



# Biofortification: Enhancing Nutritional Content in Crops through Biotechnology and Fighting Climate Change

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## Authors' contributions

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## 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; nutritional value; human health; climate-smart agriculture; environmental footprint.*

## 1. INTRODUCTION

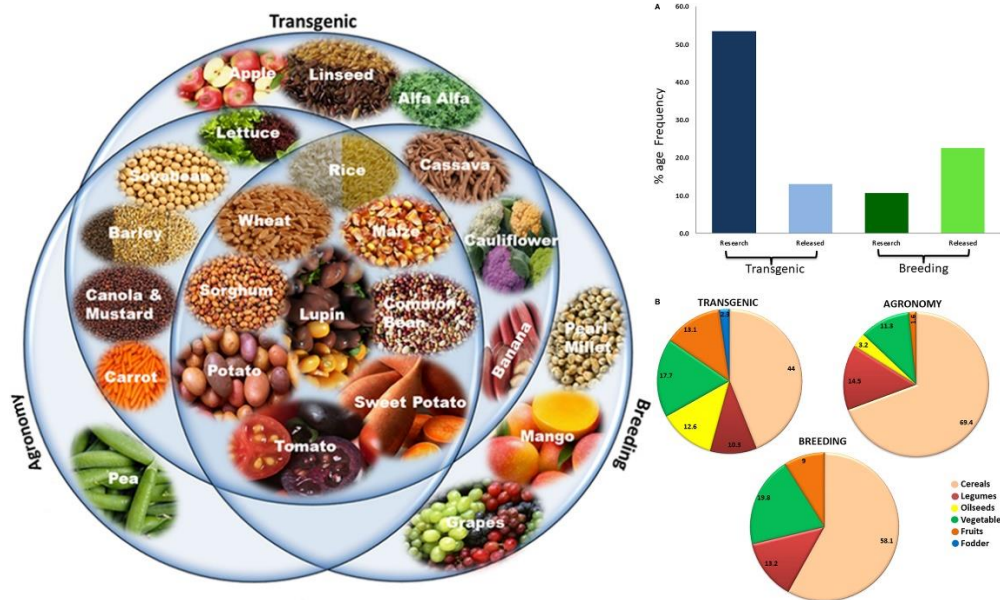
Micronutrient malnutrition is a growing problem since the world's food system isn't able to provide enough nutritious food, particularly to those who are poor and have little resources. In poor nations like India, where people rely heavily on cereal-based diets and have limited access to meat, fruits, 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].

A sustainable and cost-effective way to reduce VAD is to bio fortify essential agricultural plants using biotechnological technologies. To increase the quantities of  $\beta$ -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  $\beta$ -lycopene cyclase ( $\beta$ -lyc). The quantities of total carotenoids and  $\beta$ -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. 1. Fortified and biofortified growth**



**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]. With the help of 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 CO<sub>2</sub> 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].

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].

## 2. IMPORTANCE OF BIOFORTIFICATION

For humans to be able to live healthy and productive lives, they need around forty different nutrients. Essential nutrients, which include sodium, potassium, calcium, magnesium, phosphorous, chlorine, and sulfur, are required in the body in small amounts. On the other hand, micronutrients, which include iron, zinc, copper, manganese, iodine, selenium, molybdenum, cobalt, nickel, and vitamin A, play crucial roles in the development of both the body and the mind [14].

The major source of nutrients for people is agricultural goods, especially those that are

produced in nations that are still developing economies. On the other hand, the diets of these communities often include inadequate quantities of vital nutrients, which results in poor health, illness, a rise in morbidity and disability, impaired development, stunted growth, limited livelihoods, and lower socioeconomic development.

There is a correlation between micronutrient deficiency and childhood stunting in many poor nations. In these countries, 32 percent of pregnant women and 43 percent of pre-school children are impacted by this condition. Anemia affects more than thirty percent of the world's population, with the majority of the afflicted nations being located in South-East Asia and Africa. According to estimates, nearly half of this condition is caused by a lack of iron [15].

Another problem is the early distribution of nutrients among the various sections of the plant. For example, the iron content of rice leaves is high, while the iron content of rice grain that has been polished is low. An increasing number of people are becoming concerned about overnutrition, especially diabetes, which may lead to issues such as obesity and diabetes. The goal of biofortification is to increase the amount of required micronutrients that are present in agricultural plants. This will ensure that the edible component of the crop has all of the necessary nutrients. The impoverished population may benefit from this strategy since it can give sufficient calories to fulfill their energy requirements and increase the quantity of nutrients they take in [16].

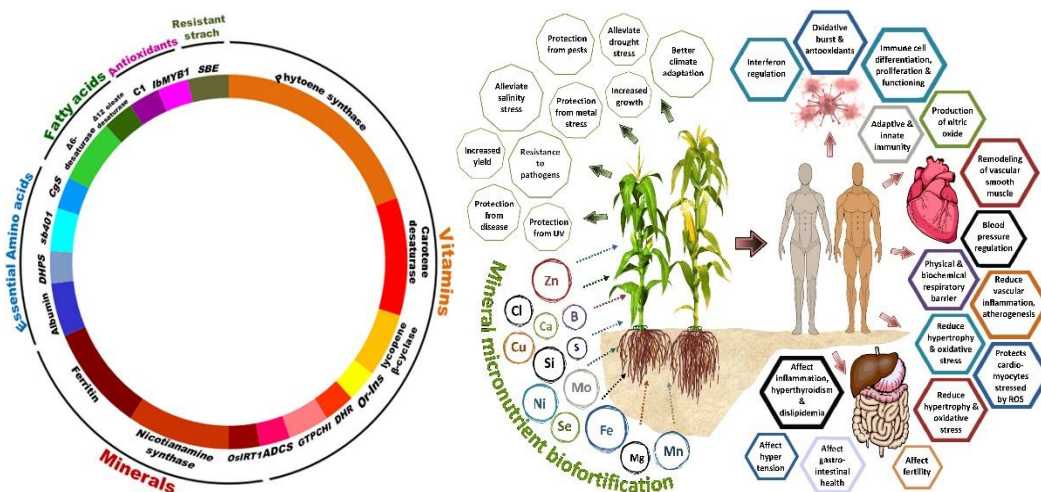


Fig. 3. Importance of biofortification



### 3. METHODS TO ACHIEVE BIOFORTIFICATION

When it comes to increasing the concentrations of micronutrients in crops, biofortification is a strategy that is both environmentally friendly and economical. Despite the fact that it is a lengthy procedure that calls for a significant amount of labor and money, conventional breeding is characterized by its own set of capabilities and limitations. Genetic modification makes it possible to increase a greater number of characteristics in a shorter amount of time and achieves better levels of nutritional improvement in comparison to traditional breeding [17].

Increasing the amounts of provitamin A carotenoids in cassava and sweet potatoes, iron in cassava, bananas, and beans, and zinc in rice, pearl millet, and wheat has been accomplished via the use of conventional breeding techniques in a number of different regions throughout the globe. A significant number of the biofortified items are already accessible on the market, and they are highly received by those living in rural areas. However, there are several restrictions that come with the use of this method. These constraints include the very restricted number of characteristics that may be enhanced simultaneously, the amount of genetic variation that is accessible for the characteristic, and the length of time that is necessary [18].

The use of transgenic methods is helpful in situations when the nutrient in question does not naturally occur in a crop or when it is not possible to efficiently breed adequate quantities of bioavailable micronutrients into the crop. Following the acquisition of a transgenic line, a number of years of conventional breeding are required in order to guarantee that the transgenes are inherited in a stable manner and to integrate the transgenic line into types that are preferred by farmers. The use of genetic modification makes it possible to improve a greater number of characteristics in a shorter amount of time and has the potential to achieve much higher levels of nutritional improvement in comparison to traditional breeding [19].

