients are lectins, saponins, and phytate in legumes and whole grains, which impede the absorption of calcium (Ca), Zn, Fe, and phosphorus (P). However, in some cases, anti-nutrients have beneficial properties too, depending on the amount consumed. To prevent the negative effects of anti-nutrients, it is recommended to avoid eating large quantities of a single food per meal. This is hardly possible for people who only have access to a limited variety of food and rely on staple foods as their main source of energy [33].

Conclusively, hidden hunger is originating from the insufficient uptake of micronutrients and is a crucial health aspect for populations in developing countries. Staple foods are often all they can afford, leading to a monotonous diet. Staple foods often lack sufficient essential nutrients and may contain anti-nutrients if consumed in large amounts. Staple food biofortification offers a sustainable solution to reduce the drawbacks of staple foods and can contribute to reducing malnutrition. However, depending on the crop and the soil where it is grown, a combination with the application of micronutrient fertilizer might be necessary to maintain soil fertility and achieve sufficient micronutrient concentrations in the produce.

4. Existing Strategies to Reduce Malnutrition in Developing Countries

Large numbers of people in the developing world are facing deficiencies in their diet, leading to not only individual health issues but also economic problems in their countries. Nutrient supplementation and food fortification are thought to be effective methods for balancing people's diet globally. Nonetheless, they are difficult to address effectively in the long term given the often limited accessibility of rural populations and inhabitants of developing countries.

Food fortification, a form of supplementation and enhancement of processed food, comes with a marginal change in already existing infrastructure of food distribution. While portion sizes remain the same, they are made more nutritious by adding crucial macro or micronutrients which are essential for physical and mental health functions. Benefits of applying food fortification strategies in aged care homes were shown and found to be cost effective [34]. With the focus on products that are already consumed in care homes, fortification strategies are very effective. A six-month consumption of fortified milk has been successfully used to achieve recommended vitamin D levels [35,36]. Consequently, this led to better bone quality (with additional vitamin supplementation), muscle strength, and digestive resorption of calcium. Thus, the dilemma of natural ageing and its accompanying undernutrition can be sustainably softened by means of food fortification. This shows that food fortification can improve quality and diversity in diets especially when combined with the nutritional potential of fruits and vegetables. However, the recipients are not always so easy to reach, particularly with canned food there is a significant loss of vitamins, and industrial food processing often includes addition of sugar, fat, and sodium, causing obesity, hypertension, and/or diabetes. Therefore, it does require great effort and investment when trying to implement fortified food especially into poor infrastructure, and there is the rising issue with obesity due to consumption of industrially processed foods [36,37].

With the outlined difficulties of supplementation and food fortification, biofortification offers a more fundamental and sustainable strategy for fighting malnutrition in developing countries [38]. Described as a feasible and affordable method of micronutrient delivery to poor people, it offers plants to the farmers that are more nutritious thanks to breeding or biotechnology. Studies claim that already more than 20 million individuals are benefiting from CIGAR's (Consultative Group for International Agricultural Research) 2004 introduced HarvestPlus program. The potential and delivery of biofortification for major crops shown in Table 3 was validated by [39] and shows the increasing number of target countries reached by the HarvestPlus program. Before eventually releasing crop varieties, they undergo different stages of screening and breeding, ensuring not only rich and tasteful nutritional potential, but also high yields as well as tolerance to drought and diseases. Table 3 demonstrates the increasing utilization of biofortified crops in developing countries within just few years of the HarvestPlus program. The Orange Sweet Potato (OSP) is now ranked as a major crop to fight vitamin A carotene deficiencies in sub-Saharan Africa and shows also growing potential as a source of income for poor households [40]. Furthermore, the immense cost effectiveness of OSP is demonstrated in [38], describing its upcoming crucial role not only to fight global malnutrition but also providing financial stability to farmers.

Table 3. Biofortified products, their release date, and spread in different countries around the globe, based on [39].

