Review Article

Biofortification: Enhancing Nutritional Content in Crops through Biotechnology and fighting climate change

Abstract

Comment [A1]: Your abstract should cover summary of introduction, objectives, method, results, conclusion and recommendation

Biofortification, a cutting-edge agricultural strategy, involves leveraging biotechnological advancements to bolster the nutritional value of crops, thereby addressing global malnutrition challenges. This innovative approach not only tackles nutritional deficiencies but also emerges as a potent tool in the fight against climate change. By employing techniques such as genetic engineering and selective breeding, biofortification enhances the concentration of crucial vitamins and minerals in staple crops. This not only improves the nutritional quality of food, especially in vulnerable populations, but also contributes to climate change mitigation. Biofortified crops exhibit increased resilience to environmental stressors, making them vital components of sustainable and climate-smart agriculture. Additionally, the reduced dependence on excessive fertilizers and other inputs minimizes the environmental footprint associated with traditional farming practices. In essence, biofortification emerges as a dual-purpose solution, promoting both human health and environmental sustainability in the face of global challenges.

Keywords: biofortification, nutritional, vitamins, environmental, minerals, malnutrition, staple

Introduction

Micronutrient malnutrition is a growing problem since the world's food system isn't able to provide enough nutritious food, particularly to those who are poor and have little resources. In poor nations like India, where people rely heavily on cereal-based diets and have limited access to meat, fruits, and vegetables, mineral (Fe, Zn) and vitamin A deficiency is a major food-related primary health concern**[1]**. Subclinical vitamin A deficiency (VAD) affects over 57% of pre-schoolers and their mothers, increasing their risk of morbidity and mortality, and is a major public health concern since it causes over 330,000 child deaths annually. The afflicted groups are not being adequately covered by sponsored nutrition programs, which are presently addressing therapeutic supplementation of vitamin A**[2]**.

Comment [A2]: citation

Fig 1. Fortified and biofortified growth

A sustainable and cost-effective way to reduce VAD is to bio fortify essential agricultural plants using biotechnological technologies. To increase the quantities of β-carotene in agricultural plants, genetic engineering is the clear choice. The creation of "golden rice" demonstrated that by manipulating the genetic code of several genes that encode essential enzymes in the carotenoids biosynthesis pathway, the whole system can be redirected. There have been several reports on the creation of transgenic crops that have higher quantities of provitaminA**[3]**. These crops include corn, tomatoes, cassava, potatoes, and wheat. Genetic transformation using Agrobacterium has been used to create transgenic groundnut and pigeon pea plants that contain either one maize phytoene synthase 1 (psy1) gene or two genes, one for psy1 and one for tomato β-lycopene cyclase (β-lyc). The quantities of total carotenoids and β-carotene were found to be significantly higher in the transgenic events, according to preliminary studies. Because of its important role in bioavailability and metabolic efficiency, vitamin A enrichment of these crops may considerably change nutrition and nutrient interactions in the impacted populations**[4]**.

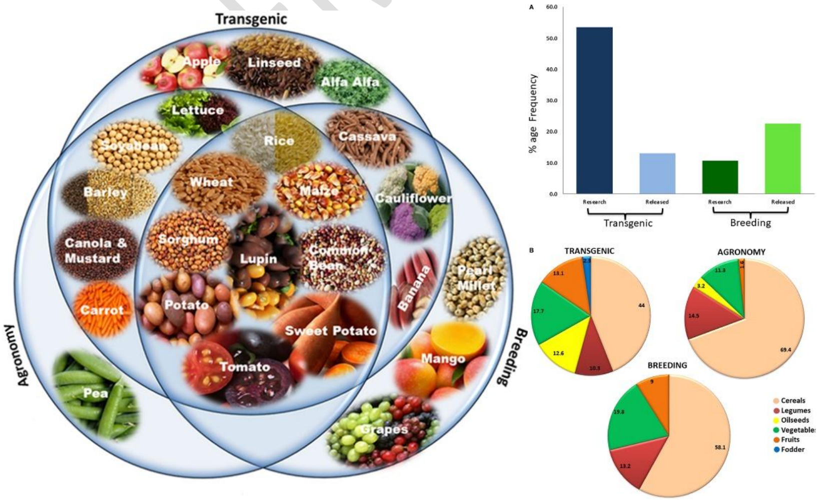

Fig 2. Bio fortify essential agricultural plants using biotechnological technologies

There is an immediate need to address the worldwide problems of food insecurity, hunger, and malnutrition. There is a lack of effective response to these issues in the present international framework for managing agriculture, nutrition, and food. Agricultural output, food quality, and nutritional value may all be improved with the help of new technologies like nanotechnology, biofortification, and green biotechnology. It is essential that plant kinds can endure and even thrive in harsh and unpredictable environments**[5]**.

To improve nutritional quality, quantity, and production economics, green biotech applies biological approaches to plants. Reduced fuel use and soil erosion are two benefits that farmers experience when they grow herbicide-tolerant (HT) genetically modified (GM) crops instead of ploughing their fields. Thus, less tillage or no-tillage systems are used, leading to decreased emissions of carbon dioxide**[6]**. Thanks to agricultural biotech crops, farmers may now embrace conservation or "no-till" farming methods that eliminate the need for ploughing and tillage. The result is better soil quality and more carbon sequestration, which in turn reduces atmospheric carbon dioxide levels**[7]**.

Comment [A3]: With the help of

Another crucial component of food security is reducing the usage of fertilizer. The annual chemical fixation of nitrogen is around 120 teragrams, with about 66% of that amount ending up in the environment. Genetically modified (GM) rice and canola are examples of nitrogen usage efficiency technologies that allow farmers to grow crops with traditional yields while using much less nitrogen fertilizer**[8]**. Finally, Green Biotechnology, Biofortification, and Nanotechnology are some of the emerging technologies that show promise in the fight against hunger, malnutrition, and food insecurity.

There is hope that we may lessen our influence on the environment by engineering cereal crops to fix nitrogen**[9]**. Nevertheless, it would be quite difficult to substitute nitrogen fertilizer without achieving nitrogen fixation levels comparable to legumes. The incorporation of the nitrogenase enzyme into plant cell organelles or the establishment of a nitrogen-fixing symbiosis in cereal roots are two of the many biotechnological strategies now under investigation. Problems with these methods include nitrogenase's complexity and its high energy requirements**[10]**.

Comment [A4]: citation

By assisting farmers in dealing with water constraint and using water more sustainably, agricultural biotechnology has the potential to greatly increase crop productivity. Lessening agricultural water loss and increasing drought tolerance are the two primary means to this end. There are hybrid crops that can withstand drought and occasional water shortages, and in the United States, drought-tolerant maize is now in the regulatory phase of research**[11]**. Biotechnological techniques may also be useful in increasing photosynthetic activity. While wheat and rice need more water and nitrogen to thrive, sugarcane and corn grow like weeds and produce more. Instead of using C3 photosynthesis, which enables plants to absorb CO2 during the day, they use C4, which allows them to collect sunlight during the day. Scientists from around the world are transferring the genes responsible for C4 photosynthesis from corn and sugarcane to rice. This should lead to wheat and rice with increased drought resistance, reduced water and fertilizer needs per calorie, and a yield that is 1.5 times higher per acre**[12]**.

Comment [A5]: citation

Comment [A6]: citation

It takes a lot of time and effort to produce sugarbeet cultivars that are resistant to cold, so that they don't bolt or blossom when exposed to cold. All things considered, biotechnology methods and better crop adaptation to water use efficiency have the potential to drastically cut down on the need of inorganic fertilizer in farming**[13]**.

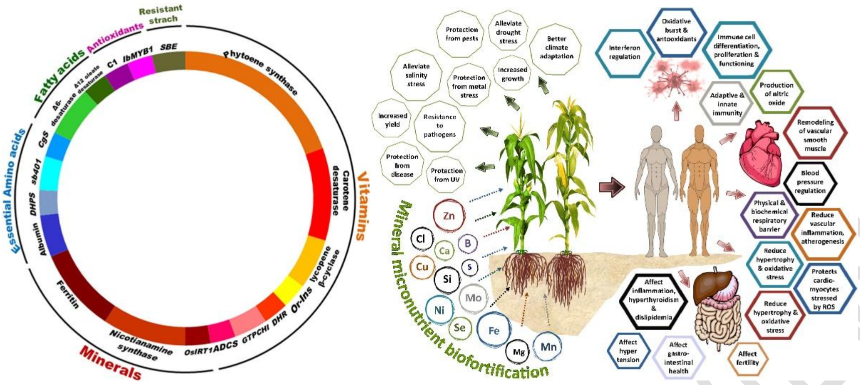

Fig 3. Importance of biofortification

Importance of biofortification

Humans require around 40 known nutrients to live healthy and productive lives. Essential nutrients, such as sodium, potassium, calcium, magnesium, phosphorous, chlorine, and sulfur, are required in small amounts in the body, while micronutrients, such as iron, zinc, copper, manganese, iodine, selenium, molybdenum, cobalt, nickel, and vitamin A, play crucial roles in physical and mental development**[14]**.

Agricultural products, particularly those in developing countries, are the primary source of nutrients for humans. However, the diets of these populations often contain insufficient amounts of essential nutrients, leading to poor health, sickness, increased morbidity and disability, impaired development, stunted growth, diminished livelihoods, and reduced socioeconomic development.

Comment [A7]: Citation

Childhood stunting is associated with micronutrient malnutrition in many developing countries, with 38% of pregnant women and 43% of pre-school children affected. Over 30% of the world's population is anemic, with Africa and South-East Asian countries being most affected. Iron deficiency is estimated to be approximately half of this**[15]**.

Early distribution of nutrients among different plant parts is another issue, with iron content high in rice leaves but low in polished rice grain. Overnutrition, particularly diabetes, is a growing concern, leading to problems like overweight and diabetes. Biofortification aims to enhance the contents of desired micronutrients in crop plants, providing essential nutrients in the edible portion of crops. This approach can provide enough calories to meet energy needs and improve the amount of nutrients consumed by the poor population**[16]**.

Methods to achieve biofortifiction

Biofortification is a sustainable and cost-effective approach to improving micronutrient concentrations in crops. Conventional breeding is a long-term process that requires substantial effort and resources, but it has unique capabilities and constraints. Genetic modification allows for more traits to be improved in shorter periods of time and attains higher levels of nutrient enhancement relative to conventional breeding**[17]**.

