

Review Article

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

Abstract

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].



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 provitamin A[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].

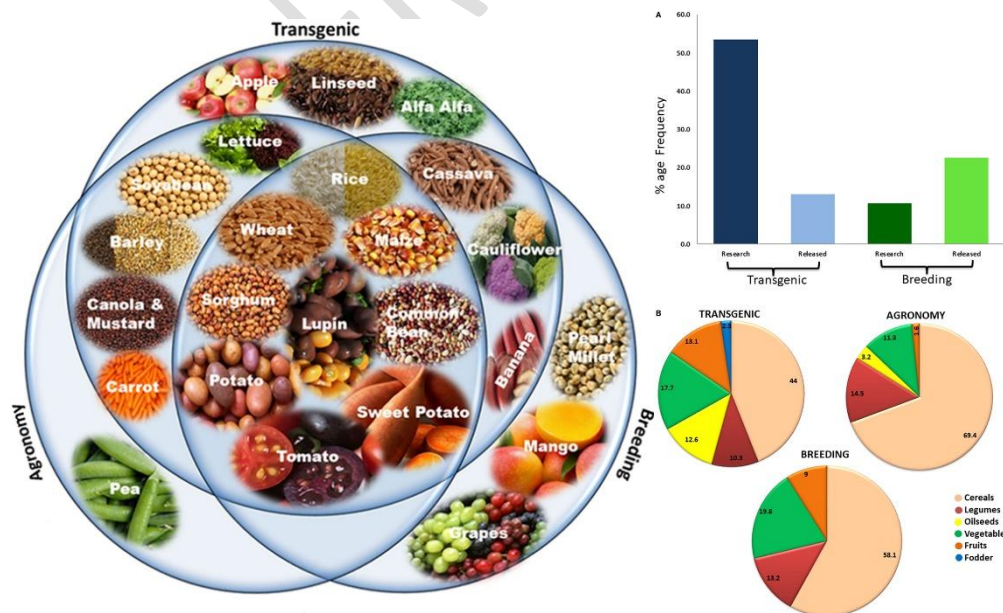


Fig 2. Bio fortify essential agricultural plants using biotechnological technologies

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].

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].

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].

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 provitamin A 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 provitamin A 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].

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].

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].

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].

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, 'BRRI dhan 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].

Challenges of climate change on environment and crop production

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 staple 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].

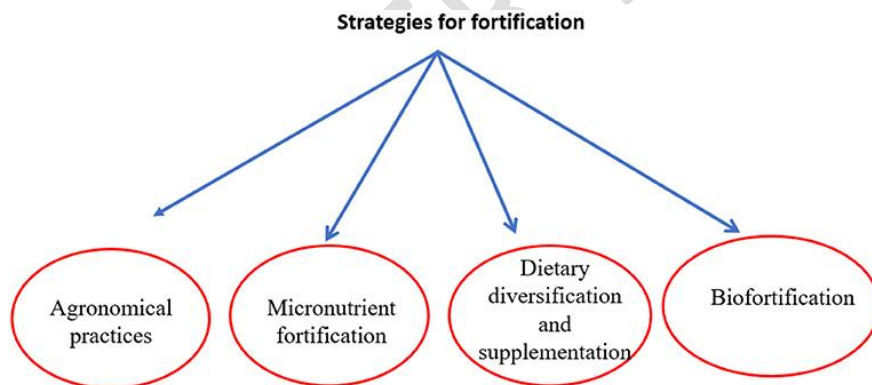


Fig 4. Fortification strategies

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 Fe^{2+}/Fe^{3+} 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 Fe^{2+}/Fe^{3+} ratio, leading to lower iron availability to the plants. Additionally, drought reduces nitrogen and phosphorus uptake in plants[38].

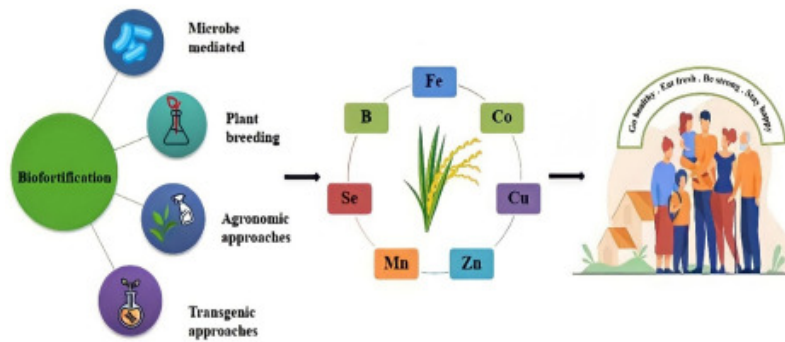


Fig 5. Biofortification technique

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].
- 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 provitamin A (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].
- 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 geranylgeranyl transferase [HGGT] and vitamin C (l-ascorbic acid), which play roles in cardiovascular function, immune cell development, and iron utilization[46].