Crops	Nutrient	Year	Biofortified Products Released in These Countries
Banana	Vitamin A	2017	Tanzania, Burundi, DR Congo
Beans	Iron	2016, 2017	Brazil, Bolivia, Colombia, El Salvador, Guatemala, Honduras, Nicaragua, Panama, Burundi, DR Congo, Rwanda, Tanzania, Uganda, Zimbabwe
Cassava	Vitamin A	2017	Brazil, Nigeria, Sierra Leone, Cameron, DR Congo, Ghana,
Irish potato	Iron, zinc	2016	China
Lentil	Iron, zinc	2017	Bangladesh, Eretria, Ethiopia, Syria, India, Nepal, Syria
Maize	Vitamin A	2017	Cameron, DR Congo, Ghana, Malawi, Mali, Nigeria, Zambia, Zimbabwe, Ruanda, Tanzania, Brazil
Maize	Zinc	2018	Bolivia, Colombia, Guatemala, Honduras, Nicaragua
Pearl millet	Iron	2017	India, Niger
Rice	Zinc	2016, 2017	India, Bangladesh, Indonesia, El Salvador
Sweet potato	Vitamin A	2016	Angola, Burkina Faso, Burundi, Cote D'Ivoire, Ethiopia, Ghana, Kenya, Madagascar, Malawi, Mozambique, Nigeria, Rwanda, South Africa, Tanzania, Uganda, Zambia, Bangladesh, China, East Timor, India, Indonesia, Brazil, Colombia, Guatemala, Nicaragua, Panama, Peru
Wheat	Zinc	2016, 2017	Pakistan, India, Bangladesh, Bolivia, Mexico

While food fortification is successfully introduced into given infrastructures where possible, biofortified crops create a hopeful future to millions of people currently facing micronutrient deficiencies. Potential damage caused by losses of micronutrient fertilizers used in agronomic biofortification needs to be further researched to maintain not only healthy human individuals but also a sustainable environment.

5. Biofortification of Cereals

Increases of a few milligrams of essential minerals' concentrations in staple cereals can affect millions of people around the world by making their life healthy and more productive. Significant health, social, and economic benefits can be achieved by biofortification of cereal crops which can provide an increased supply of minerals to plants and ultimately to consumers. An overview of major cereal and non-cereal crops biofortified with Fe, Zn, protein, amino acids, and/or provitamin A is given in Table 4.

5.1. Wheat

Wheat grain makes a major contribution to the human diet as it provides many nutrients and minerals. Therefore, wheat production is required to double by 2050 for global food security [41]. Wheat germplasm has been extensively screened for its mineral contents of Fe, Zn, Se, Mn, Mg [42–46], proteins [47,48], and vitamins [49]. The screening also included phytic acid which is important due to its role in limiting the bioavailability of nutrients [50–52]. Breeding as well as agronomic and genetic solutions have been dissected for the objective of wheat biofortification in recent decades. The dedication of the International Maize and Wheat Improvement Center (CIMMYT) gene bank and the Harvest Plus project set the basis by breeding competitive bread wheat cultivars with 40% higher Zn concentration in South Asia [53–55]. Following this process, five biofortified wheat cultivars have been released, cv. Zincol 2016 in Pakistan, cv. Bari Gom 33 in Bangladesh and cv. Zinc Shakti (Chitra), WB02 and HPBW-01 in India. Ranges of Fe concentrations of 20–60 mg kg^{-1} and Zn concentrations from 15 to 35 mg kg^{-1} in a set of high yielding genotypes were reported [44]. This confirmed that sufficient genetic variation exists within the wheat gene pool that can be explored for substantial increases in grain micronutrient concentrations. In addition, up to 3-fold enhancements of Fe and Zn concentrations in wheat grains through soil and foliar application methods have been reported [42,56]. Creating awareness for balanced fertilization among farmers in the developing world will further contribute to meet micronutrient concentration targets to combat hidden hunger. Enhancing the concentration of Zn and Fe in the most edible part, the endosperm, is not simple to achieve through agronomic practices, nevertheless, an increase in concentration of Fe and Zn through soil application has been reported [57–59].

5.2. Rice

Rice is particularly highlighted for micronutrient improvement due to its global role as one of the main staple food crops, giving rice biofortification a huge potential for alleviation of malnutrition globally. In 2013, the first high Zn rice cultivar (with 20–22 mg kg^{-1} Zn in brown rice) was spread through Harvest Plus and the Bangladesh Rice Research Institute. Increases of 17.4, 0.123, and 14.2 mg kg^{-1} for Zn, Se, and Fe, respectively, have been reported in rice by [60]. Provitamin A biofortified "Golden Rice" has proved itself as a cost-effective intervention in the areas where rice is the staple crop [61]. Recently, [62] screened 484 rice lines and found co-localized QTL regions for Fe and Zn along with high yield attributes. The composition of rice grains, including localization of Fe and Zn, their chelators, transporters, promoters, and inhibitors, needs to be considered in order to improve the bioavailability of micronutrients in rice and consequently the nutrition and health of consumers [62]. Zinc management in soil has also significantly improved the grain Zn content in aromatic rice [63,64].