Conventional breeding has been employed in various parts of the world to enhance the levels of provitaminA carotenoids in cassava, sweet potato, iron in cassava, banana and beans, and zinc in rice, pearl millet, and wheat. Many of the biofortified products are now available in the market and are well accepted in rural populations. However, there are limitations to the use of this technique, including the very limited number of traits that can be improved at the same time, available genetic variation for the trait, and the long period required**[18]**.

Transgenic approaches are advantageous when the nutrient does not naturally exist in a crop or when sufficient amounts of bioavailable micronutrients cannot be effectively bred into the crop. Once a transgenic line is obtained, several years of conventional breeding are needed to ensure that the transgenes are stably inherited and to incorporate the transgenic line into varieties that farmers prefer. Genetic modification allows more traits to be improved in much shorter periods of time and attains much higher levels of nutrient enhancement relative to conventional breeding**[19]**.

Examples of biofortification projects include increasing protein content, combating vitamin A deficiency, and developing other crops engineered for higher β-carotene content. Orange sweet potato (OSP), rich in vitamin A, is the first biofortified crop to be released, and golden rice, a rice variety rich in provitamin A, has been developed and distributed in parts of Africa where prevalence of vitamin A deficiency is high**[20]**.

In addition to rice, other crops engineered for higher β-carotene content include potato, canola, tomato, carrot, and cauliflower. The first generation of provitamin A-rich orange open-pollinated maize varieties developed using conventional breeding was released by the Institute for Agricultural Research in Nigeria in June 2012. A human bioavailability study using transgenic provitaminA banana began in late 2013, and trials have commenced in Uganda. Provitamin A bananas are expected to be released in 2019**[21]**.

In conclusion, biofortification is a sustainable and cost-effective approach to improving micronutrient concentrations in crops. While conventional breeding has made significant strides, genetic modification offers a more efficient and sustainable method for improving crop health and nutrition**[22]**.

Comment [A8]: remove

Iron deficiency anemia affects over 2 billion people worldwide, making it the most common micronutrient deficiency. Iron is found in vegetables, grains, and red meat, but its bioavailability in plants is low. Biofortification of common beans is advantageous because the baseline grain iron content is high at 55 ppm (mg/kg) and the trait has great variability, allowing initial breeding attempts to be more successful than in cereals. The target areas for biofortified beans are in iron deficiency anemia prone areas of Latin America and eastern and southern Africa where the crop is important and consumption is high**[23]**.

Comment [A9]: remove and put comma (,)

The bioavailability of iron in rice is very low, aggravated by the presence of phytate, a potent inhibitor of iron resorption, and the lack of iron resorption-enhancing factors. Scientists have had to increase the iron content in grains, reduce the level of phytate, and add resorption-enhancing factors. Expression of the iron storage protein ferritin from French bean and soybean in the endosperm of rice results in a 3-fold increase of iron in seeds. To decrease the level of phytate, an enzyme that degrades it (known as phytase) has also been transformed into rice**[24]**. Over-expression of a cysteine-rich protein that transports metals in rice can improve the rate of iron resorption during digestion. A transgenic high-iron rice variety has been developed by the University of Melbourne and IRRI that contains 14 ppm iron in the white rice grain and translocates iron to accumulate in the endosperm, where it is unlikely to be bound by phytic acid and therefore likely to be bioavailable**[25]**.

Comment [A10]: write in full

Efforts to increase iron concentrations in wheat by conventional breeding have not been successful, and there are currently no iron-biofortified wheat varieties available for farmers. The University of Melbourne, Australia, has been employing an approach that has proven highly effective in rice, using NAS to increase iron concentrations in wheat and produce biofortified wheat varieties with 52 ppm iron in whole grain. The John Innes Centre, Norwich UK, has been investigating several independent strategies to increase iron concentration and bioavailability in wheat grains through transgenic means**[26]**.

Comment [A11]:

Comment [A12]: write in full

A number of zinc rice advanced breeding lines for both Boro (irrigated) and T. Aman (rainfed) seasons are under development through a breeding program at the Bangladesh Rice Research Institute. The first zinc rice aman variety, 'BRRIdhan 62,' contains 19 ppm of zinc and 9% protein and yields 4.2 tons per hectare. At least one zinc rice boro variety with 22-24 ppm is expected to be released in 2014. In India, the first varieties are expected to be commercialized in 2015**[27]**.

Comment [A13]: write in full

Challenges of climate change on environment and crop production

Comment [A14]: remove underline and make it capital letters

Climate change is a growing concern due to the emission of greenhouse gases such as methane, carbon dioxide, and chlorofluorocarbons. Agriculture researchers are increasingly focusing on the impact of environmental changes on crop production, with increased attention from national and international bodies, non-government organizations, corporations, and individual levels. In 2000, 1.8 billion people lived in areas with severe water scarcity, and by 2025, the human population in these areas would have increased to over 2 billion**[28]**.

Climate change has directly affected agriculture due to the direct interaction of crops with climate. Climatic conditions like air and soil temperature have a greater influence on crop growth and development. Climate-related disasters, such as floods and droughts, have adversely affected crop production and food security. The Gangotri glacier, one of the Himalayas' largest glaciers, is deteriorating annually, leading to flooding. Climate change is not only the result of increasing mean annual air temperature but also the increase in the concentration of greenhouse gases in the atmosphere. Over the past 100 years, the global increase in mean annual temperature is 0.74°C. The frequency of climate events such as floods and droughts has also been enhanced by climate change. The Gangotri glacier is deteriorating around 12-13 meters annually, resulting in flooding**[29]**.

The increase in sea-level rise (SLR) affects food security and food production, as it can lead to saltwater intrusion into crop fields and freshwater, leading to land degradation and unfit soil for cropping. In Bangladesh, an SLR of 1.5 meters may flood about 16% of the country suitable for rice cultivation, affecting rice production. Standard crops are sensitive to fluctuations in temperature and precipitation, and an increase in global mean temperatures by 2°C can disrupt agricultural practices and crop production periods. Increased temperature can also affect crop production period, rainfall patterns, hydrological cycle, cultivar selection, quality, and quantity of food crops**[30]**.

Climate change is also affecting groundwater levels and air temperature, leading to decreased crop production. High CO2 levels in the presence of other climate change impacts can decrease plant growth and nutrient status. Climate change has a significant impact on crop yields, with some species experiencing the highest pressure while others are less affected. For example, C4 plants have been believed to be less affected negatively or positively by increasing atmospheric CO2 levels. However, all prediction models depict a high level of yield loss in all stable food crops, leading to food insecurity, hunger, and poverty**[31]**. Climate change could significantly affect wheat yield, which accounts for 21% of global food, cultivated on 200 million hectares worldwide. While global warming may be helpful in some regions where optimal temperature exists, it will be harmful for regions where optimal temperature always exists. High temperatures will decrease seed yield, making it vital to develop heat-tolerant wheat germplasm to handle the yield loss by climate change. Climate change has also led to desertification, which results in the loss of arable land due to water shortages, making it unsuitable for crop cultivation. Climate change influences water considerably, changing physical and chemical circumstances within water bodies and altering the bio-environment, such as the decrease in marine life and reallocation of distribution and abundance of marine species. The lack of water for cultivation will lead to the death of crops**[32]**.

Comment [A15]: CO_2, do same for the others

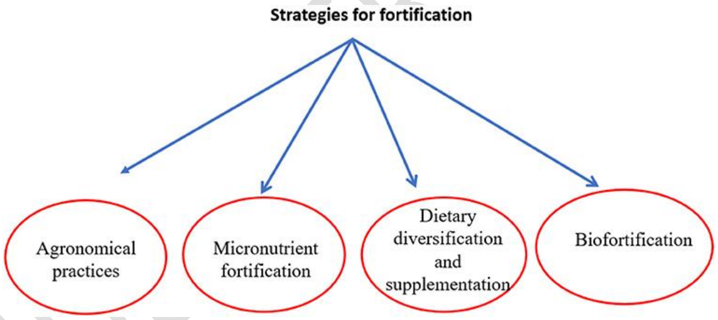

Fig 4. Fortification strategies

In 2003, maize yield decreased significantly in some European countries due to climate change. Additionally, increasing global temperatures induced by climate change could lead to an increase in weed growth and development, resulting in the use of herbicides. Plants are more susceptible to the intensive attack of insect-pest and plant diseases due to increasing temperatures. This is because increasing temperatures usually generate more ideal conditions for disease-causing bacteria and pests, which can adversely influence crop development, quality, and quantity. As a result, farmers will be forced to use more synthetic insecticides and pesticides in the field**[33]**.

The threats related to climate change threaten world agriculture production and create food insecurity for upcoming generations, especially in developing countries where the poor will suffer greatly as they cannot afford upmarket prices for food. Climate change is predicted to lead to increased global temperatures and water scarcity, affecting nutrient uptake and crop yield. Iron is an essential micronutrient involved in various metabolic processes, including photosynthesis and respiration. Defects in iron mobilization can result in severe yield losses in crops, making iron homeostasis crucial for proper growth and development**[34]**. High soil pH, which is high in iron, hinders iron uptake in plants. Despite high pH, high temperature also reduces iron uptake by plants.

High temperature stress can also affect the uptake of nitrogen and phosphorus in plants. An increase in temperature enhances phosphorus uptake and translocation in wheat under heat stress, while soybean plants show more root and shoot iron content under water stress. Low nutrient uptake due to high temperature stress may be due to factors such as reduction in rhizosphere or nutrient acquisition per unit root**[35]**. This decrease in nutrient acquisition per unit root may be due to the reduction of labile carbon (total non-structural carbohydrates) or direct root damage due to high temperature stress. Drought is another abiotic stress that affects plant nutritional quality by decreasing the efficiency of nutrient uptake. Most nutrients uptake from the soil to the roots depends on the amount of water present in the soil. Drought reduces the diffusion rate of nutrients in the soil towards the roots, root nutrient uptake, and then translocation into the shoot due to decreased transpiration flux, active transport, and membrane permeability. Drought has variable effects on Zn, Fe, Mn, or Cu uptake and translocation among various plant species. Drought increases leaf Mn and Cu content while decreasing Fe content in the leaves**[36]**.