Examples of initiatives that include biofortification include the enhancement of protein content, the prevention of vitamin A deficiency, and the development of additional crops that are modified to have a larger amount of  $\beta$ -carotene. The first

biofortified crop to be released is orange sweet potato (OSP), which is abundant in vitamin A. Additionally, golden rice, a rice variety that is abundant in provitamin A, has been created and disseminated in regions of Africa where the incidence of vitamin A insufficiency is high [20].

Rice is not the only crop that has been designed to have a greater  $\beta$ -carotene concentration; other crops that have been modified include potato, canola, tomato, carrot, and cauliflower. In June of 2012, the Institute for Agricultural Research in Nigeria made available the first generation of provitamin A-rich orange open-pollinated maize varieties that were generated via the use of conventional breeding techniques. An investigation of the human bioavailability of transgenic provitamin A banana was initiated in the latter part of 2013, and trials have already begun in Uganda. It is anticipated that provitamin A bananas will be made available in the year 2019 [21].

When it comes to increasing the concentrations of micronutrients in crops, biofortification is a strategy that is both environmentally friendly and economical. In spite of the fact that traditional breeding has made great progress, genetic modification provides an approach that is both more effective and more environmentally friendly for enhancing the health and nutrition of crops [22].

There are approximately 2 billion individuals throughout the globe who suffer from iron deficiency anemia, making it the most prevalent form of micronutrient imbalance. Vegetables, cereals, and red meat are all good sources of iron; nevertheless, the bioavailability of iron in plants is rather low. Due to the fact that the baseline grain iron content of common beans is high, at 55 parts per million (mg/kg), and the trait has a significant deal of variability, biofortification of these beans is desirable. This allows for early breeding efforts to be more successful than they would be in cereals. In regions of Latin America, eastern and southern Africa, and other regions where the crop is significant and consumption is high, the areas that are targeted for biofortification of beans are those that are prone to iron deficiency anemia [23].

Because to the presence of phytate, which is a powerful inhibitor of iron resorption, and the absence of iron resorption-enhancing substances, the bioavailability of iron in rice is

very poor. This is made even worse by the food's composition. Scientists have been forced to raise the amount of iron that is present in grains, decrease the amount of phytate that is there, and add elements that enhance resorption. Within the endosperm of rice, the expression of the iron storage protein ferritin, which originates from French bean and soybean, leads to a threefold increase in the amount of iron found in the seeds. Additionally, an enzyme that breaks down phytate, which is known as phytase, has been included into rice in order to reduce the amount of phytate that is present [24]. Excessive production of a protein that is rich in cysteine and transports metals in rice has the potential to increase the pace at which iron is absorbed during digestion. The University of Melbourne and the International Rice Research Institute (IRRI) have collaborated to produce a transgenic high-iron rice variety. This variety features a white rice grain that has 14 parts per million (ppm) of iron and translocates iron to concentrate in the endosperm, where it is less likely to be bound by phytic acid and is hence more likely to be bioavailable [25].

Conventional breeding methods have not proven effective in increasing the amount of iron found in wheat, and there are presently no wheat varieties that have been biofortified with iron that are accessible to farmers. Utilizing NAS to raise iron concentrations in wheat and generate biofortified wheat types with 52 ppm iron in whole grain, the University of Melbourne in Australia has been applying a method that has been shown to be particularly efficient in rice development. Through the use of transgenic methods, the John Innes Centre in Norwich, United Kingdom, has been doing research on a number of different independent ways to boost the iron content and bioavailability in wheat grains [26].

At the Bangladesh Rice Research Institute, a breeding program is now in the process of developing a number of advanced breeding lines of zinc rice for both the Boro (irrigated) and T. Aman (rainfed) seasons. With a yield of 4.2 tons per hectare, the first zinc rice aman variety, known as 'BRRI dhan 62,' has a zinc content of 19 parts per million and a protein content of 9 percent. In 2014, it is anticipated that at least one zinc rice boro type with 22-24 ppm would be made available to the public. The first varieties are anticipated to be made available for commercial sale in India in the year 2015 [27].

#### **4. CHALLENGES OF CLIMATE CHANGE ON ENVIRONMENT AND CROP PRODUCTION**

As a result of the release of greenhouse gases including methane, carbon dioxide, and chlorofluorocarbons, the issue of climate change is becoming an increasingly pressing problem. Agriculture researchers are increasingly concentrating their attention on the influence that changes in the environment have on crop output. This is a topic that is receiving growing attention from national and international governments, non-governmental organizations, companies, and individual levels simultaneously. When the year 2000 rolled around, there were 1.8 billion people living in regions that were severely lacking in water. By the year 2025, the human population in these regions would have expanded to more than 2 billion [28].

Agricultural production has been directly impacted by climate change as a result of the direct relationship between crops and climate. There is a stronger impact on the growth and development of crops that comes from climatic circumstances such as the temperature of the air and the soil. Disasters that are caused by climate change, such as floods and droughts, have had a negative impact on agricultural output and the availability of food. The Gangotri glacier, which is one of the greatest glaciers in the Himalayas, is eroding on a yearly basis, which eventually leads to floods. Not only is the rise in the average annual air temperature the cause of climate change, but the rise in the concentration of greenhouse gases in the atmosphere is also a contributing factor. The rise in the average yearly temperature throughout the globe over the last century has been 0.74 degrees Celsius. climatic change has also increased the frequency of climatic events such as floods and droughts. This is another adverse effect of climate change. The Gangotri glacier is degrading at a rate of around 12-13 meters per year, which means that flooding is occurring [29].

The increase in sea-level rise (SLR) has an impact on both food production and food security since it may cause seawater to seep into agricultural fields and freshwater, which in turn causes land degradation and an inability to cultivate soil that is suitable for that purpose. It is possible that an SLR of 1.5 meters might flood around 16% of the land in Bangladesh that is suitable for rice cultivation, which would have an impact on rice output. An rise in global mean

temperatures of two degrees Celsius may cause disruptions to agricultural operations and crop production times. Standard crops are sensitive to changes in temperature and precipitation, and such variations can have a significant impact. Additionally, an increase in temperature may have an impact on the duration of agricultural production, the patterns of rainfall, the hydrological cycle, the selection of cultivars, as well as the quality and quantity of food crops [30].

The levels of groundwater and the temperature of the air are both being impacted by climate change, which is resulting in a drop in agricultural productivity. When combined with other effects of climate change, high amounts of carbon dioxide may have a negative influence on the development and nutritional status of plants. There is a considerable influence that climate change has on agricultural yields, with some species being subjected to the greatest amount of pressure while others are less impacted. For instance, it has been hypothesized that C4 plants are less likely to be adversely impacted, either favorably or negatively, by rising amounts of CO<sub>2</sub> in the atmosphere. Nevertheless, every single prediction model paints a picture of a significant amount of yield loss in every single staple food crop, which ultimately results in food insecurity, hunger, and poverty [31].

Climate change has the potential to have a large impact on wheat output, which is responsible for 21 percent of the world's food supply and is grown on 200 million hectares around the globe. Although there is a possibility that global warming would be beneficial in some locations due to the presence of ideal temperatures, it will

be detrimental to regions that always have perfect temperatures. High temperatures will result in a drop in seed production; thus, it is essential to produce wheat germplasm that is heat-tolerant in order to deal with the lack of yield that is caused by climate change. Additionally, climate change has resulted in desertification, which leads to the loss of arable land as a consequence of water shortages, rendering the area unfit for vegetable and crop growth. As a result of climate change, water is subject to significant changes, including alterations in the physical and chemical conditions that exist inside water bodies and modifications to the bioenvironment. These changes include a reduction in the number of marine species and a reallocation of their distribution and abundance. It is certain that crops would perish if there is insufficient water available for cultivation [32].