5.3. Maize

Maize is often considered a cash crop, but it is also a staple in many countries and provides food for humans and animals globally. Exogenous application of Zn in the form of seed priming, foliar spray, or incorporated in the soil enhance the germination of maize seed, seedling vigor, and tolerance against different stresses [65,66]. Maqbool and Bashir [67] reported high accumulation of Zn, i.e., 36 mg kg^{-1}, in maize grains with application of ZnO nano-particulates. Significant genome-wide association between micronutrient concentration in maize kernel and yield has been reported previously, suggesting that biofortification of maize is achievable using specialized phenotyping tools and conventional plant breeding techniques [68–70]. Among 1000 CIMMYT maize lines, concentration ranges of Zn, Fe, and provitamin A have been reported, and maize lines with 15–35 mg kg^{-1} Zn, an average of 20 mg kg^{-1} Fe, and about 0–15 mg kg^{-1} total provitamin A concentration have been identified [71].

5.4. Pearl Millet

Pearl millet (*Pennisetum glaucum* L., R. Br.) is a major warm-season cereal grown in the arid and semi-arid tropical regions of Asia and Africa and is used as food and fodder. It is a staple food for millions of people in Africa and Asia and contains higher levels of micronutrients such as Zn and Fe than wheat and rice. Variation in Fe concentration (35–116 mg kg^{-1}), Zn (21–80 mg kg^{-1}), and protein (6–18%) were reported in 281 advanced breeding lines bred at ICRISAT by [72]. Pearl millet has also been shown to exhibit great genetic variation (30–140 mg kg^{-1} Fe and 20–90 mg kg^{-1} Zn) which can be used to breed new cultivars that have high contents of Zn and Fe and are high yielding. Pujar et al. [72] found highly significant and positive correlations between general performance and Fe/Zn density in pearl millet populations. Incorporation of parental lines with a high degree of average heterosis could prove to be beneficial in breeding programs with a focus to enhance Fe/Zn in pearl millet [73]. The high iron and zinc pearl millet varieties AIMP92901 and ICMR312 have been developed by [71]. In India, open pollinated pearl millet varieties (Dhanashakti) and hybrids (ICMH 1202, ICMH 1203, and ICMH 1301) with high concentrations of iron (70–75 mg kg^{-1}) and zinc (35–40 mg kg^{-1}) have been introduced [74,75].

Table 4. List of major cereals and non-cereal crops biofortified with Fe, Zn, protein, amino acids, and/or pro vitamin A along with concentration achieved in edible parts.

Crop	Mineral/Nutrients	Increase in Nutrients	Reference
Wheat	Iron	>54 mg kg^{-1}	[57]
	Zinc	>38 mg kg^{-1}	
	Protein	>18 g kg^{-1}	
Rice	Zinc	>20.0 mg kg^{-1}	[76]
	Protein	>10.0%	
Maize	Lysine	>2.5%	[77–79]
	Tryptophan	>0.6%	
	Provitamin A	>8.0 mg kg^{-1}	
Pearl millet	Zinc	>40.0 mg kg^{-1}	[75]
	Iron	>70.0 mg kg^{-1}	
Cassava	Provitamin A	0–19 mg kg^{-1}	[80]
	Iodine	83.6 mg kg^{-1}	[81]
Potato	Iron	37 mg kg^{-1}	[82]
	Zinc	20 mg kg^{-1}	
Common beans	Iron	58–81 mg kg^{-1}	[83]
	Zinc	17–57 mg kg^{-1}	[84]
	Selenium	0.4–0.5 mg kg^{-1}	[85]
	Folates	232 mg g^{-1}	[86]
Sweet potato	Anthocyanin	>0.531 mg g^{-1}	[87–89]
	β-carotene	>130 mg kg^{-1}	
	Provitamin A	>32 mg kg^{-1}	

6. Biofortification of Non-Cereals

There are a number of non-cereal crops that are contributing to food security worldwide, especially in many African countries. Both agronomic and genetic biofortification are applicable for non-cereals as well. Few of them have been mentioned yet, nevertheless, biofortification of other crops such as pulses also has significant potential such as biofortification of chickpea [90].

6.1. Cassava

In many African countries, cassava (Manihot esculenta) is a staple, but it contains only low concentrations of Zn, Fe, I, and vitamin A. Therefore, there is a need to biofortify this crop for Fe, Zn, I, and vitamin A in poor resource countries to reduce micronutrient deficiencies. Outside of Africa, cassava is also used as a staple crop in Latin America and Caribbean countries. It is being considered an important crop to biofortify with beta carotene to enhance the vitamin A level of its consumers [81]. Cassava is tolerant to various stresses and poor soils, therefore, an important crop for tropical and sub-tropical climatic conditions.