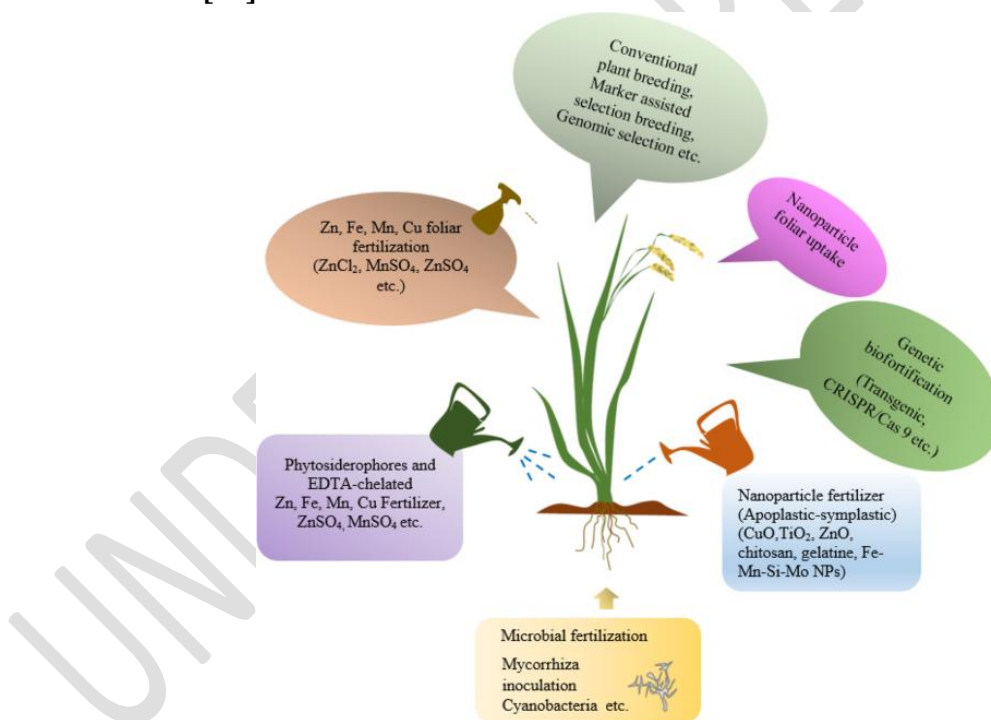


Fig 6. Biofortified crops recent developments

- Bioavailability of micronutrients is hindered by antinutrient components, such as expressing soybean ferritin and *Aspergillus* phytase, soybean ferritin, *Aspergillus niger* 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 MaverTM 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].
- Sorghum is one of the most important staple foods for millions of poor rural people, and it has been targeted to improve provitamin A (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 RNAi silencing 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 provitamin A (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].
- 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-acetylserine sulfhydrylase, 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 Δ 6-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 Δ 6 desaturase and Δ 15 desaturase[52].
- 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; TreusTM, PlenishTM (DP305423) in Australia, Canada, China, European Union, Japan, Mexico, New Zealand, Philippines, Singapore, South Africa, South Korea, Taiwan, USA; and TreusTM (DP 305423 \times

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 (*Lupinus angustifolius*) 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 (*Solanum tuberosum*) 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, phytoene desaturase, 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 zeaxanthin epoxidase 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 coexpressing cystathionine γ -synthase (*CgS Δ 90*) 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 cyclodextrin glycosyltransferases (CGT) gene, which results in the production of multipurpose dietary fiber cyclodextrins from starch. Potato tubers have also been targeted to increase phenolic acid, anthocyanins contents by single-gene overexpression or by simultaneous expression of CHS, chalcone isomerase (CHI), and dihydroflavonol reductase. 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].

- 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 $\Delta 6$ 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, phytoene desaturase, 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 $\Delta 6$ 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 $\Delta 12$ or $\Delta 6$ 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 $\Delta 6$ FAD3 that led to the production of gamma linoleic acid in the transgenic mustard.
- Transgenic tomato (*Solanum lycopersicum*) is a popular fruit that provides essential vitamins, micronutrients, and phytonutrients. Its color comes from isoprenoid 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 (*Medicago sativa*) 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 arbuscular mycorrhizal 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 as selenate. 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 se biofortification has been achieved by applying KIO_3 and Na_2SeO_4 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].

- 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,

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 provitamin A (beta-carotene) rich orange coloured cauliflower variety (Pusa BetaKesari; 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].

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 provitamin A 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.

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