



Bio fortification: Biotechnological tool for crop improvement to fight back hunger

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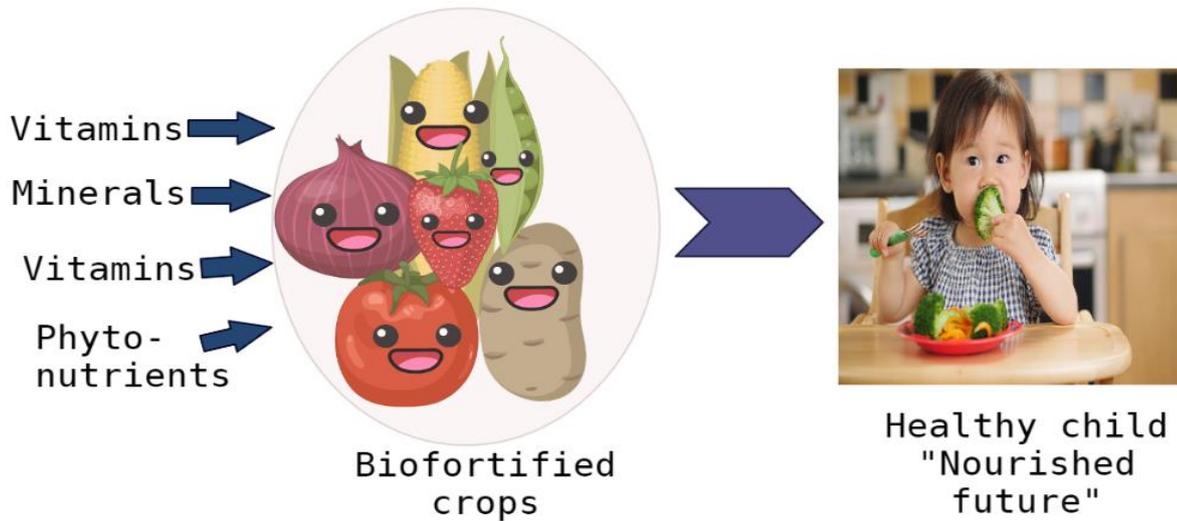
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Abstract

Under nutrition is a difficult undertaking, especially in Africa and Asia, where it affects hundreds of millions of pregnant women and school-aged children. As a result of their use of carbohydrate-rich yet dietary plant-based meals, impoverished folks are far more vulnerable to nutrient problems and mental deprivation. The availability of genomic sequences across multiple crops, and also advances in genetic manipulation tools, has created an opportunity to propagate for nearly any desirable characteristic. Bio-fortification has lately emerged as a self-targeting method as well as a non-recurring reaction to dietary deficits. Most traditional breeding procedures were restricted due to a lack of genetic variation in traversable genetic traits. Furthermore, it appears to lack the reduction of expression of genes that is required for nutritional retention. Bio-fortification genetically based is a viable strategy to tackle hidden shortages of food, especially in areas where propagation isn't really successful due to a shortage of phenotypic variation. When compared to other tactics, genetic manipulation is indeed a clear and precise way of developing improved nutritional cultivars without repeatedly engaging. This review emphasizes plant biotechnologists' alternatives for improving crop utilising genome engineering methods that are based on bio-fortification, which could also help to address global issues.

Keywords: Poverty, Food security, Gene editing, bio-fortification, gene expression

Graphical Abstract



1. Introduction

Feeding the world's ever-growing population, which is predicted to reach billion dollars by 2050, is one of the most daunting challenges humanity will confront in the twenty-first century (Van *et al.*, 2017). Plants provide the majority of the nutrients in the human diet; however all of our major food crops are deficient in certain essential micronutrients (vitamins and minerals). The vast majority of the world's population relies on plant-based meals, which are frequently deficient in important micronutrients (Waters and Grusak, 2008). Micronutrients, iron (Fe), zinc (Zn), selenium (Se), iodine (I), carotenoids, and folates are critical nutrients for developmental psychology because they assist to a variety of metabolic functions (Zhu *et al.*, 2007). Nutritional deficiencies can cause major diseases such as growth retardation, cognitive disabilities, prenatal difficulties, and a higher likelihood of incidence and death; they can also exacerbate infectious and non - communicable diseases, reducing standard of living substantially (Tulchinsky, 2010; Bailey *et al.*, 2015).