Ortiz-Monasterio et al. [89] investigated and introduced transgenic cassava that can accumulate a high concentration of beta carotene in roots based on nptII, crtB, and DXS genes. It has been described that transgenic cassava is high in concentration of carotenoid through overexpressing a *PSY* transgene [91]. However, the natural variability of carotene contents in cassava is also high and linked to the color of roots. It is reported that higher carotene content is observed in orange varieties (12.6 $\mu g\ g^{-1}$) while low carotene content is found in white varieties (1.3 $\mu g\ g^{-1}$).

In African countries, cassava has been utilized for mitigation of beta carotene deficiency by the partnership of Harvest plus with the International Institute of Tropical Agriculture (IITA), and they are utilizing beta-carotene biofortified cassava for mitigation of vitamin A deficiency in rural populations. Until 2014, this partnership has produced six vitamin A fortified varieties of cassava in Nigeria, i.e., TMS 01/1368-UMUCASS 36 and TMS 01/1412-UMUCASS 37 in2011, TMS 01/1371-UMUCASS 38, NR 07/0220-UMUCASS 44, TMS 07/0593-UMUCASS 45, and TMS 07/539-UMUCASS 46. Meanwhile, biofortified cassava has been introduced in many more countries (Table 2). Cassava also has a wide variety of genotype modifications for minerals (Fe and Zn) and proteins that resulted in the development of enhanced nutritional standards for cassava [92] and this diversity is a potential asset for the development of Fe, Zn, and protein enriched cassava varieties.

6.2. Potato

Potato (*Solanum tuberosum*) is an important vegetable and a good source of energy and calories for people of all ages. As it is being used globally, it has great potential to improve human nutrition through various biofortification strategies. Using transgenic techniques, the beta-carotene contents of potato have been enhanced by adding the PSY gene [93].

Field experiments were conducted to enhance Zn content in potato tubers by foliar application of Zn fertilizers which increased tuber Zn content significantly. It was also observed that zinc sulfate and zinc oxide were more productive than zinc nitrate for foliar applications targeted at enhanced Zn concentrations and improved yield [94].

Similarly, it was shown that the selenium (Se) concentration in potato tubers was enhanced by foliar application of selenite and selenate [95]. The Se content of potato tuber also improved by foliar application of Se with humic acid [96]. In addition, potato is also a good source of antioxidants for human health. Additionally, the contribution of potatoes as a source of antioxidants and nutritive properties was related to the natural variation of red and purple pigments in cultivated potato germplasm. Thus, breeders are paying serious attention to the breeding of these variants [97]. In summary, potatoes have considerable genetic diversity for micronutrient concentration that can be utilized for conventional breeding of varieties with enhanced Fe and Zn concentrations for human nutrition [98].

6.3. Sweet Potato

Orange-fleshed sweet potato (*Ipomea batatas* L., *Lam*) varieties have a higher beta carotene content than white fleshed varieties. The main objective of the sweet potato biofortification initiative is replacing white fleshed varieties with orange fleshed plants. The target level of beta carotene set by the Harvest Plus project for sweet potato is 32 mg kg^{-1} but cultivars with higher concentrations of up to 100 mg kg^{-1} have been reported by HarvestPlus [89] and Nestel et al. [99]. In addition to the value of sweet potato as an essential source of natural antioxidants and bioenergy, it is also enriched with several phytochemicals, vitamin C, carbohydrates, anthocyanin, and dietary fiber [87]

The nutritive value of sweet potato can be improved by enhancing the contents of lutein, carotene, and total carotenoids, through overexpression of "orange" *IbOr-Ins* genes in white fleshed sweet potato [100]. The orange fleshed sweet potato beta carotene content can also be enhanced by irrigation and chemical fertilizer applications [88].

To overcome malnutrition issues, developing countries grow about 95% of the global sweet potato crops, with a major portion being grown in China. Therefore, sweet potato was selected for the amelioration of vitamin A deficiency. Harvest Plus and the International Potato Centre have improved various cultivars of orange sweet potato through high vitamin A content. Six varieties of the sweet potato were released in Uganda, i.e., Ejumula, Kakamega, Vita, Kabode, Naspot 12O, and Naspot 13O, and three in Zambia, i.e., Twatasha, Kokota, and Chiwoko. The orange sweet potato developed by Harvest Plus has already shown a remarkable impact on nutrition and food security in Africa, which was acknowledged with the World Food Prize, 2016.