In 2003, the yield of maize in a number of European nations saw a considerable decline as a result of climate change. Additionally, the rise in global temperatures that is caused by climate change may result in an increase in the growth and development of weeds, which may lead to an increase in the use of herbicides at this time. Increasing temperatures make plants more vulnerable to the intense onslaught of insect pests and plant diseases. This is because plants are more responsive to heat. This is due to the fact that rising temperatures often provide more favorable circumstances for germs that cause diseases and pests, which may have a negative impact on the growth, quality, and quantity of crops. Farmers will be compelled to employ a greater quantity of synthetic insecticides and pesticides in the field as a consequence of this [33].

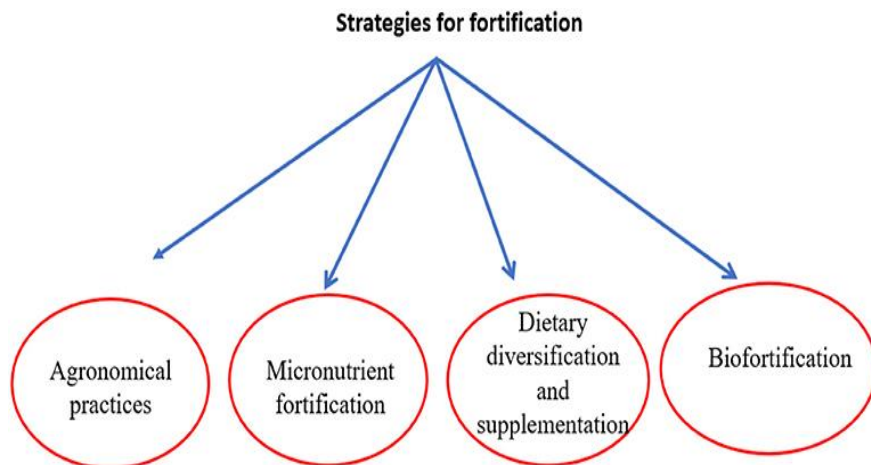


Fig. 4. Fortification strategies

Especially in developing nations, where the poor will suffer significantly because they are unable to pay premium rates for food, the dangers associated to climate change pose a threat to the output of agriculture across the globe and generate food insecurity for future generations. It is anticipated that climate change would result in higher temperatures around the globe and a shortage of water, which will have an impact on the absorption of nutrients and agricultural productivity. There are several metabolic activities that need iron, including photosynthesis and respiration. Iron is an important micronutrient that plays a role in many processes. As a consequence of the fact that deficiencies in iron mobilization may lead to significant yield losses in crops, iron homeostasis is an essential component for the normal growth and development of plants [34]. The process of iron absorption in plants is hampered by a high soil pH, which is also rich in iron. The absorption of iron by plants is decreased by high temperature, despite the fact that the pH is high.

Stress caused by high temperatures may also have an effect on the amount of nitrogen and phosphorus that plants take in. When wheat is subjected to heat stress, a rise in temperature leads to an increase in phosphorus absorption and translocation. On the other hand, when soybean plants are subjected to water stress, they exhibit an increase in root and shoot iron content. It is possible that variables such as a decrease in rhizosphere or nutrient absorption per unit root are to blame for low nutrient uptake experienced by plants as a result of high temperature stress [35]. One possible explanation for this drop in nutrient absorption per unit root is the loss of labile carbon, which refers to total non-structural carbohydrates, or it might be the result of direct root injury caused by high temperature stress. Drought is another kind of abiotic stress that has an impact on the nutritional quality of plants because it reduces the efficiency with which they absorb nutrients. The quantity of water that is present in the soil is related to the majority of the nutrients that are taken up by the roots from the soil. As a result of lower transpiration flux, active transport, and membrane permeability, drought slows down the pace at which nutrients in the soil are transported to the roots, as well as the rate at which roots take in nutrients and subsequently translocate those nutrients into the foliage of the plant. Depending on the type of plant, drought may have a variety of impacts on the absorption and translocation of zinc, iron, manganese, or

copper. When there is a drought, the amount of manganese and copper in the leaves rises, whereas the amount of iron in the leaves decreases [36].

The development of solutions to mitigate the negative impacts of abiotic stressors and altered nutritional homeostasis may be facilitated by a comprehensive knowledge of the processes of nutrient intake and translocation under a variety of abiotic environmental conditions. Boron shortage and leaf damage occurring in plants as a result of low temperature stress have been proven to be connected; however, the reasons underlying this association at the molecular, biochemical, and physiological stages are still unknown. There is some evidence to indicate that a low temperature in the rhizosphere makes it more difficult for the shoots to absorb B and reduces the efficiency with which they supply and utilize B. Boron intake enables plants to transfer sugar more effectively, which in turn improves seed germination and grain development for the plant. As a result, it is able to restore the yield by reducing the impact of the low temperature phenomenon [37].

A plant's iron homeostasis may be disrupted by the water in the soil. The ratio of Fe<sup>2+</sup> to Fe<sup>3+</sup> is larger in soils that are moist, which results in increased iron availability or absorption by the plants from the soil. Under drought circumstances, however, a greater presence of oxygen results in a decrease in the ratio of Fe<sup>2+</sup> to Fe<sup>3+</sup>, which in turn leads to a decrease in the amount of iron that is available to the plants. Furthermore, dryness decreases the amount of nitrogen and phosphate that plants are able to absorb [38].

## 5. BIOFORTIFIED CROPS RECENT DEVELOPMENTS AND EXAMPLES

- Transgenic methods offer a feasible option for the development of biofortified crops in situations when there is little to no genetic diversity in the nutritional content of different plant kinds. For the transfer and expression of desired genes from one plant species to another, they rely on the infinite genetic pool, regardless of the evolutionary and taxonomic standing of the plant species in question [39]. The simultaneous insertion of genes that are involved in the augmentation of micronutrient concentration and their bioavailability, as well as the decrease in the concentration of antinutrients that restrict the bioavailability of nutrients in plants, is something



that may be accomplished via the use of transgenic crops. The redistribution of micronutrients across tissues, the enhancement of the micronutrient concentration in the edible sections of commercial crops, the enhancement of the efficiency of biochemical processes in edible tissues, and even the reconstruction of specific pathways are all possible outcomes that may be anticipated with the application of genetic changes [40].

Transgenic crops that have increased levels of micronutrients have the potential to lower the prevalence of micronutrient deficiency among consumers, particularly among the economically disadvantaged population in developing nations. There have been several crops that have been genetically engineered in order to increase the amount of micronutrients that they contain. These micronutrients include vitamins, minerals, essential amino acids, and essential fatty acids. Some examples of successful transgenic technologies are high-lysine maize, soybeans with a high level of unsaturated fatty acids, cassava with a high level of provitamin A and iron, and golden rice with a high proportion of provitamin A. Cereals, legumes, vegetables, oilseeds, fruits, and fodder crops that have been biofortified are all included in the reports that are accessible [41].

- The worldwide problem of undernutrition, iron deficiency anemia, quality protein, seed oil quality, polyunsaturated fatty acid, flavonoids, overnutrition, and obesity has been the focus of efforts to develop transgenic rice as a solution to these problems. Rice has also been targeted for improving the quality of seed oil by raising the quantity of polyunsaturated fatty acid, which has the potential to assist in the reduction of levels of negatively correlated cholesterol in the body and to enhance human nutrition [42].