A proper understanding of nutrient uptake and translocation under various abiotic stresses can help develop strategies to reduce the harmful effects caused by abiotic stresses and disturbed nutrient homeostasis. Boron deficiency and leaf injury due to low temperature stress in plants have been shown to be related, but the reasons behind this relationship at molecular, biochemical, and physiological stages remain unclear. Partial evidence suggests that low temperature in the rhizosphere restricts B uptake capacity and B supply/use efficiency in the shoots. Boron uptake enhances sugar transportation in plants, improving seed germination and grain formation. Thus, it can recover the yield by decreasing the effect of low temperature**[37]**.

Soil water can affect iron homeostasis in plants. In wet soils, the Fe2+/Fe3+ ratio is higher, leading to more iron availability or uptake by the plants. However, more presence of oxygen under drought conditions causes a reduction in the Fe2+/Fe3+ ratio, leading to lower iron availability to the plants. Additionally, drought reduces nitrogen and phosphorus uptake in plants**[38]**.

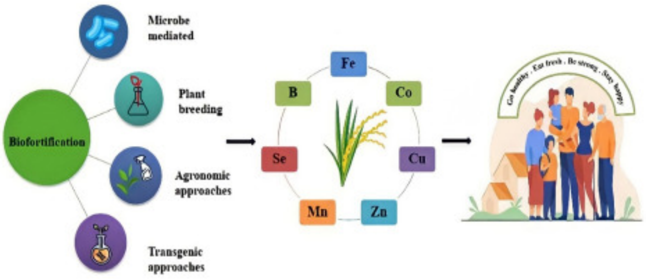

Fig 5.Bifortification technique

Biofortified crops recent developments and examples

- Transgenic approaches are a viable alternative for developing biofortified crops when there is limited or no genetic variation in nutrient content among plant varieties. They rely on the unlimited genetic pool for the transfer and expression of desirable genes from one plant species to another, independent of their evolutionary and taxonomic status**[39]**. Transgenic crops can be used for the simultaneous incorporation of genes involved in the enhancement of micronutrient concentration, their bioavailability, and reduction in the concentration of antinutrients which limit the bioavailability of nutrients in plants. Genetic modifications can be targeted to redistribute micronutrients between tissues, enhance the micronutrient concentration in the edible portions of commercial crops, increase the efficiency of biochemical pathways in edible tissues, or even the reconstruction of selected pathways**[40]**.
- Transgenic crops with enhanced micronutrient contents hold a potential to reduce micronutrient malnutrition among consumers, especially poor people in developing countries. Numerous crops have been genetically modified to enhance their micronutrient contents, including vitamins, minerals, essential amino acids, and essential fatty acids. Successful examples of transgenic methods include high lysine maize, high unsaturated fatty acid soybean, high provitamin A and iron rich cassava, and high provitamin A Golden rice. Reports are available for biofortified cereals, legumes, vegetables, oilseeds, fruits, and fodder crops**[41]**.
- Transgenic rice has been targeted to address the global challenge of undernutrition, iron deficiency anemia, quality protein, seed oil quality, polyunsaturated fatty acid, flavonoids, overnutrition, and obesity. Rice has also been targeted for seed oil quality by increasing the amount of polyunsaturated fatty acid that can help in the reduction of bad cholesterol levels in the body and improve human nutrition**[42]**.
- To address the challenge of overnutrition and obesity, the content of less digestible and resistant amylose starch has been enhanced by expression of antisense waxy genes and antisense RNA inhibition of starch-branching enzymes (SBE). The expression of functional human milk protein (lactoferrin) in rice grains has opened the possibility for creating value-added cereal-based ingredients that can be introduced into infant formula and baby food**[43]**.

- In conclusion, transgenic approaches offer a cost-effective and sustainable approach to biofortification, particularly in the context of undernutrition and iron deficiency. By targeting specific genes and incorporating micronutrients, these crops can contribute to a more sustainable and nutritious diet for the global population**[44]**.

Comment [A16]: Remove , all your conclusion should be at the session of the article

- Transgenic wheat, maize, barley, sorghum, and soybean are all staple food crops that have been genetically modified to address various nutritional challenges. Wheat has been enhanced with the expression of bacterial PSY and carotene desaturase genes, while maize has been enriched with provitaminA (carotenoids) and multiple carotenogenic genes. Vitamin E and its analog are potent antioxidants with implications for human health, and many research groups are emphasizing on biofortification of these components in maize crop**[45]**.

Comment [A17]: write in full then in (PSY)

- Maize endosperm has been enriched with provitamin A (carotenoids) by expressing bacterial crtB and multiple carotenogenic genes. Vitamin E and its analog play roles in cardiovascular function, immune cell development, and iron utilization. Tocotrienol and tocopherol content in maize has been increased by overexpression of homogentisic acid geranylgeranyltransferase [HGGT] and vitamin C (l-ascorbic acid), which play roles in cardiovascular function, immune cell development, and iron utilization**[46]**.

Comment [A18]: MEANING?

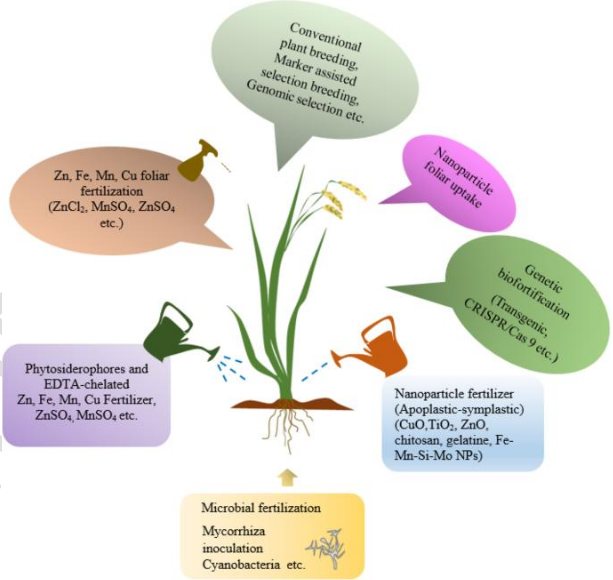

Fig 6. Biofortified crops recent developments

- Bioavailability of micronutrients is hindered by antinutrient components, such as expressing soybean ferritin and Aspergillusphytase, soybean ferritin, Aspergillusniger phyA2, and silencing the expression of ATP-binding cassette transporter and multidrug resistance-associated protein. Maize varieties rich in lysine have been targeted with significant achievement, such as Mavrea™YieldGard Maize by

Monsanto in Japan and Mexico and Mavera™ Maize (LY038) by Renessen LLC in Australia, Columbia, Canada, Japan, Mexico, New Zealand, Taiwan, USA**[47]**.

- Barley, being a model cereal crop, has been targeted to improve its micronutrient content. Zinc content has been improved by overexpression of zinc transporters, and phytase activity has been increased in barely seeds by expression of phytase gene. Essential amino acid lysine has been enhanced in barley by expressing DHPS gene **[48].**

Comment [A19]: write in full then put in bracket

- Sorghum is one of the most important staple foods for millions of poor rural people, and it has been targeted to improve provitaminA (beta-carotene) by expressing Homo188-A (108). The content of essential amino acid lysine has been improved in sorghum by the introduction of a high lysine protein [HT12]. One of the issues with sorghum consumption is that its grains are less digestible than the other major staple crops. The digestibility index of transgenic sorghum has been increased by RNAisilencing of the γ-kafirin and combined suppression involving three genes [γ-kafirin-1, γ-kafirin-2, and α-kafirin A1]**[49]**.
- Transgenic legumes and pulses have also been developed to address nutritional challenges. Soybean, a global source of vegetable oil and high-quality protein, has been targeted to increase provitaminA (beta-carotene), a monounsaturated ω-9 fatty acid (oleic acid), and seed protein contents by expressing bacterial PSY gene. Provitamin A (Canthaxanthin) was enhanced by expressing bacterial PSY [crtB, crtW, bkt1**[50]**.

Comment [A20]: write in full then in bracket

- Soybeans contain approximately 40% protein, but they are deficient in one or more of the essential amino acids, especially the sulfur-containing amino acids, cysteine and methionine. The cysteine content of soybean seeds has been increased through overexpression of the sulfur assimilatory enzyme, O-acetylserinesulfhydrylase, and the methionine content of soybean has been increased by expressing cystathionine γ-synthase**[51]**.
- Soybean, a grain with approximately 20% oil content, is rich in healthy oil but contains unstable fatty acids that contribute to reduced oil quality. To enhance the agronomic value of soybean seed oil by reducing the levels of α-linolenic acids (18:3), siRNA-mediated gene silencing-based approach has been utilized. In another experiment, γ-linolenic acid (GLA) and STA (ω-3 fatty acids) content in soybean oil has been increased by expression of Δ6-desaturase gene responsible for the conversion of linoleic acid and α-linolenic acid to GLA and STA. Similarly, STA content has been increased by simultaneous expression of Δ6 desaturase and Δ15 desaturase**[52]**.

Comment [A21]: not clear. Make it understandable for public read

- Importance of improvement in ω-3 fatty acid content in soybean is evident from the release of a large number of cultivars with improved oleic, linoleic, and STA. Transgenic soybean varieties rich in oleic acid have been released in Australia, Canada, Japan, New Zealand, USA; Treus™, Plenish™ (DP305423) in Australia, Canada, China, European Union, Japan, Mexico, New Zealand, Philippines, Singapore, South Africa, South Korea, Taiwan, USA; and Treus™ (DP 305423 ×

GTS 40-3-2) in Argentina, Canada, China, Japan, Mexico, Philippines, South Africa, South Korea, Taiwan, USA**[53]**.