6.4. Common Beans

The common bean (Phaseolus vulgaris) is an essential grain legume, consumed by humans in all parts of the world. It is an annual herbaceous plant and its dry grains are edible. The beans are a rich source of amino acids, i.e., threonine, valine, leucine, isoleucine, and lysine, but its nutritive value is insufficient due to low concentrations of the essential amino acids methionine and cysteine. However, the methionine concentration in beans can be enhanced through expression of methionine-rich storage albumin protein from seeds of the Brazil nut [101].

Common beans also have a potential for Zn biofortification through foliar application of Zn fertilizer [102,103]. It has been reported that in common beans N, P, K, Mn, Cu, and Zn concentrations can be enhanced by the administration of organic and chemical fertilizers [104]. Furthermore, it has been shown that in common beans the Fe concentration can be enhanced by 60–80% and Zn concentration by around 50%, using different strategies. High genetic diversity in common beans has been discovered for Fe and Zn concentration [105,106] and genes have been reported in navy bean that are related to Zn accumulation [107]. Generally, staple crops are poor sources of dietary folates but legumes and particularly beans were shown to be a good source of dietary folates [86].

Thus, biofortification of common beans with minerals and amino acids can play a significant role to uplift the nutritional status of resource poor people of developing countries and promising work is in progress.

7. Effectiveness of Biofortification and the Way Forward

Considering current developments and future predictions, mineral and vitamin deficiencies are expected to be more threatening across the globe, but especially in developing countries, due to fast growing populations, resource limitations, decline in natural resources, lack of awareness, and social behaviors. Balanced diets and/or provision of limiting nutrients including minerals, vitamins, and protein is crucial to keep upcoming generations healthy. Due to lack of education and economic deteriorations in many developing countries, it will be very difficult to accomplish the target of zero hunger declared in the United Nations Sustainable Development Goals (SDGs). However, the significant progress made by biofortification during the last two decades provides hope for developing countries to combat their nutritional issues. Biofortification is a low-cost crop-based approach holding great promise to achieve the targets of healthy nutrition in the developing world. As described, significant impact has been achieved and future strategic research and appropriate policy could lead to great success in biofortification in upcoming years.

Crop biofortification offers the economic, easily accessible, and scalable solution for improved crop varieties with nutritionally dense grains. A great resource of exploitable genetic variation has been documented across gene pools in a wide range of crops. The accelerated selection or incorporation of gene(s) that enhance the nutrient content in staple crops will largely determine the success of various nutritional breeding schemes. Collaborative research efforts involving breeders, biotechnologists, physiologists, biochemists and, importantly, nutritionists are needed to strengthen biofortification programs to meet the challenge of attaining global nutritional security.

The biofortified cropping system is highly sustainable as nutritionally improved varieties will continue to be grown and consumed year after year, even if government attention and international funding for micronutrient issues fade. Combined with the use of mineral fertilizers, such systems can enhance the mineral content in cereals, vegetables, and fruits durably in a very cost-effective way without the need for costly centralized programs. The staple crops enriched through fertilizer applications in soils or on foliage can be as effective as varieties developed by breeding or genetic engineering although the combination of both technologies is probably the best approach. Moreover, biofortification provides a truly feasible means of reaching malnourished populations in relatively remote rural areas, delivering naturally fortified food to people with limited access to commercially marketed fortified foods, which are more readily available in urban areas. Thus, there is a strong case to spend more resources to promote and augment strategic research on biofortification sustaining the available resources and exploiting the available potential wisely and sustainably. It complements work for higher yielding cropping systems, the conservation of soil fertility and improvement of soil health by sustaining and improving soil organic matter, effective application of fertilizers and development of nutrient enriched staple crops using conventional breeding and, where necessary, transgenic approaches.

However, the impact of biofortification will also depend on the development of sustainable markets for biofortified seeds and products. Post-harvest handling, storage losses of biofortified nutrients, and market segregation of biofortified crops are research topics that still need more attention. Seed producers must have access to biofortified varieties and be made aware of the market opportunity. Consumers need to receive information on the nutritional benefits of biofortified crops as well as their characteristics to actively choose biofortified crops over comparable non-biofortified varieties. Further on, biofortified products need to be advocated to food processing companies to mainstream biofortified crops beyond small rural markets. Behavior change communication and effective promotional efforts are therefore essential to achieve acceptance by farmers, consumers, policy makers, and other stakeholders. We conclude that for many "new" biofortified varieties, there is still need for context-specific, innovative solutions to achieve widespread adoption.

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