- The production of antisense waxy genes and antisense RNA suppression of starch-branching enzymes (SBE) has been used to increase the amount of less digestible and resistant amylose starch. This is being done in order to address the problem of overnutrition and obesity. Lactoferrin, a functional human milk protein, has been expressed in rice grains, which has opened the door to the potential of developing cereal-based components with additional value that may be included into infant formula and baby food [43].

- The use of transgenic methods provides a method of biofortification that is both cost-

effective and sustainable, especially in the setting of iron deficiency and undernutrition. Through the targeting of certain genes and the incorporation of micronutrients, these crops have the potential to provide a diet that is both more sustainable and more nutritious for the whole world's population [44].

- Transgenic wheat, maize, barley, sorghum, and soybeans are all examples of basic food crops that have been genetically modified in order to solve a variety of nutritional issues. Wheat has been improved by the expression of bacterial PSY and carotene desaturase genes, while maize has been enriched with provitamin A (carotenoids) and numerous carotenogenic genes. Both of these improvements have been made. A significant number of research organizations are putting an emphasis on the biofortification of these components in maize crop [45]. Vitamin E and its analogue are powerful antioxidants that have potentially beneficial effects on human health.

- The expression of bacterial crtB and numerous carotenogenic genes has resulted in an increase in the amount of provitamin A (carotenoids) found in maize endosperm. The operation of the cardiovascular system, the formation of immunological cells, and the consumption of iron are all affected by vitamin E and its analogue. The overexpression of homogentisic acid geranylgeranyl transferase (HGGT) and vitamin C (l-ascorbic acid) in maize has resulted in an increase in the amount of tocotrienol and tocopherol. These two enzymes are involved in the formation of immune cells, the function of the cardiovascular system, and the use of iron [46].

- Antinutrient components, such as the production of soybean ferritin and *Aspergillus* phytase, soybean ferritin, and *Aspergillus niger* phyA2, as well as the suppression of the expression of ATP-binding cassette transporter and multidrug resistance-associated protein, are known to impede the bioavailability of micronutrients. Mavrea™ YieldGard Maize, developed by Monsanto in Japan and Mexico, and Maverat™ Maize (LY038), developed by Renesse LLC in Australia, Columbia, Canada, Japan, Mexico, New Zealand, Taiwan, and the United States of America [47], are two examples of maize types that have been targeted with substantial success by biotechnology companies. Due to the fact that barley is a model cereal crop, efforts have been made to enhance the amount of micronutrients it contains. The overexpression

of zinc transporters has resulted in an increase in zinc content, while the expression of the phytase gene has led to a rise in phytase activity in barely seeds. Expression of the DHPS gene in barley has resulted in an increase in the amount of the essential amino acid lysine [48].

In order to enhance provitamin A (beta-carotene) levels, sorghum has been targeted for expression of Homo188-A (108). Sorghum is one of the most essential staple crops for millions of people living in rural areas who are poverty-stricken. The addition of a protein that is rich in lysine, known as HT12, has resulted in an increase in the amount of the important amino acid lysine that is present in sorghum. On the other hand, the grains of sorghum are less digestible than the grains of the other main staple crops, which is one of the problems with

consuming sorghum. Through the use of RNA interference (RNAi) to silence the  $\gamma$ -kafirin gene and the combined suppression of three genes, namely  $\gamma$ -kafirin-1,  $\gamma$ -kafirin-2, and  $\alpha$ -kafirin A1, the digestibility index of transgenic sorghum has been enhanced, as reported in reference [49].

• In order to address the nutritional problems that have been identified, transgenic beans and pulses have also been produced. By expressing the bacterial PSY gene, soybeans, which are a worldwide supply of vegetable oil and high-quality protein, have been targeted for the purpose of increasing the levels of provitamin A (beta-carotene), a monounsaturated  $\omega$ -9 fatty acid (oleic acid), and seed protein. It was shown that the expression of bacterial PSY [crtB, crtW, and bkt1 [50] increased the amount of provitamin A (canthaxanthin).

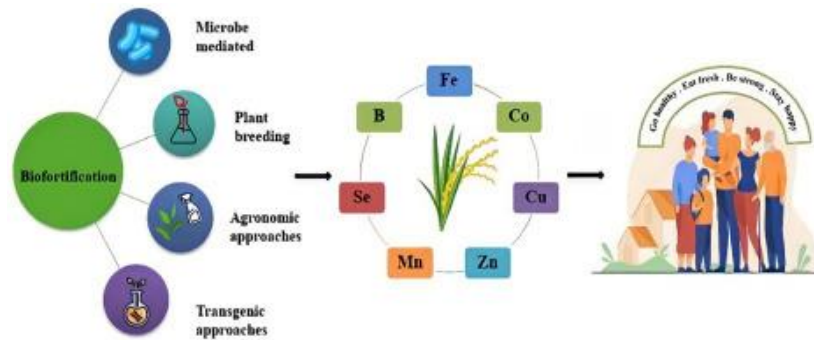


Fig. 5. Biofortification technique

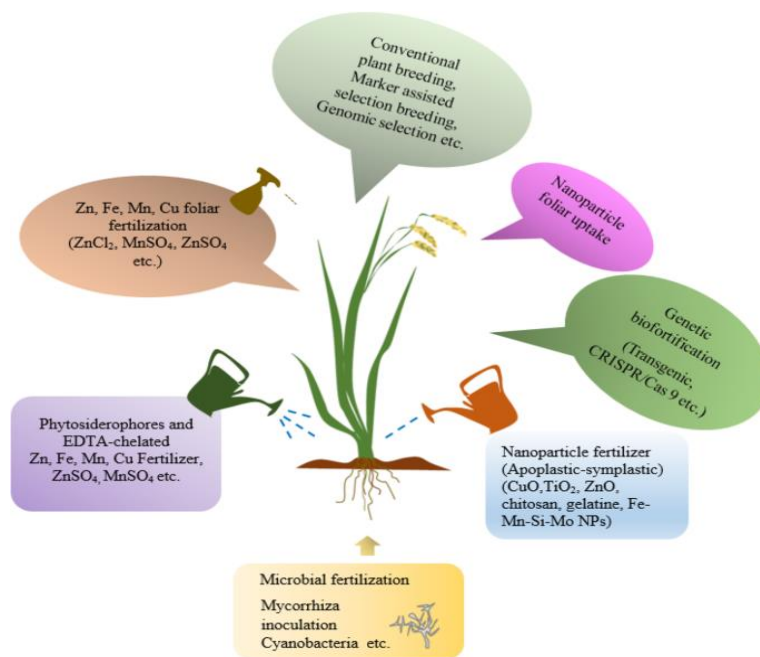


Fig. 6. Biofortified crops recent developments

- Although soybeans contain roughly forty percent protein, they are lacking in one or more of the important amino acids, particularly cysteine and methionine, which are sulfur-containing amino acids. Overexpression of the sulfur assimilatory enzyme, O-acetylserine sulfhydrylase, has resulted in an increase in the cysteine content of soybean seeds. Additionally, the expression of cystathionine  $\gamma$ -synthase has led to an increase in the methionine content of soybeans [51].

- Although soybeans, which are a grain that contain around twenty percent oil, are abundant in beneficial oil, they also include unstable fatty acids, which lead to a decrease in the quality of the oil. An strategy that is based on siRNA-mediated gene silencing has been applied in order to increase the agronomic value of soybean seed oil by decreasing the quantities of  $\alpha$ -linolenic acids (18:3). During a different experiment, the expression of the  $\Delta 6$ -desaturase gene, which is responsible for the conversion of linoleic acid and  $\alpha$ -linolenic acid to GLA and STA, resulted in an increase in the concentration of  $\gamma$ -linolenic acid (GLA) and  $\omega$ -3 fatty acids (STA) in soybean oil. Similar to the previous example, the expression of  $\Delta 6$  desaturase and  $\Delta 15$  desaturase simultaneously has been shown to enhance the amount of STA present [52].