- Transgenic Common Beans (Phaseolus vulgaris) are among the most important grain legumes used for human consumption. However, their nutritional value is limited due to the small amounts of the essential amino acid methionine and cysteine. Common bean methionine content has been increased by the expression of methionine-rich storage albumin from Brazil nut**[54]**.
- Transgenic Lupines (Lupinusangustifolius) are the major grain legumes. Their methionine content has been increased by the expression of sunflower seed albumin gene**[55]**.
- Transgenic Vegetables such as Potato (Solanumtuberosum) have been targeted for nutritional enhancement. In potato tuber, provitamin A (carotenoid forms) have been increased by incorporating PSY gene and by simultaneous incorporation of three genes: PSY, phytoenedesaturase, and lycopene β-cyclase. Beta-carotene content in tubers has been enhanced by using RNAi to silence the beta-carotene hydroxylase gene (bch), which converts beta-carotene to zeaxanthin and by regulation of beta-carotene synthesis through expression of lycopene β-cyclase [StLCYb]. Zeaxanthin, another form of carotenoid, has also been increased by expressing zeaxanthinepoxidase genes in transgenic potato tuber**[55]**.
- Potato has been targeted for enhancement of vitamin C (ascorbic acid) by overexpressing strawberry GalUR. Potato tubers are very poor in essential amino acid, methionine, which has been targeted for its enhancement by coexpressingcystathionine γ-synthase (CgSΔ90) and methionine-rich storage protein. Methionine content has been enhanced by overexpressing the gene encoding the seed storage protein from Perilla [PrLeg polypeptide] and cystathionine γ-synthase (CgS) genes. Transgenic potatoes expressing Amaranth albumin (ama1) result in an increase in total protein content in tubers along with the significant increase in the concentration of several essential amino acids including methionine **[56]**.
- High value carbohydrate rich potato tubers have been synthesized by expressing cyclodextringlycosyltransferases (CGT) gene, which results in the production of multipurpose dietary fibercyclodextrins from starch. Potato tubers have also been targeted to increase phenolic acid, anthocyanins contents by single-gene overexpression or by simultaneous expression of CHS, chalconeisomerase (CHI), and dihydroflavonolreductase. Transgenic potato varieties engineered for starch quality have been released by BASF viz., Starch Potato (AM 04—1020) in the USA and Amflora™ (EH 92-527-1) in the European Union**[57]**.
- Transgenic sweet potato, cassava, carrot, lettuce, cauliflower, linseed, canola, and mustard are all potential sources of bioenergy and natural antioxidants. Sweet potato is rich in phytochemicals, anthocyanins, vitamin C, carbohydrates, potassium, and dietary fiber, while cassava is deficient in essential nutrients like provitamin A, vitamin E, iron, and zinc. Cassava biofortification has been developed to reduce their deficiency among undernourished communities**[58]**.

Comment [A22]: write in full then in bracket.
Do same for all abbreviations

- Carrots are popular vegetables with high levels of beta-carotene, vitamins, and minerals but are low in calcium content. Transgenic carrots have been improved for iron content, yield, and growth rate by expressing the Arabidopsis H+/Ca2+ transporter. Lettuce has been improved for iron content, yield, and growth rate by expressing a soybean ferritin gene**[60]**.
- Cauliflower is a popular vegetable with antioxidant phytonutrients and has been enhanced by increasing beta-carotene content in mutant orange cauliflower. Linseed edible oil is in demand as a nutritional supplement, but it is highly susceptible to auto-oxidation, generating toxic derivatives. Genetically modified flax plants with increased antioxidant potential, stable, and healthy oil production have been generated by suppressing the CHS gene that results in hydrolyzable tannin accumulation. Researchers have intended to enhance the accumulation of Δ6 desaturated C18 fatty acids and C20 polyunsaturated fatty acids by seed-specific expression of cDNAs encoding fatty acyl-desaturases and elongases in linseed**[61]**.
- Canola is an important oilseed crop for millions of people worldwide. To further enhance its health benefits, its carotenoid content (mainly alpha and beta-carotenes) has been increased by overexpressing bacterial PSY. Higher β-carotenoid content has been achieved by simultaneous expression of PSY, phytoenedesaturase, and lycopene cyclase genes, and simultaneous expression of seven bacterial genes; idi, crtE, crtB, crtI, crtY, crtW, and crtZ**[62]**.
- Canola normally does not have any Δ6 desaturase activity and thus lacks GLA. To produce GLA more economically and make it more readily available, transgenic lines rich in GLA have been developed by expression of Δ12 or Δ6 desaturases genes. Phytic acid is known as a food inhibitor, which chelates micronutrients and prevents their bioavailability. Transgenic canola varieties viz., Phytaseed™ Canola (MPS 961-965) engineered for phytase degradation to enhance the availability of phosphorus in canola have been produced and released by BASF in the USA**[63]**.
- Mustard is an economically significant crop and extensively cultivated for oil throughout the world. It has been targeted for improving the nutritionally important unsaturated fatty acids by the expression of the enzyme Δ6 FAD3 that led to the production of gamma linoleic acid in the transgenic mustard.
- Transgenic tomato (Solanumlycopersicum) is a popular fruit that provides essential vitamins, micronutrients, and phytonutrients. Its color comes from isopernoid lycopene, which plays a crucial role in membrane structure, free radical scavenging, redox chemistry, defense mechanisms, and growth regulation. Several attempts have been made to increase the isoprenoid content in tomato, such as sterol expression, phytoene and beta-carotene content, and beta-carotene content by expression of the PSY gene**[64]**.
- Apple has also been bioengineered with a stilbene synthase gene from the grapevine, leading to the synthesis of resveratrol in transgenic apple, expanding its antioxidant capacity. Transgenic banana (Super Banana) has been developed by expressing the PSY gene of Asupina banana, which is naturally high in beta-carotene**[65]**.

- Transgenic alfalfa (Medicagosativa) is an important feed legume crop in many countries. Attempts have been made to improve its nutritional status through enhancement of isoflavonoids, essential amino acids, and improve its digestibility. Isoflavonoids are a predominantly legume-specific subclass of flavonoid secondary metabolites. Transgenic alfalfa has been generated by constitutively expressing IFS, which is correlated with its increased isoflavonid composition**[66]**.
- Biofortification through agronomic approaches requires physical application of nutrients to temporarily improve the nutritional and health status of crops and consumption of such crops. Organic minerals like nitrogen, phosphorus, and potassium (NPK) make an important contribution to the attainment of higher crop yields. In the late 1960s, agricultural productivity increased in many countries, leading to the Green Revolution and saving them from starvation**[67]**.
- Microminerals like iron, zinc, copper, manganese, I, Se, Mo, Co, and Ni are found in varying degrees in the edible portion of certain plants and are usually absorbed from the soil. Improvement of the soil micronutrient status by their application as fertilizers can contribute to decrease in micronutrient deficiency in humans. When crops are grown in soils where mineral elements become immediately unavailable in the soil and/or not readily translocated to edible tissues, targeted application of soluble inorganic fertilizers to the roots or leaves is practiced**[68]**.
- Agronomic biofortification is simple and inexpensive, but needs special attention in terms of source of nutrient, application method, and effects on the environment. These should be applied regularly in every crop season and thus are less cost-effective in some cases. The use of mineral fertilizers is evidently feasible in the developed world, as exemplified by the success of Se fertilization of crops in Finland, zinc fertilization in Turkey, and I fertilization in irrigation water in China**[69]**.
- Plant growth-promoting soil microorganisms can be used to enhance nutrient mobility from soil to edible parts of plants and improve their nutritional status. Soil microorganisms like Bacillus, Pseudomonas, Rhizobium, and Azotobacter can also be utilized to increase the phytoavailability of mineral elements. N2-fixing bacteria play an important role in increasing crop productivity in nitrogen limited conditions. Many crops are associated with mycorrhizal fungi that can release organic acids, siderophores, and enzymes capable of degrading organic compounds and increasing mineral concentrations in edible produce**[70]**.
- Cereals have been targeted through agronomical biofortification to improve human nutritional status. Rice has been effectively biofortified by foliar spray of iron to promote iron concentration in rice grains. Foliar application of zinc has been reported as an effective agronomic practice to promote rice grain zinc concentration and zinc bioavailability. Selenium, an essential trace element for human health and potent antioxidant, has been increased by the application of selenate as a foliar spray or as fertilizer in rice**[71]**.
- Wheat has been efficiently utilized in wheat grain quality improvement. Inclusion of iron in foliar urea fertilizers has been positively correlated with high iron accumulation. Application of foliar zinc has reduced human zinc deficiency in regions

with potentially zinc-deficient soil and improved its bioavailability by reducing antinutrient factors like phytic acid. The total amount of zinc-containing NPK fertilizers increased from 0 in 1994 to a record level of 400,000 t per annum in 10–15 years in Turkey**[72]**.

- Maize is required for obtaining nutrient-enriched grain and optimum yield in maize. Various zinc fertilizer treatments and foliar applications have been carried out in maize crop. Plant growth-promoting rhizobacteria have led to nutrient enrichment in the plants and have been included in agronomic approaches to develop effective biofortification strategies for the staple crops. One of the effective examples is the maize crop with increased zinc content**[73]**.
- Barley has been improved by the application of various organic and inorganic biofertilizers. The concentration of zinc and iron in grains has been enhanced by the application of biofertilizers along with inorganic fertilizers and vermicompost.
- Sorghum is cultivated worldwide for grain and fodder, often suffering from the challenge of growing in nutrient poor and contaminated soil. Its nutrient profile has been promoted by the application of fertilizers (both organic and inorganic) that have an additive effect on the yield. Researchers have intended to improve the nutrient uptake and alter the metabolic profile of sorghum by using the combination of plant growth-promoting bacteria and arbuscularmycorrhizal fungi (AMF)**[74]**.
- Legumes have been targeted for zinc biofortification, with selenium-enriched soybean produced by the foliar application of selenium complex salts as fertilizers. Chickpea has been targeted for mineral deficiencies, especially mineral iron, zinc, calcium, copper, manganese, and Mg by using plant growth-promoting actinobacteria. Field peas are the second largest legume crop worldwide, and enrichment for zinc has been obtained with foliar zinc applications alone or in combination with soil zinc applications**[75]**.
- Oilseeds have been targeted for Se enhancement, with rhizosphere bacteria from a seleniferous area enhancing plant uptake of Se asselenate. Vegetables have also been targeted for biofortification, with field experiments showing significant increases in zinc concentrations in potato tubers. Carrot and lettuce have been used for agronomic biofortification, supplementing their leaves and storage roots with iron and selenium as fertilizers. Consumption of 100g of carrots fertilized with iron and selenium can provide 100% of the recommended daily allowance. Lettuce I and sebiofortification has been achieved by applying KIO3 and Na2SeO4 as foliar spray and nutrient medium. Tomato is an excellent crop for iodine biofortification programs when treated with iron fertilizers**[76]**.
- Conventional breeding is the most accepted method of biofortification, offering a sustainable, cost-effective alternative to transgenic- and agronomic-based strategies. Breeding programs can utilize this variation to improve the levels of minerals and vitamins in crops. However, breeding strategies sometimes rely on limited genetic variation present in the gene pool. In some cases, crossing to distant relatives and moving the trait slowly into commercial cultivars or introducing new traits directly into commercial varieties by mutagenesis can overcome this limitation**[77]**.