The emergence of a significant number of cultivars that have enhanced oleic, linoleic, and STA content is a clear indication of the significance of enhancing the  $\omega$ -3 fatty acid content in soybeans. There have been releases of transgenic soybean varieties that are abundant in oleic acid in the following countries: Australia, Canada, Japan, New Zealand, and the United States of America; Treus™, Plenish™ (DP305423) in Australia, Canada, China, European Union, Japan, Mexico, New Zealand, Philippines, Singapore, South Africa, South Korea, Taiwan, and the United States of America; and Treus™ (DP 305423 x GTS 40-3-2) in Argentina, Canada, Europe, Japan, Mexico, Philippines, South Africa, South Korea, Taiwan, and the United States of America [53]. The transgenic Common Beans, also known as *Phaseolus vulgaris*, are considered to be among the most significant grain legumes that are consumed by humans. The fact that they contain only trace levels of the important amino acids cysteine and methionine, however, means that their nutritional value is restricted. It has been shown that the expression of methionine-rich storage albumin from Brazil nut contributed to an

increase in the methionine content of common bean [54].

- The most important grain legumes are transgenic lupines, which belong of the genus *Lupinus angustifolius*. The expression of the gene for sunflower seed albumin has resulted in an increase in the amount of methionine that they contain [55].

There has been considerable emphasis placed on transgenic vegetables, such as the potato (*Solanum tuberosum*), in order to improve their nutritional value. Provitamin A (carotenoid forms) have been raised in potato tuber by the integration of the PSY gene as well as the simultaneous insertion of three genes: PSY, phytoene desaturase, and lycopene  $\beta$ -cyclase. via the use of RNA interference (RNAi) to suppress the beta-carotene hydroxylase gene (bch), which is responsible for the conversion of beta-carotene to zeaxanthin, and via the control of beta-carotene synthesis through the expression of lycopene  $\beta$ -cyclase (StLCYb), the amount of beta-carotene found in tubers has been increased. Increasing the expression of zeaxanthin epoxidase genes in transgenic potato tuber has also been shown to increase the amount of zeaxanthin, which is another kind of carotenoid [55].

- The overexpression of strawberry GalUR has been identified as a potential method for increasing the amount of vitamin C (ascorbic acid) in potatoes. Potato tubers are characterized by a deficiency in the important amino acid methionine, which has been specifically targeted for improvement by the coexpression of cystathionine  $\gamma$ -synthase (CgS $\Delta 90$ ) and methionine-rich storage protein. Through the process of overexpressing the gene that encodes the seed storage protein from *Perilla* (PrLeg polypeptide) and the cystathionine  $\gamma$ -synthase (CgS) genes, the amount of methionine in the organism has been increased. Amaranth albumin (ama1) is expressed in transgenic potatoes, which leads to a rise in the overall protein content of the tubers. Additionally, there is a considerable increase in the concentration of numerous important amino acids, including methionine [56].

As a consequence of the expression of the cyclodextrin glycosyltransferases (CGT) gene, high-value carbohydrate-rich potato tubers have been created. This process leads to the creation of multifunctional dietary fiber cyclodextrins from

starch. It has also been attempted to enhance the amount of phenolic acid and anthocyanins found in potato tubers by the overexpression of a single gene or with the combined expression of CHS, chalcone isomerase (CHI), and dihydroflavonol reductase. According to BASF, transgenic potato types that have been altered for starch quality have been released. These varieties include Amflora™ (EH 92-527-1) in the European Union and Starch Potato (AM 04—1020) in the United States of America [57].

In addition to being possible sources of bioenergy and natural antioxidants, transgenic sweet potatoes, cassava, carrots, lettuce, cauliflower, linseed, canola, and mustard are also potential suppliers of these nutrients. While cassava is lacking in vital nutrients such as provitamin A, vitamin E, iron, and zinc, sweet potatoes are abundant in phytochemicals, anthocyanins, vitamin C, carbs, potassium, and dietary fiber. On the other hand, sweet potatoes are high in nutritional fiber. Cassava biofortification has been created in order to lessen the amount of deficiencies that are present in populations that are undernourished [58,59].

- Carrots are the most often consumed vegetables because they have a large amount of beta-carotene, vitamins, and minerals, although they have a relatively low calcium content. Through the expression of the Arabidopsis H<sup>+</sup>/Ca<sup>2+</sup> transporter, transgenic carrots have been enhanced in terms of their iron content, yield, and growth rate. Through the expression of a soybean ferritin gene, lettuce has shown improvements in terms of its iron content, yield, and growth rate [60].

The beta-carotene concentration of mutant orange cauliflower has been increased, which has resulted in an increase in the food's popularity. Cauliflower is a popular vegetable that contains antioxidant phytonutrients. There is a great need for linseed edible oil as a nutritional supplement; yet, it is particularly vulnerable to auto-oxidation, which results in the production of detrimental derivatives. Suppressing the CHS gene, which causes hydrolyzable tannin buildup, has resulted in the generation of genetically engineered flax plants that have a higher antioxidant capacity, steady oil output, and healthy oil production. Through the use of seed-specific production of cDNAs that encode fatty acyl-desaturases and elongases in linseed, researchers have aimed to increase the

accumulation of  $\Delta 6$  desaturated C18 fatty acids and C20 polyunsaturated fatty acids [61].

For millions of people all over the globe, canola is an essential crop for the production of oilseeds. Through the process of overexpressing bacterial PSY, the carotenoid concentration of the substance, mostly consisting of alpha and beta-carotenes, has been raised in order to further boost its health advantages. Through the simultaneous expression of PSY, phytoene desaturase, and lycopene cyclase genes, as well as the simultaneous expression of seven bacterial genes, namely *idi*, *crtE*, *crtB*, *crtI*, *crtY*, *crtW*, and *crtZ*, it has been possible to produce a higher  $\beta$ -carotenoid content [62].

As a general rule, canola does not possess any  $\Delta 6$  desaturase activity, and hence, it does not possess GLA. Genes that express  $\Delta 12$  or  $\Delta 6$  desaturases have been used to establish transgenic lines that are abundant in GLA. This has been done in order to make the production of GLA more economically feasible and to make it more easily accessible. The food inhibitor known as phytic acid chelates micronutrients and limits their bioavailability. Phytic acid is also known as a food inhibitor. Phytase™ Canola (MPS 961-965) is one of the transgenic canola varieties that have been generated and released by BASF in the United States of America [63]. These kinds have been altered for phytase breakdown in order to increase the amount of phosphorus that is available in canola.

Throughout the globe, mustard is a crop that is widely farmed for oil production and is considered to be economically important. Because of the expression of the enzyme  $\Delta 6$  FAD3, which resulted in the formation of gamma linoleic acid in the transgenic mustard, it has been targeted for the purpose of enhancing the nutritionally significant unsaturated fatty acids.

The transgenic tomato, also known as *Solanum lycopersicum*, is a well-liked fruit that is rich in phytonutrients, micronutrients, and vitamins that are necessary to human health. Isoprenoid lycopene, which is responsible for its color, is an important component in membrane construction, the elimination of free radicals, redox chemistry, defense mechanisms, and the control of development. Increasing the amount of isoprenoid content in tomato has been attempted on several occasions, including the expression of sterol, the content of phytoene and beta-

carotene, and the expression of the PSY gene, which results in the production of beta-carotene molecule [64].

- Apple has also been bioengineered with a stilbene synthase gene from the grapevine, which has resulted in the production of resveratrol in transgenic apple, which has increased the apple's potential to act as an antioxidant. By expressing the PSY gene of the Asupina banana, which is naturally rich in beta-carotene, a transgenic banana known as the Super Banana has been generated [65]. In many countries, transgenic alfalfa, also known as *Medicago sativa*, is a significant crop for the production of feed legumes. There have been efforts made to increase its digestibility and nutritional quality by increasing the amount of important amino acids and isoflavonoids that it contains. The subclass of flavonoid secondary metabolites known as isoflavonoids is mostly comprised of legume-specific compounds. Constitutively expressing IFS has resulted in the generation of transgenic alfalfa, which has been shown to have a correlation with the increased isoflavonoid composition of the plant [66].

- The administration of nutrients by physical means is necessary for biofortification using agronomic methods. This is done in order to temporarily enhance the nutritional and health condition of crops as well as the consumption of such foods. The use of organic minerals such as nitrogen, phosphorus, and potassium (NPK) is a significant factor that contributes to the achievement of increased crop yields. As the 1960s came to a close, agricultural output grew in a number of nations, which ultimately resulted in the Green Revolution and prevented those countries from starving to death [67].

- Minerals such as iron, zinc, copper, manganese, I, Se, Mo, Co, and Ni are typically absorbed from the soil and may be found in variable degrees in the edible part of some plants. These minerals are found in the edible component of certain plants. Enhancing the micronutrient status of soil via the use of these substances as fertilizers has the potential to contribute to a reduction in the prevalence of micronutrient deficiencies in human beings. For the purpose of cultivating crops in soils in which mineral elements become instantly unavailable in the soil and/or are not rapidly translocated to edible tissues, it is common practice to apply soluble inorganic fertilizers to the roots or leaves of the plant [68].

However, agronomic biofortification requires specific care in terms of the source of the nutrients, the application technique, and the consequences on the environment. Although it is easy and affordable, it requires special attention. Because they need to be sprayed on a consistent basis throughout each and every crop season, they are, in certain instances, less cost-effective. The successful use of mineral fertilizers in the industrialized world is shown by the fact that the use of selenium fertilization on crops in Finland, zinc fertilization in Turkey, and iodine fertilization in irrigation water in China [69] are all examples of successful applications of mineral fertilizers.

Enhancing the movement of nutrients from the soil to the edible sections of plants and improving their nutritional status may be accomplished via the use of soil microorganisms that promote plant development. The phytoavailability of mineral elements may also be increased by the use of soil microbes such as *Bacillus*, *Pseudomonas*, *Rhizobium*, and *Azotobacter*. Increasing crop yield under nitrogen-deficient circumstances is largely dependent on the presence of microorganisms that fix nitrogen dioxide (N<sub>2</sub>). Mycorrhizal fungi are connected with a wide variety of crops. These fungi have the ability to emit organic acids, siderophores, and enzymes that are capable of decomposing organic compounds and raising mineral concentrations in edible product [70].

The goal of agronomical biofortification is to enhance the nutritional condition of humans, and cereals have been targeted for this purpose. Using foliar sprays of iron, rice has been successfully biofortified in order to increase the amount of iron that is present in the grains of rice. Rice grain zinc concentration and zinc bioavailability have both been shown to be improved by the use of foliar zinc treatment, which has been described as a successful agronomic approach. The use of selenate as a foliar spray or as a fertilizer in rice has resulted in an increase in the amount of selenium, which is a trace element that is an important component for human health and a powerful antioxidant [71]. The quality of wheat grain has been significantly improved by the effective use of wheat. The presence of iron in foliar urea fertilizers has been shown to have a favorable correlation with the buildup of much more iron. The application of foliar zinc has brought to a reduction in human zinc insufficiency in areas that have soil that may be zinc-deficient. Additionally, the bioavailability of zinc has been increased via the reduction of



antinutrient factors such as phytic acid. Within a span of ten to fifteen years, the total quantity of zinc-containing NPK fertilizers in Turkey reached a record level of 400,000 t per year, having climbed from zero in the year 1994 [72].

- In order to acquire grain that is rich in nutrients and to achieve the highest possible yield in maize, maize is necessary. The maize crop has been subjected to a number of different zinc fertilizer treatments and foliar sprays. Rhizobacteria that promote plant development have been shown to result in the enrichment of nutrients in plants. These rhizobacteria have also been included into agronomic procedures in order to produce efficient biofortification solutions for staple crops. The maize crop, which has a higher zinc content, is an example of an effective example [73].

A number of different organic and inorganic biofertilizers have been used to barley, which has resulted in its improvement. The use of biofertilizers, in addition to inorganic fertilizers and vermicompost, has resulted in an increase in the content of zinc and iron in grains.

Sorghum is grown all over the globe for the purpose of producing grain and fodder, but it often faces the issue of growing on soil that is deficient in nutrients and is polluted. Its nutritional profile has been improved as a result of the use of fertilizers, both organic and inorganic, which have an impact that is additive to the yield. By using a mix of plant growth-promoting bacteria and arbuscular mycorrhizal fungus (AMF), researchers have the intention of enhancing the nutrient intake of sorghum and enhancing the metabolic profile of the plant [74].

- Zinc biofortification has been addressed for legumes, along with the production of selenium-enriched soybeans by the foliar application of selenium complex salts as fertilizers. Through the use of plant growth-promoting actinobacteria, chickpea has been targeted for the treatment of mineral deficiencies, particularly deficits in the minerals iron, zinc, calcium, copper, manganese, and magnesium. Field peas are the second most important legume crop in the world, and it has been shown that foliar zinc treatments, either on their own or in conjunction with soil zinc applications, may enrich the crop with zinc [75].

- Se (Selenium) augmentation has been targeted for oilseeds, with rhizosphere bacteria from a seleniferous location boosting plant absorption of

Se as selenate. This has been accomplished by targeting oilseeds. Additionally, vegetables have been the focus of biofortification efforts; field trials have shown that there is a large rise in zinc concentrations in potato tubers and other vegetables. Both carrots and lettuce have been used for the purpose of agronomic biofortification. This practice involves the addition of iron and selenium as nutrients to the leaves and store roots of the plants. The consumption of one hundred grams of carrots that have been fertilized with iron and selenium may offer one hundred percent of the daily necessary amount. The biofortification of lettuce with iodine and selenium has been accomplished by the use of KIO<sub>3</sub> and Na<sub>2</sub>SeO<sub>4</sub> as foliar spray and nutrient medium, respectively. The tomato is a good crop for iodine biofortification programs [76], especially when it is treated with iron fertilizers beforehand.

Conventional breeding is the most widely acknowledged way of biofortification. It provides an option that is both sustainable and cost-effective in comparison to agronomic and transgenic-based methods respectively. By using this variety, breeding techniques have the potential to enhance the quantities of minerals and vitamins that are present in crops. On the other hand, breeding tactics often depend on the limited genetic variety that is available in the gene pool because of this. Under some circumstances, it is possible to circumvent this restriction by crossing to distant relatives and gradually introducing the trait into commercial cultivars, or by introducing novel characteristics directly into commercial varieties by the process of mutagenesis [77].