Comment [A23]: write in full

Comment [A24]:

Comment [A25]: write in full

Comment [A26]: write in full

Comment [A27]: write in full then in bracket

Comment [A28]: write in full then in bracket

- Several international organizations have initiated programs to improve the nutritional content of crops through breeding programs. The Health grain Project (2005–2010) in the European Union aimed to develop health-promoting and safe cereal foods and ingredients of high eating quality. The CGIAR, along with the International Center for Tropical Agriculture (CIAT) and the International Food Policy Research Institute, launched the HarvestPlus program to breed biofortified staple food crops in Asia and Africa. The program aims to produce staple food crops with enhanced levels of bioavailable essential minerals and vitamins that will have measurable impact on improving the micronutrient status of target populations, primarily resource-poor people in the developing world**[78]**.
- Rice is highly emphasized for micronutrient enhancement, as it is one of the most consumed staple food crops and its biofortification can have a significant effect on malnutrition challenges. Different old rice varieties with high iron and zinc content in grain have been screened and combined with improved agronomic traits by breeding methods. The world's first zinc enriched rice varieties developed by HarvestPlus were released in 2013 by the Bangladesh Rice Research Institute**[79]**.
- Wheat as a staple crop is the first and foremost target for biofortification. Wide variation in grain iron and zinc concentrations in wheat and its closely related wild species has been observed that it can be exploited for improvement of modern elite cultivars. HarvestPlus has released several varieties of wheat with 4-10 ppm higher zinc content. Yellow pigment content (YPC) in durum wheat is an important quality trait and antioxidant. A large number of recent durum wheat varieties released in different countries in the past decade show significantly higher YPC than the old varieties released before the 1970s**[80]**.
- Coloured wheat (black, blue, and purple) trait has been used in several breeding programs in different countries. Black-grained wheat cultivar has been released in China after more than 20 years of running effort in breeding and has been reported to be high in protein content and selenium. The purple wheat cultivar Indigo has been released in Austria in 2006, and the purple wheat cultivar PS Karkulka has been registered in Slovakia in 2014. The importance of coloured wheat can be adjudged from the patent on functional foods from coloured wheat in China**[81]**.
- Maize is a cash crop grown for animal feed, industrial purposes, and human consumption. The vast genetic diversity of maize has led to breeding programs that have generated high-yielding varieties of biofortified maize with higher levels of provitamin A to combat vitamin A deficiency. HarvestPlus is using these lines to breed high-yielding varieties of biofortified orange maize to combat vitamin A deficiency. Biofortified orange maize varieties have been grown commercially in Zambia, Nigeria, Ghana, Malawi, Zimbabwe, and Tanzania since 2013**[82]**.
- Quality protein maize (QPM) has also been developed by breeders by incorporating opaque-2 (o2) mutant gene from naturally occurring maize into the maize cultivars. International Maize and Wheat Improvement Center (CIMMYT) has released hybrid varieties in India, China, Vietnam, Mexico, South Africa, Ghana, Guinea, Uganda,

Comment [A29]: write in full

Benin, Mozambique, Brazil, Venezuela, Peru, Colombia, Honduras, El Salvador, Guatemala, and Nicaragua**[83]**.

- Sorghum breeding focuses on micronutrients and beta-carotene rich sorghums. Sorghum varieties have been screened for high minerals, protein, lutein, zeaxanthin, and beta-carotene contents. Biofortified iron rich sorghum lines and hybrids have been bred by ICRISAT and released in India. New nutritionally high (Fe) sorghum varieties (12KNICSV-22 and 12KNICSV-188) have been released in Nigeria, potentially boosting malnourished populations, especially children in Nigeria**[84]**.
- Pearl millet is the cheapest source of iron and zinc, and large variation has been seen in its germplasm for these micronutrients. In India, biofortified pearl millet variety “Dhanashakti” and a hybrid ICMH 1201 (Shakti-1201) have been released by ICRISAT, HarvestPlus in 2014. Various well-adapted commercial varieties, their progenies, and hybrids containing high content of iron and zinc in grain have been reported**[85]**.
- Legumes and pulses include lentil breeding, which has been directed by ICARDA, HarvestPlus for biofortification of iron and zinc using genetic diversity stored in gene banks. Lentil varieties with higher iron, zinc, and protein content can be developed together. Cow pea, also known as poor man meat, rich in protein content, has been biofortified for iron content through breeding methods**[86]**.
- Bean breeding studies suggest that the iron content of the common bean (P. vulgaris) could be increased by 60–80%, while zinc content would be more modest, perhaps around 50%. HarvestPlus is working in this direction and promoting iron biofortified beans in several developing countries. They have released 10 Fe-biofortified common bean varieties in Rwanda and ten biofortified iron bean varieties in the Democratic Republic of Congo.
- Potato breeding is a crucial aspect of human nutrition, with the natural variation of cultivated potato germplasm containing red and purple pigments potentially representing the contribution of potatoes to antioxidants. Breeders focus on breeding such variants to increase iron and zinc levels in human diets. A genetically diverse sample of potato cultivars native to the Andes of South America has been obtained from a collection of nearly 1,000 genotypes and evaluated as a source of antioxidants and minerals (copper, iron, manganese, and zinc)**[87]**.
- Sweet potato breeding is another area of focus, with developing countries growing 95% of the world's sweet potato crop, where malnutrition is the biggest problem. HarvestPlus and International Potato Centre (CIP) have developed and released several varieties of orange sweet potato with high vitamin A. Six varieties have been released in Uganda and three in Zambia, while the Zambia Agriculture Research Institute has successfully completed the development of 15 new varieties of vitamin A fortified sweet potatoes**[88]**.
- Cauliflower breeding has also been screened for genetic variation of zinc concentration, and sufficient natural variation has been identified. The Indian Agricultural Research Institute (IARI) has released provitaminA (beta-carotene) rich orange coloured cauliflower variety (PusaBetaKesari; 800–1,000 μg/100g) and

coloured cauliflower varieties, Purple Graffiti and Orange Cheddar, have been developed by Cornell University, USA**[89]**.

- Cassava breeding is a staple vegetable root crop in developing countries, especially in Africa, Latin America, and the Caribbean. HarvestPlus in collaboration with the International Institute of Tropical Agriculture has released six vitamin A fortified varieties in Nigeria and one in DRC-Democratic Republic of Congo. Cassava also has a wide range of genotype differences for total carotene, proteins, and minerals (iron and zinc), leading to the development of improved nutritive value cassava crops**[90]**.
- Tomato breeding is another area of focus, with genetically diverse wild populations of tomato being investigated intensively for specific traits and exploited in breeding. Anthocyanin biofortified tomato "Sun Black" with deep purple fruit pigmentation due to high anthocyanin content in the peel has been developed by conventional breeding approach**[91]**.
- Banana breeding is difficult and expensive due to commercial varieties being sterile triploids and high degree of cross incompatibility among fertile groups. Biodiversity International (BI) in collaboration with HarvestPlus has carried out large-scale screening of several banana germplasm for the identification of high levels of provitamin A in the Democratic Republic of Congo (DRC) and Burundi**[92]**.
- Mango breeding offers a natural source of beta-carotene, vitamin C, and valuable antioxidants, but their nutrient levels vary with mango variety. The Mexican-grown Ataulfo variety ranked highest in both vitamin C (ascorbic acid) and beta-carotene. In India, IARI introduced many varieties with enhanced nutritional and agronomical important characters**[93]**.

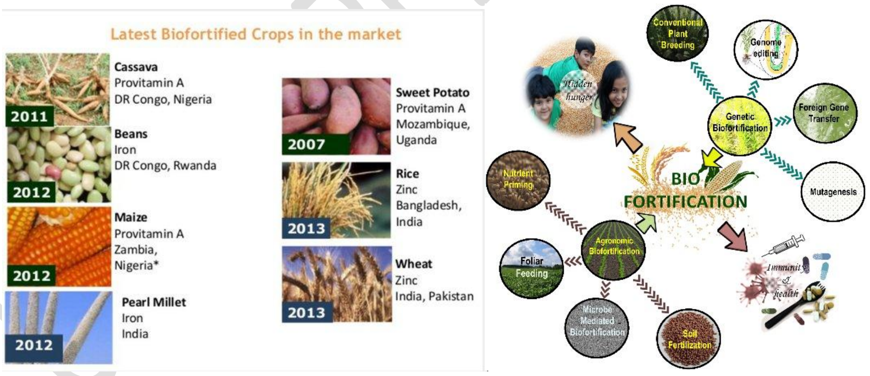

Fig 7. Biofortified crops available in market

Economics for biofortification

Two research streams were conducted to assess the cost-effectiveness of biofortification interventions in improving health outcomes and comparing them to alternative investments. The first was developed early in the discovery phase, using educated guesses about intervention costs and possible benefits. The second was developed to estimate the health benefits of biofortified staple crops from increased intake of micronutrients, such as iron, zinc, and vitamin A. The Disability-adjusted life years (DALYs) approach was adopted to calculate the number of years lost due to disease burden, weighing adverse health outcomes according to severity and duration. A DALY tool was developed for each micronutrient, allowing for a comparison of the cost per DALY saved for biofortification interventions with other micronutrient interventions. The tool measures the reduction in micronutrient deficiency prevalence among target populations and calculates the cost-effectiveness of the biofortification intervention, allowing comparison with other micronutrient interventions**[97]**.

Ex ante analyses have shown that biofortification is highly cost-effective for reducing micronutrient deficiencies, according to the World Bank criteria. The Copenhagen Consensus also found that every USD invested in biofortification resulted in $17 of benefits. Biofortification is more cost-effective than other interventions targeting micronutrient deficiencies, such as supplementation and fortification. HarvestPlus has focused its investments and efforts on South Asia and Sub-Saharan Africa regions following an ex ante analysis that revealed countries in Asia and Africa could benefit most from biofortification, especially for mineral and vitamin A biofortification of crops**[98]**.