For the purpose of enhancing the nutritional value of crops via breeding initiatives, a number of international organizations have launched projects. The objective of the Health grain Project, which was carried out in the European Union between the years 2005 and 2010, was to create cereal meals and components that were both safe and beneficial to one's health. With the help of the International Center for Tropical Agriculture (CIAT) and the International Food Policy Research Institute, the Center for Global Initiatives for Agricultural Research (CGIAR) initiated the HarvestPlus initiative with the objective of breeding biofortified staple food crops throughout Asia and Africa. In order to improve the micronutrient status of target populations, the initiative intends to create staple food crops that have increased amounts of

bioavailable critical minerals and vitamins. This will have a demonstrable influence on improving the micronutrient status of the target populations, who are resources-poor people in developing countries [78].

Due to the fact that rice is one of the most widely eaten staple food crops and that its biofortification has the potential to have a large impact on nutritional issues, there is a strong emphasis placed on rice as a means of enhancing micronutrients. A number of older rice varieties that have a high amount of iron and zinc in their grain have been screened and paired with enhanced agronomic features via the use of certain breeding techniques. HarvestPlus was responsible for developing the world's first zinc-enriched rice cultivars, which were made available to the public in 2013 by the Bangladesh Rice Research Institute [79].

The first and most important objective for biofortification is to focus on wheat since it is a staple crop. It has been noted that wheat and its closely related wild species exhibit a wide variety in grain iron and zinc contents. Utilizing this diversity for the purpose of improving current elite cultivars is something that may be done. There are various wheat types that have been introduced by HarvestPlus that have a zinc level that is 4-10 ppm greater. YPC, or yellow pigment content, is an essential quality feature and antioxidant that is found in durum wheat. In comparison to the older varieties that were issued prior to the 1970s, a considerable proportion of the more recent durum wheat varieties that have been produced in various nations over the course of the last decade have shown a much higher YPC [80].

- The colored wheat trait, which includes black, blue, and purple varieties, has been used in a number of breeding efforts across a variety of nations. It has been stated that the black-grained wheat cultivar that has been launched in China is said to have a high protein content and selenium content. This comes after more than twenty years of persistent work in breeding. In the year 2006, the purple wheat cultivar Indigo was made available for cultivation in Austria, while in the year 2014, the purple wheat cultivar PS Karkulka was recorded as being registered in Slovakia. The patent on functional meals made from colored wheat in China [81] provides evidence that may be used to determine the significance of colored wheat product.

- Corn is a cash crop that is farmed for the purpose of providing animal feed, industrial manufacturing, and human consumption. In order to tackle vitamin A deficiency, breeding programs have been developed to produce high-yielding varieties of biofortified maize that contain greater quantities of provitamin A. These efforts have been made possible by the immense genetic variety of maize. In order to tackle vitamin A deficiency, HarvestPlus is utilizing these lines to produce high-yielding varieties of orange maize that have been biofortified against the disease. Beginning in 2013, commercial cultivation of biofortified orange maize cultivars has been carried out in the countries of Zambia, Nigeria, Ghana, Malawi, Zimbabwe, and Tanzania [82].

- Breeders have also generated quality protein maize (QPM) by integrating the opaque-2 (o2) mutant gene from naturally existing maize into maize cultivars. This mutant gene was derived from naturally occurring maize. The International Maize and Wheat Improvement Center (CIMMYT) has distributed hybrid cultivars in the following countries: India, China, Vietnam, Mexico, South Africa, Ghana, Guinea, Uganda, Benin, Mozambique, Brazil, Venezuela, Peru, Colombia, Honduras, El Salvador, Guatemala, and Nicaragua [83].

- Sorghum breeding places an emphasis on producing sorghums that are rich in beta-carotene and minerals. Various types of sorghum have been examined to see whether or not they contain high levels of minerals, protein, lutein, zeaxanthin, and beta-carotene. ICRISAT has taken the initiative to breed biofortified iron-rich sorghum lines and hybrids, which have then been distributed across India. New sorghum varieties that are rich in iron have been produced in Nigeria, and they are called 12KNICSV-22 and 12KNICSV-188, respectively. These varieties have the potential to improve the nutritional status of impoverished people, particularly children in Nigeria [84].

There is a significant amount of variety in the germplasm of pearl millet for the micronutrients iron and zinc, and pearl millet is the most cost-effective source of these minerals. The biofortified pearl millet variety known as "Dhanashakti" and a hybrid known as ICMH 1201 (Shakti-1201) were both introduced by ICRISAT, HarvestPlus in the year 2014. [85] There have been reports of a number of commercial varieties that have been well-adapted, as well as their offspring and hybrids,

which have a high concentration of iron and zinc in their grain.

- Lentil breeding, which has been led by ICARDA and HarvestPlus for the purpose of biofortification of iron and zinc via the use of genetic variety preserved in gene banks, is included in the category of legumes and pulses. Combining efforts may result in the development of lentil types that contain greater levels of iron, zinc, and protein. Through the use of breeding techniques, cow pea, which is sometimes referred to as "poor man meat" and has a high amount of protein, has been biofortified to increase its iron level [86].

The iron concentration of the common bean (*P. vulgaris*) might be boosted by sixty to eighty percent, according to research on bean breeding. On the other hand, the zinc level would be much lower, perhaps somewhere around fifty percent. HarvestPlus is working in this area and marketing beans that have been biofortified with iron in a number of underdeveloped nations. They have introduced ten types of common beans that have been biofortified with iron in Rwanda, and ten varieties of iron beans that have been biofortified in the Democratic Republic of the Congo.

- The breeding of potatoes is an essential component of human nutrition. The natural variety of farmed potato germplasm that contains red and purple pigments may be a possible representation of the contribution that potatoes provide to antioxidants. Breeders are concentrating their efforts on developing such varieties in order to raise the quantities of iron and zinc in human diets. As a source of antioxidants and minerals (copper, iron, manganese, and zinc), a genetically varied sample of potato cultivars native to the Andes of South America has been acquired from a collection of nearly one thousand genotypes and analyzed [87]. This sample was taken from the collection of Nearly One Thousand genotypes.

- Sweet potato breeding is another area of concern, since developing nations are responsible for cultivating 95% of the world's sweet potato crop. Malnutrition is the most significant issue in these countries of the globe. HarvestPlus and the International Potato Centre (CIP) have collaborated to create and distribute a number of orange sweet potato cultivars that are rich in vitamin A. The Zambia Agriculture Research Institute has successfully finished the

creation of 15 new varieties of vitamin A enriched sweet potatoes [88]. In Uganda, six types have been released, while in Zambia, three kinds have been released.

In addition, the breeding of cauliflower has been examined for the presence of genetic variation in zinc content, and it has been shown that there is significant natural variation. Provitamin A (beta-carotene) rich orange colored cauliflower variety (Pusa BetaKesari; 800–1,000 µg/100g) has been produced by the Indian Agricultural Research Institute (IARI). Additionally, Pink Graffiti and Orange Cheddar, two coloured cauliflower cultivars, have been created by Cornell University in the United States of America [89].

Cassava breeding is a primary vegetable root crop in underdeveloped nations, particularly in Africa, Latin America, and the Caribbean. Cassava is a product of the cassava plant. In Nigeria, six kinds that have been fortified with vitamin A have been issued by HarvestPlus in partnership with the International Institute of Tropical Agriculture. In the Democratic Republic of the Congo, one of these types has also been released. Additionally, there is a broad range of genotype variances for total carotene, proteins, and minerals (iron and zinc) in cassava, which has led to the production of cassava crops with increased nutritional value [90].

- Another area of interest is tomato breeding, which involves the careful investigation of genetically varied wild populations of tomatoes for particular features and the use of these populations in breeding. Anthocyanin biofortified tomato "Sun Black" has been created using a traditional breeding strategy [91]. This tomato has a deep purple fruit color owing to the high anthocyanin concentration in the peel.

On account of the fact that commercial banana types are sterile triploids and that there is a significant degree of cross incompatibility across fertile groups, banana breeding is a challenging and costly endeavor. In the Democratic Republic of the Congo (DRC) and Burundi, Biodiversity International (BI) and HarvestPlus have conducted a large-scale screening of different banana germplasms in order to identify high amounts of provitamin A [92]. This screening was carried out in conjunction with HarvestPlus.

It is true that mango breeding provides a natural supply of beta-carotene, vitamin C, and other beneficial antioxidants; however, the quantities of

these nutrients vary according on the type of mango. Both vitamin C (ascorbic acid) and beta-carotene were found in the greatest concentrations in the Ataulfo variety, which was farmed in Mexico. In India, the Indian Agricultural Research Institute (IARI) created a large number of cultivars that had improved nutritional and agronomically significant characteristics [93-96].

## 6. ECONOMICS FOR BIOFORTIFICATION

Two different lines of inquiry were carried out in order to evaluate the cost-effectiveness of biofortification initiatives in terms of enhancing health outcomes and drawing comparisons between these interventions and alternative investments. During the early stages of the discovery phase, the first one was formed by making educated assumptions about the costs of intervention and the potential benefits of intervention. Increased consumption of micronutrients including iron, zinc, and vitamin A was the focus of the second study, which was designed to determine the extent to which biofortified staple crops contribute to improved health. In order to determine the number of years that have been lost as a result of the burden of illness, the Disability-adjusted life years (DALYs) method was established. This method takes into account the severity and length of bad health

consequences. A DALY tool was built for each micronutrient, which made it possible to compare the cost per DALY saved by biofortification therapies with the cost per DALY saved by other micronutrient interventions. The tool evaluates the cost-effectiveness of the biofortification intervention and measures the decrease in the prevalence of micronutrient deficiencies among the target groups. Additionally, it allows for comparison with other micronutrient therapies [97].

According to the criteria established by the World Bank, ex ante evaluations have shown that biofortification is a very cost-effective method for eliminating deficiencies in micronutrients among populations. In addition, the Copenhagen Consensus discovered that for every dollar spent in biofortification, there was a seventeen dollar return on investment. Different therapies that address micronutrient deficiencies, such as supplementation and fortification, are more expensive than biofortification, which is a more cost-effective alternative. An ex ante research found that nations in Asia and Africa may profit the most from biofortification, particularly for the biofortification of crops with minerals and vitamin A [98]. As a result, HarvestPlus has concentrated its investments and efforts on the areas of South Asia and Sub-Saharan Africa.



Fig. 7. Biofortified crops available in market

To assess low- and middle-income countries (LMICs) according to their potential for effect, the biofortification priority index (BPI) was established in 2013. The BPI makes use of data collected at the national level and computes the geometric mean of three sub-indices. These sub-indices include production and consumption indices for biofortifiable staples, as well as a micronutrient deficiency index for the micronutrient that the crop may be biofortified with. Crop breeders, agricultural, nutrition, and health departments of international financial institutions, national decision makers, humanitarian organizations, international non-governmental organizations, and commercial seed businesses have all used the tool in order to make investment choices about biofortification [99].

It is well recognized that the BPI tool is useful for informing investment choices relevant to biofortification. It is also renowned for assisting crop breeders, agricultural departments, non-governmental organizations, and commercial seed businesses in designing their investments. The World Food Programme (WFP) is able to get a better understanding of which nations would benefit the most from biofortification at the population level, particularly for vulnerable populations such as women and children, by using the population-weighted version of the BPI [100].

## 7. CONCLUSION

The agricultural practice of biofortification has been acknowledged for a considerable amount of time as a realistic and economically viable way for meeting the nutritional requirements of undernourished people on a worldwide scale. In order to address the issue of mineral insufficiency in people, biofortification techniques may be used. These techniques may involve crop breeding, targeted genetic alteration, and/or the application of mineral fertilizers. Micronutrients such as iron, zinc, selenium, and provitamin A are often absent from meals throughout the developed and developing nations. However, because to the introduction of biofortified food crops, individuals in these areas are now able to ensure that they are receiving the nutrients that they need. One of the only ways that these objectives may be accomplished is with the assistance of national and international initiatives such as the HarvestPlus program. It is possible that the mineral content and bioavailability of some of the most important

cereal crops farmed for human consumption, such as wheat, maize, cassava, beans, sweet potatoes, and millets, have been enhanced as a consequence of these efforts. The process of biofortifying crops, on the other hand, is not without its challenges. In order to accomplish this goal, it is essential for individuals with expertise in molecular biology, genetic engineering, nutrition, and plant breeding to collaborate. Traditional breeding practices have been effectively applied to boost the nutritional content of meals, and they are swiftly gaining popularity as a result of this success. Breeding-based procedures have a far better track record of success than transgenic technologies, which are now receiving a lot of attention. This is due to the fact that transgenically improved agricultural plants face challenges such as consumer acceptability concerns and the many regulatory clearance processes that are both expensive and time-consuming, which have been enacted by various nations. In spite of these challenges, biofortified crops are the wave of the future. They have the potential to eliminate micronutrient deficiency in developing countries and improve the lives of billions of people who are living in poverty.

## 8. LIMITATIONS OF BIOFORTIFICATION

Biofortification is a technique that seeks to enhance the nutritional value of crops by including micronutrients into their production. The effectiveness of this method, on the other hand, is very varied owing to variances in mineral mobility, mineral accumulation across plant species, and soil compositions in particular geographical regions. Because it requires continual inputs in the form of the delivery of micronutrients to the soil or plant on a regular basis, agronomic biofortification is known to be less cost-effective and labor-intensive than other methods. There are occasions when it is not feasible to focus the micronutrient into edible plant components such as seeds or fruits, which may occasionally result in the accumulation of the targeted nutrients in leaves or other sections of the plant that are not edible [94].

Breeding techniques that are considered conventional have been shown to be successful, as well as sustainable and cost-effective over the long term. However, there are constraints regarding the amount of genetic variety for the micronutrients that are present in the plant gene pool, as well as the length of time that is required to develop cultivars that possess the trait(s) that



are most desired. Crossing to distant cousins and so introducing features into commercial cultivars is one method that may be used to circumvent this issue in certain circumstances. However, in many cases, it would be difficult to breed for a particular feature using traditional methods, and the amount of time and effort that would be required may be rather impractical.

Despite the fact that transgenic technologies are able to overcome the constraint of limited genetic variety among plants, they are not widely accepted by the general public. For the purpose of enhancing the overall nutritional health of a particular community, it is essential that the biofortified crops be easily adopted by farmers and communities in sufficient quantities to achieve the desired results. There are a variety of regulatory procedures that have been implemented by various nations in order to facilitate the adoption and marketing of transgenic crops. These procedures are both time-consuming and costly. In India, for instance, the Golden Rice, which was produced by Mahyco, was not made available to the public because of concerns voiced by scientists, farmers, and activists against genetically modified organisms [95].

In addition, the postharvest processing of each crop in order to maximize the effectiveness of biofortification procedures is another restriction. It is possible for milling or polishing cereal seeds to remove significant amounts of minerals from the diet. The degree to which these losses occur is determined on the genotype of the individual. In addition, the presence of certain antinutrients in fruits and vegetables decreases the bioavailability of particular nutrients that are included in such foods. For the purpose of enhancing food production in the context of global environmental change, it is necessary to enhance the capacity of a crop to retain yields despite a decrease in the quantity and quality of water available. When it comes to managing the quantity of a mineral element that is absorbed by roots, transferred to shoots, remobilized from vegetative tissues, and deposited in edible regions of seeds and grains in forms that are utilizable by those who consume the crop, a large number of genes are involved [96].

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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