The biofortification priority index (BPI) was developed in 2013 to rank LMICs according to their impact potential. The BPI uses country-level data and calculates the geometric mean of three sub-indices: production and consumption indices for biofortifiable staples and a micronutrient deficiency index for the micronutrient with which the crop can be biofortified. The tool has been used by crop breeders, agriculture, nutrition, and health departments of international financial institutions, national decision makers, humanitarian agencies, international NGOs, and private seed companies to make investment decisions pertaining to biofortification**[99]**.

The BPI tool is known to be helpful in informing investment decisions pertaining to biofortification, helping crop breeders, agricultural departments, NGOs, and private seed companies plan their investments. The population-weighted version of the BPI helps WFP understand in which countries biofortification would have the biggest population-level impact, especially for vulnerable populations like women and children**[100]**.

Comment [A30]: use time new roman

Limitations of biofortification

Biofortification is a method that uses micronutrients to improve the nutritional content of crops. However, its success is highly variable due to differences in mineral mobility, mineral accumulation among plant species, and soil compositions in specific geographical locations. Agronomic biofortification is less cost-effective and labour-intensive as it demands continuous inputs through the application of micronutrients to the soil or plant regularly. It is not always possible to target the micronutrient into edible plant parts like seed or fruit and can sometimes result in the accumulation of desired nutrients in leaves or other non-edible portions of plants **[94]**.

Conventional breeding methods have proven successful and are sustainable and cost-effective in the long run. However, there are limitations with respect to the amount of genetic variability for the micronutrients in the plant gene pool and the time needed to generate cultivars with the desired trait(s). In some cases, this can be overcome by crossing to distant relatives and thus introgressing traits into commercial cultivars. However, in many instances, it would be impossible to breed for a specific trait using conventional means, and the timescale and effort involved may be quite unrealistic.

Transgenic methods overcome the limitation of restricted genetic variation among plants but have low acceptance among masses. It is important that the biofortified crops be readily adapted by farmers and communities in significant enough numbers to improve the general nutritional health of a given community. Different countries have adopted different regulatory processes for the acceptance and commercialization of these transgenic crops, which are expensive and time-consuming. For example, the Golden Rice, developed by Mahyco, was not released in India due to concerns raised by scientists, farmers, and anti-GMO activists**[95]**.

Other limitations include the postharvest processing of each crop to optimize biofortification strategies. Milling or polishing cereal seeds can remove large quantities of minerals from the diet, and the extent of these losses is genotype dependent. Additionally, the presence of certain antinutrients in crops reduces the bioavailability of certain nutrients in crops. In the context of global environmental change, improving food production requires improvements in a crop's ability to maintain yields with lower water supply and quality. Numerous genes are involved in controlling the amount of a mineral element that is absorbed by roots, translocated to shoot, remobilized from vegetative tissues, and deposited in edible portions of seeds and grains in forms that are utilizable in persons consuming the crop**[96]**.

Conclusion

Biofortification has long been recognized as a viable and economically viable agricultural method for addressing the nutritional needs of undernourished people on a global scale. Mineral deficiency in humans might be addressed by biofortification procedures that include crop breeding, targeted genetic modification, and/or the use of mineral fertilizers. Micronutrients like iron, zinc, selenium, and provitaminA are often missing from diets throughout the developed and developing worlds, but thanks to biofortified food crops, people in both regions are getting the nutrients they need. These goals can only be attained with the help of national and international programs like the HarvestPlus program. Some of the most important cereal crops grown for human consumption—wheat, corn, cassava, beans, sweet potatoes, and millets—may have had their mineral content and bioavailability improved as a result of these efforts. However, the process of biofortifying crops is not without its difficulties. To do this, it is crucial for molecular biologists, genetic engineers, nutritionists, and plant breeders to work together. In order to improve the nutritional value of meals, traditional breeding methods have been successfully used, and they are quickly gaining appeal. While transgenic methods are getting a lot of attention, breeding-based approaches have a far better track record of success. This is because transgenically enhanced crop plants encounter problems like consumer acceptance issues and the various costly and time-consuming regulatory approval procedures that different countries have implemented. In spite of these obstacles, biofortified crops are the wave of the future, with the ability to end micronutrient deficiency in emerging nations and the lives of billions of impoverished people.

Comment [A31]: with the of

References

1. McGuire S. FAO, IFAD, and WFP. The state of food insecurity in the world 2015: meeting the 2015 international hunger targets: taking stock of uneven progress. Rome: FAO. *AdvNutr* (2015) 6(5):623–4. doi:10.3945/an.115.009936

2. Hodge J. Hidden hunger: approaches to tackling micronutrient deficiencies. In: Gillespie S, Hodge J, Yosef S, Pandya-Lorch R, editors. *Nourishing Millions: Stories of Change in Nutrition*. Washington: International Food Policy Research Institute (IFPRI) (2016). p. 35–43.

3. Muthayya A, Rah JH, Sugimoto JD, Roos FF, Kraemer K, Black RE. The global hidden hunger indices and maps: an advocacy tool for action. *PLoS One* (2013) 8(6):e67860. doi:10.1371/journal.pone.0067860

4. Gould J. Nutrition: a world of insecurity. *Nat Outlook* (2017) 544:S7. doi:10.1038/544S6a

5. Khush GS, Lee S, Cho JI, Jeon JS. Biofortification of crops for reducing malnutrition. *Plant Biotechnol Rep* (2012) 6:195–202. doi:10.1007/s11816-012-0216-5

6. Gilani GS, Nasim A. Impact of foods nutritionally enhanced through biotechnology in alleviating malnutrition in developing countries. *J AOAC Int* (2007) 90(5):1440–4.

7. Perez-Massot E, Banakar R, Gomez-Galera S, Zorrilla-Lopez U, Sanahuja G, Arjo G, et al. The contribution of transgenic plants to better health through improved nutrition: opportunities and constraints. *Genes Nutr* (2013) 8(1):29–41. doi:10.1007/s12263-012-0315-5

8. Bouis HE. Economics of enhanced micronutrient density in food staples. *Field Crops Res* (1999) 60:165–73. doi:10.1016/S0378-4290(98)00138-5

9. Nestel P, Bouis HE, Meenakshi JV, Pfeiffer W. Biofortification of staple food crops. *J Nutr* (2006) 136:1064–7. doi:10.1093/jn/136.4.1064

10. Pfeiffer WH, McClafferty B. HarvestPlus: breeding crops for better nutrition. *Crop Sci* (2007) 47:S88–100. doi:10.2135/cropsci2007.09.0020IPBS

11. Qaim M, Stein AJ, Meenakshi JV. Economics of biofortification. *Agric Econ* (2007) 37:119–33. doi:10.1111/j.1574-0862.2007.00239.x

12. Hirschi KD. Nutrient biofortification of food crops. *Annu Rev Nutr* (2009) 29:401–21. doi:10.1146/annurev-nutr-080508-141143

13. Meenakshi JV, Johnson NL, Manyong VM, DeGroote H, Javelosa J, Yanggen DR, et al. How cost-effective is biofortification in combating micronutrient malnutrition? An ex ante assessment. *World Dev* (2010) 38(1):64–75. doi:10.1016/j.worlddev.2009.03.014

14. Hefferon KL. Can biofortified crops help attain food security? *CurrMolBiol Rep* (2016) 2(4):180–5. doi:10.1007/s40610-016-0048-0

15. Bazuin S, Azadi H, Witlox F. Application of GM crops in Sub-Saharan Africa: lessons learned from green revolution. *BiotechnolAdv* (2011) 29:908–12. doi:10.1016/j.biotechadv.2011.07.011

16. Das JK, Kumar R, Salam RA, Bhutta ZA. Systematic review of zinc fortification trials. *Ann NutrMetab* (2013) 62(1):44–56. doi:10.1159/000348262

17. Bouis HE. Enrichment of food staples through plant breeding: a new strategy for fighting micronutrient malnutrition. *Nutrition* (2000) 16:701–4. doi:10.1016/S0899-9007(00)00266-5

18. Prashanth L, Kattapagari KK, Chitturi RT, Baddam VR, Prasad LK. A review on role of essential trace elements in health and disease. *J NTR Univ Health Sci* (2015) 4:75–85. doi:10.4103/2277-8632.158577

19. White J, Broadley MR. Biofortifying crops with essential mineral elements. *Trends Plant Sci* (2005) 10:586–93. doi:10.1016/j.tplants.2005.10.001

20. Welch RM, Graham RD. Breeding for micronutrients in staple food crops from a human nutrition perspective. *J Exp Bot* (2004) 55:353–64. doi:10.1093/jxb/erh064

21. Graham RD, Welch RM, Bouis HE. Addressing micronutrient malnutrition through enhancing the nutritional quality of staple foods: principles, perspectives and knowledge gaps. *AdvAgron* (2001) 70:77–142. doi:10.1016/S0065-2113(01)70004-1

22. McGuire J. Addressing micronutrient malnutrition. *SCN News* (1993) 9:1–10.

23. Schneeman BO. Linking agricultural production and human nutrition. *J Sci Food Agri* (2001) 81:3–9. doi:10.1002/1097-0010(20010101)81:1<3::AID-JSFA743>3.0.CO;2-Q

24. Chizuru N, Ricardo U, Shiriki K, Prakash S. The joint WHO/FAO expert consultation on diet, nutrition and the prevention of chronic diseases: process, product and policy implications. *Public Health Nutr* (2003) 7(1a):245–50. doi:10.1079/PHN2003592

25. Branca F, Ferrari M. Impact of micronutrient deficiencies on growth: the stunting syndrome. *Ann NutrMetab* (2002) 46:8–17. doi:10.1159/000066397

26. Golden MHN. The nature of nutritional deficiencies in relation to growth failure and poverty. *ActaPaediatrScand* (1991) 374:95–110. doi:10.1111/j.1651-2227.1991.tb12012.x

27. Grantham-McGregor SM, Ani CC. The role of micronutrients in psychomotor sad cognitive development. *Br Med Bull* (1999) 55:511–27. doi:10.1258/0007142991902583

28. Ramakrishnan U, Manjrekar R, Rivera J, Gonzales-Cossio T, Martorell R. Micronutrients and pregnancy outcome: a review of the literature. *Nutr Res* (1999) 19:103–59. doi:10.1016/S0271-5317(98)00178-X

29. Caballero B. Global patterns of child health: the role of nutrition. *Ann NutrMetab* (2002) 46:3–7. doi:10.1159/000066400

30. Stevens GA, Finucane MM, De-Regil L, Paciorek CJ, Flaxman SR, Branca F, et al. Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for 1995–2011: a systematic analysis of population-representative data. *Lancet Glob Health* (2013) 1(1):e16–25. doi:10.1016/S2214-109X(13)70001-9

31. Brotanek JM, Halterman JS, Auinger P, Flores G, Weitzman M. Iron deficiency, prolonged bottle-feeding, and racial/ethnic disparities in young children. *Arch PediatrAdolesc Med* (2005) 159:1038–42. doi:10.1001/archpedi.159.11.1038

32. Zhu C, Naqvi S, Gomez-Galera S, Pelacho AM, Capell T, Christou P. Transgenic strategies for the nutritional enhancement of plants. *Trends Plant Sci* (2007) 12:548–55. doi:10.1016/j.tplants.2007.09.007

33. Welch RM, Graham RD. A new paradigm for world agriculture: meeting human needs-productive, sustainable, nutritious. *Field Crops Res* (1999) 60:1–10. doi:10.1016/S0378-4290(98)00129-4

34. Saltzman A, Birol E, Bouis HE, Boy E, De Moura FF, Islam Y, et al. Biofortification: progress toward a more nourishing future. *Glob Food Secur* (2014) 2(1):9–17. doi:10.1016/j.gfs.2012.12.003

35. Brinch-Pedersen H, Borg S, Tauris B, Holm PB. Molecular genetic approaches to increasing mineral availability and vitamin content of cereals. *J Cereal Sci* (2007) 46:308–26. doi:10.1016/j.jcs.2007.02.004

36. Christou P, Twyman RM. The potential of genetically enhanced plants to address food insecurity. *Nutr Res Rev* (2004) 17:23–42. doi:10.1079/NRR200373

37. Newell-McGloughlin M. Nutritionally improved agricultural crops. *Plant Physiol* (2008) 147:939–53. doi:10.1104/pp.108.121947

38. Shewmaker CK, Sheehu JA, Daley M, Colburn S, Ke DY. Seed-specific overexpression of phytoene synthase: increase in carotenoids and metabolic effects. *Plant J* (1999) 20:41–412. doi:10.1046/j.1365-313x.1999.00611.x

39. Agrawal PK, Kohli A, Twyman RM, Christou P. Transformation of plants with multiple cassettes generates simple transgene integration patterns and high expression levels. *Mol Breed* (2005) 16:247–60. doi:10.1007/s11032-005-0239-5

40. Yang SH, Moran DL, Jia HW, Bicar EH, Lee M, Scott MP. Expression of a synthetic porcine alpha-lactalbumin gene in the kernels of transgenic maize. *Transgenic Res* (2002) 11:11–20. doi:10.1023/A:1013996129125

41. Ye X, Al-Babili S, Kloti A, Zhang J, Lucca P, Beyer P, et al. Engineering the provitamin A (β-carotene) biosynthetic pathway into (carotenoids-free) rice endosperm. *Science* (2000) 287:303–5. doi:10.1126/science.287.5451.303

42. Beyer P, Al-Babili S, Ye X, Lucca P, Schaub P, Welsch R, et al. Golden rice: introducing the β-carotene biosynthesis pathway into rice endosperm by genetic engineering to defeat vitamin A deficiency. *J Nutr* (2002) 132(3):506S–10S. doi:10.1093/jn/132.3.506S

43. Datta K, Baisakh N, Oliva N, Torrizo L, Abrigo E, Tan J, et al. Bioengineered 'golden' Indica rice cultivars with beta-carotene metabolism in the endosperm with hygromycin and mannose selection systems. *Plant Biotechnol J* (2003) 1:81–90. doi:10.1046/j.1467-7652.2003.00015.x

44. Paine JA, Shipton CA, Chaggar S, Howells RM, Kennedy MJ, Vernon G, et al. Improving the nutritional value of golden rice through increased pro-vitamin A content. *Nat Biotechnol* (2005) 23:482–7. doi:10.1038/nbt1082

45. Burkhardt PK, Beyer P, Wuenn J, Kloeti A, Armstrong GA, Schledz M, et al. Transgenic rice (*Oryzasativa*) endosperm expressing daffodil (*Narcissus pseudonarcissus*) phytoene synthase accumulates phytoene, a key intermediate of provitamin A biosynthesis. *Plant J* (1997) 11:1071–8. doi:10.1046/j.1365-313X.1997.11051071.x

46. Storozhenko S, De Brouwer V, Volckaert M, Navarrete O, Blancquaert D, Zhang GF, et al. Folate fortification of rice by metabolic engineering. *Nat Biotechnol* (2007) 25(11):1277–9. doi:10.1038/nbt1351

47. Blancquaert D, Van daele J, Strobbe S, Kiekens F, Storozhenko S, De Steur H, et al. Improving folate (vitamin B9) stability in biofortified rice through metabolic engineering. *Nat Biotechnol* (2015) 33:1076–8. doi:10.1038/nbt.3358

48. Takahashi M, Nakanishi H, Kawasaki S, Nishizawa NK, Mori S. Enhanced tolerance of rice to low iron availability in alkaline soils using barley nicotianamine aminotransferase genes. *Nat Biotechnol* (2001) 19:466–9. doi:10.1038/88143

49. Lee S, An G. Over-expression of OsIRT1 leads to increased iron and zinc accumulations in rice. *Plant Cell Environ* (2009) 32:408–16. doi:10.1111/j.1365-3040.2009.01935.x

50. Zheng L, Cheng Z, Ai C, Jiang X, Bei X, Zheng Y, et al. Nicotianamine, a novel enhancer of rice iron bioavailability to humans. *PLoS One* (2010) 5(4):e10190. doi:10.1371/journal.pone.0010190

51. Lee S, Kim YS, Jeon US, Kim YK, Schjoerring JK, An G. Activation of rice nicotinamine synthase 2 (OsNAS2) enhances iron availability for biofortification. *Mol Cell* (2012) 33:269–75. doi:10.1007/s10059-012-2231-3

52. Trijatmiko K, Duenas C, Tsakirpaloglou N, Torrizo L, Arines FM, Adeva C, et al. Biofortifiedindica rice attains iron and zinc nutrition dietary targets in the field. *Sci Rep* (2016) 6:19792. doi:10.1038/srep19792

53. Goto F, Yoshihara T, Shigemoto N, Toki S, Takaiwa F. Iron fortification of rice seed by the soybean ferritin gene. *Nat Biotechnol* (1999) 17:282–6. doi:10.1038/7029

54. Vasconcelos M, Datta K, Oliva N, Khalekuzzaman M, Torrizo L, Krishnan S, et al. Enhanced iron and zinc accumulation in transgenic rice with the ferritin gene. *Plant Sci* (2003) 164:371–8. doi:10.1016/S0168-9452(02)00421-1

55. Lucca P, Hurrell R, Potrykus I. Fighting iron deficiency anemia with iron-rich rice. *J Am CollNutr* (2002) 21:184S–90S. doi:10.1080/07315724.2002.10719264

56. Wirth J, Poletti S, Aeschlimann B, Yakandawala N, Drosse B, Osorio S, et al. Rice endosperm iron biofortification by targeted and synergistic action of nicotianamine synthase and ferritin. *Plant Biotechnol J* (2009) 7:631–44. doi:10.1111/j.1467-7652.2009.00430.x

57. Masuda H, Ishimaru Y, Aung MS, Kobayashi T, Kakei Y, Takahashi M, et al. Iron biofortification in rice by the introduction of multiple genes involved in iron nutrition. *Sci Rep* (2012) 2:534. doi:10.1038/srep00543

58. Masuda H, Kobayashi T, Ishimaru Y, Takahashi M, Aung MS, Nakanishi H, et al. Iron-biofortification in rice by the introduction of three barley genes participated in mugineic acid biosynthesis with soybean ferritin gene. *Front Plant Sci* (2013) 4:132. doi:10.3389/fpls.2013.00132

59. Hurrell R, Egli I. Iron bioavailability and dietary reference values. *Am J ClinNutr* (2010) 91:1461S–7S. doi:10.3945/ajcn.2010.28674F

60. Masuda H, Suzuki M, Morikawa KC, Kobayashi T, Nakanishi H, Takahashi M, et al. Increase in iron and zinc concentrations in rice grains via the introduction of barley genes involved in phytosiderophore synthesis. *Rice* (2008) 1:100–8. doi:10.1007/s12284-008-9007-6

61. Zheng A, Sumi K, Tanaka K, Murai N. The bean seed storage protein β-phaseolin is synthesized, processed and accumulated in the vacuolar type-II protein bodies of transgenic rice endosperm. *Plant Physiol* (1995) 109:777–86. doi:10.1104/pp.109.3.777

62. Sindhu AS, Zheng Z, Murai N. The pea seed storage protein legumin was synthesized, processed, and accumulated stably in transgenic rice endosperm. *Plant Sci* (1997) 130:189–96. doi:10.1016/S0168-9452(97)00219-7

63. Lee TTT, Wang MMC, Hou RCW, Chen LJ, Su RC, Wang CS, et al. Enhanced methionine and cysteine levels in transgenic rice seeds by the accumulation of sesame 2S albumin. *BiosciBiotechnolBiochem* (2003) 67:1699–705. doi:10.1271/bbb.67.1699

64. Katsube T, Kurisaka N, Ogawa M, Maruyama N, Ohtsuka R, Utsumi S, et al. Accumulation of soybean glycinin and its assembly with the glutelins in rice. *Plant Physiol* (1999) 120:1063–73. doi:10.1104/pp.120.4.1063

65. Yang QQ, Zhang CQ, Chan ML, Zhao DS, Chen JZ, Wang Q, et al. Biofortification of rice with the essential amino acid lysine: molecular characterization, nutritional evaluation, and field performance. *J Exp Bot* (2016) 67(14):4285–96. doi:10.1093/jxb/erw209

66. Lee SI, Kim HU, Lee YH, Suh SC, Lim YP, Lee HY, et al. Constitutive and seed-specific expression of a maize lysine-feedback-insensitive dihydrodipicolinate synthase gene leads to increased free lysine levels in rice seeds. *Mol Breed* (2001) 8:75–84. doi:10.1023/A:1011977219926

67. Wakasa K, Hasegawa H, Nemoto H, Matsuda F, Miyazawa H, Tozawa Y, et al. High-level tryptophan accumulation in seeds of transgenic rice and its limited effects on agronomic traits and seed metabolite profile. *J Exp Bot* (2006) 57:3069–78. doi:10.1093/jxb/erl068

68. Zhou Y, Cai H, Xiao J, Li X, Zhang Q, Lian X. Over-expression of aspartate aminotransferase genes in rice resulted in altered nitrogen metabolism and increased amino acid content in seeds. *TheorAppl Genet* (2009) 118:1381–90. doi:10.1007/s00122-009-0988-3

69. Anai T, Koga M, Tanaka H, Kinoshita T, Rahman SM, Takagi Y. Improvement of rice (*Oryzasativa* L.) seed oil quality through introduction of a soybean microsomal omega-3 fatty acid desaturase gene. *Plant Cell Rep* (2003) 21(10):988–92. doi:10.1007/s00299-003-0609-6

70. Shin YM, Park HJ, Yim SD, Baek NI, Lee CH, An G, et al. Transgenic rice lines expressing maize C1 and R-S regulatory genes produce various flavonoids in the endosperm. *Plant Biotechnol J* (2006) 4:303–15. doi:10.1111/j.1467-7652.2006.00182.x

71. Ogo Y, Ozawa K, Ishimaru T, Murayama T, Takaiwa F. Transgenic rice seed synthesizing diverse flavonoids at high levels: a new platform for flavonoid production with associated health benefits. *Plant Biotechnol J* (2013) 11:734–46. doi:10.1111/pbi.12064

72. Liu Q, Wang Z, Chen X, Cai X, Tang S, Yu H, et al. Stable inheritance of the antisense waxy gene in transgenic rice with reduced amylose level and improved quality. *Transgenic Res* (2003) 12(1):71–82. doi:10.1023/A:1022148824018

73. Itoh K, Ozaki H, Okada K, Hori H, Takeda Y, Mitsui T. Introduction of Wx transgene into rice wx mutants leads to both high- and low-amylose rice. *Plant Cell Physiol* (2003) 44(5):473–80. doi:10.1093/pcp/pcg068

74. Wei C, Zhang J, Chen Y, Zhou W, Xu B, Wang Y, et al. Physicochemical properties and development of wheat large and small starch granules during endosperm development. *ActaPhysiol Plant* (2010) 32:905–16. doi:10.1007/s11738-010-0478-x

75. Nandi S, Suzuki YA, Huang J, Yalda D, Pham P, Wu L, et al. Expression of human lactoferrin in transgenic rice grains for the application in infant formula. *Plant Sci* (2002) 163:713–22. doi:10.1016/S0168-9452(02)00165-6

76. Wang C, Zeng J, Li Y, Hu W, Chen L, Miao Y, et al. Enrichment of provitamin A content in wheat (*Triticumaestivum* L.) by introduction of the bacterial carotenoid biosynthetic genes *CrtB* and *CrtI*. *J Exp Bot* (2014) 65(9):2545–56. doi:10.1093/jxb/eru138

77. Cong L, Wang C, Chen L, Liu H, Yang G, He G. Expression of phytoene synthase1 and carotene desaturasecrtI genes result in an increase in the total carotenoids content in transgenic elite wheat (*Triticumaestivum* L.). *J Agric Food Chem* (2009) 57(18):8652–60. doi:10.1021/jf9012218

78. Xiaoyan S, Yan Z, Shubin W. Improvement Fe content of wheat (*Triticumaestivum*) grain by soybean ferritin expression cassette without vector backbone sequence. *J AgricBiotechnol* (2012) 20:766–73.

79. Borg S, Brinch-Pedersen H, Tauris B, Madsen LH, Darbani B, Noeparvar S, et al. Wheat ferritins: improving the iron content of the wheat grain. *J Cereal Sci* (2012) 56:204–13. doi:10.1016/j.jcs.2012.03.005

80. Brinch-Pederson H, Olesen A, Rasmussen SK, Holm PB. Generation of transgenic wheat (*Triticumaestivum* L.) for constitutive accumulation of an *Aspergillus* phytase. *Mol Breed* (2000) 6:195–206. doi:10.1023/A:1009690730620

81. Bhati KK, Alok A, Kumar A, Kaur J, Tiwari S, Pandey AK. Silencing of ABCC13 transporter in wheat reveals its involvement in grain development, phytic acid accumulation and lateral root formation. *J Exp Bot* (2016) 67(14):4379–89. doi:10.1093/jxb/erw224

82. Tamas C, Kisgyorgy BN, Rakszegi M, Wilkinson MD, Yang MS, Lang L, et al. Transgenic approach to improve wheat (*Triticumaestivum* L.) nutritional quality. *Plant Cell Rep* (2009) 28(7):1085–94. doi:10.1007/s00299-009-0716-0

83. Doshi KM, Eudes F, Laroche A, Gaudet D. Transient embryo specific expression of anthocyanin in wheat. *In Vitro Cell DevBiol Plant* (2006) 42:432–8. doi:10.1079/IVP2006778

84. Sestili F, Janni M, Doherty A, Botticella E, D’Ovidio R, Masci S, et al. Increasing the amylose content of durum wheat through silencing of the SBEIIa genes. *BMC Plant Biol* (2010) 10:144. doi:10.1186/1471-2229-10-144

85. Aluru M, Xu Y, Guo R, Wang Z, Li S, White W, et al. Generation of transgenic maize with enhanced provitaminA content. *J Exp Bot* (2008) 59(13):3551–62. doi:10.1093/jxb/ern212

86. Decourcelle M, Perez-Fons L, Baulande S, Steiger S, Couvelard L, Hem S, et al. Combined transcript, proteome, and metabolite analysis of transgenic maize seeds engineered for enhanced carotenoid synthesis reveals pleotropic effects in core metabolism. *J Exp Bot* (2015) 66(11):3141–50. doi:10.1093/jxb/erv120

87. Cahoon EB, Hall SE, Ripp KG, Ganzke TS, Hitz WD, Coughlan SJ. Metabolic redesign of vitamin E biosynthesis in plants for tocotrienol production and increased antioxidant content. *Nat Biotechnol* (2003) 21:1082–7. doi:10.1038/nbt853

88. Levine M, Dhariwal KR, Welch RW, Wang Y, Park JB. Determination of optimal vitamin C requirements in humans. *Am J ClinNutr* (1995) 62:1347S–56S. doi:10.1093/ajcn/62.6.1347S

89. Chen Z, Young TE, Ling J, Chang SC, Gallie DR. Increasing vitamin C content of plants through enhanced ascorbate recycling. *ProcNatlAcadSci U S A* (2003) 100:3525–30. doi:10.1073/pnas.0635176100

90. Naqvi S, Zhu C, Farre G, Ramessar K, Bassie L, Breitenbach J, et al. Transgenic multivitamin corn through biofortification of endosperm with three vitamins representing three distinct metabolic pathways. *ProcNatlAcadSci U S A* (2009) 106(19):7762–7. doi:10.1073/pnas.0901412106

91. Drakakaki G, Marcel S, Glahn RP, Lund EK, Pariagh S, Fischer R, et al. Endosperm-specific co-expression of recombinant soybean ferritin and *Aspergillus* phytase in maize results in significant increases in the levels of bioavailable iron. *Plant MolBiol* (2005) 59(6):869–80. doi:10.1007/s11103-005-1537-3

92. Aluru MR, Rodermel SR, Reddy MB. Genetic modification of low phytic acid 1-1 maize to enhance iron content and bioavailability. *J Agric Food Chem* (2011) 59(24):12954–62. doi:10.1021/jf203485a

93. Chen R, Xue G, Chen P, Yao B, Yang W, Ma Q, et al. Transgenic maize plants expressing a fungal phytase gene. *Transgenic Res* (2008) 17(4):633–43. doi:10.1007/s11248-007-9138-3

94. Shi J, Wang H, Schellin K, Li B, Faller M, Stoop JM, et al. Embryo-specific silencing of a transporter reduces phytic acid content of maize and soybean seeds. *Nat Biotechnol* (2007) 25(8):930–7. doi:10.1038/nbt1322

95. Yu J, Peng P, Zhang X, Zhao Q, Zhu D, Sun X, et al. Seed-specific expression of the lysine-rich protein gene sb401 significantly increases both lysine and total protein content in maize seeds. *Food Nutr Bull* (2005) 26(4):427–31. doi:10.1177/156482650502604S311

96. Ambikapathi R., Schneider K. R., Davis B., Herrero M., Winters P., Fanzo J. C. (2022). Global food systems transitions have enabled affordable diets but had less favourable outcomes for nutrition, environmental health, inclusion and equity. *Nat. Food* 3, 764–779. doi: 10.1038/S43016-022-00588.

97. Arimond M., Ball A.-M., Bechoff A., Bosch D., Bouis H. (2010). Reaching and engaging end users (REU) orange fleshed sweet potato (OFSP) in East and southern Africa.

98. Biofortification MEL Collective . (2022). *(HarvestPlus, International Potato Center [CIP], Global Alliance for Improved Nutrition [GAIN], Wageningen University and Research, CGIAR Monitoring Evaluation and Learning and Impact Assessment Communities of Practice and the Standing Panel on Impact Assessment [SPIA]). Monitoring, evaluation, learning and impact assessment systems (MELIAS) for large scale biofortification programs: a blueprint*.

99. Birol E., Meenakshi J. V., Oparinde A., Perez S., Tomlins K. (2015. a). Developing country consumers' acceptance of biofortified foods: a synthesis. *Food Secur* 7, 555–568. doi: 10.1007/S12571-015-0464-7/TABLES/3

100. Bouis H., Birol E., Boy E., Gannon B., Haas J. D., Low J. W., et al.. (2020). Food biofortification: Reaping the benefits of science to overcome hidden hunger: A paper in the series on the need for agricultural innovation to sustainably feed the world by 2050 Council for Agricultural Science and Technology (CAST) Issue Paper 69. Available at: https://www.cast-science.org/wp-content/uploads/2020/10/CAST_IP69_Biofortification-1.pdf