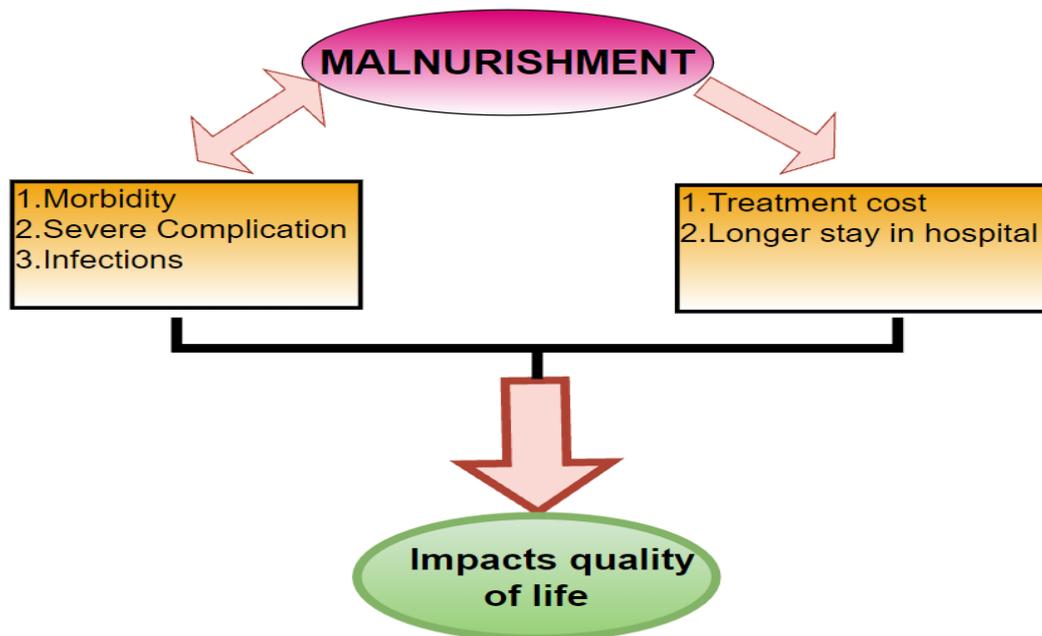


Figure 1: Malnutrition causes and associated with healthcare

For the first time in 25 years, chronic malnourishment fell from over 1 billion to less than 800 million in 2015. Although caloric starvation (acute hunger) has declined, micronutrient deficiencies, or "hidden hunger" caused by the lack of minerals and vitamins, continues to impact two billion people and is thus the more prevalent serious health problem (Van *et al.*, 2017). Hidden hunger, also known as "Micronutrient malnutrition," is a pervasive issue in Asian, African, and Latin American countries, impacting over half of the worldwide people (FAO, 2013). Fe, Zn, folic acid, and β -carotene deficiencies are global concerns (Tulchinsky, 2010, Darnton-Hill *et al.*, 2006). Pregnant women' micronutrient insufficiency and malnutrition have an impact, potentially leading to intrauterine growth retardation, premature birth, protein-energy starvation, and severe energy shortfall (Ahmed *et al.*, 2012). Four common micronutrients, Fe, I, Zn, and vitamin A, are responsible for around 12% of all fatalities in people under the age of 5 nationwide (Ahmed *et al.*, 2012). Nutrient-rich staple crops may treat micronutrients shortages and thus give a long-term solution to global health issues (Welch, 2002). Iron deficiency (ID) causes severe anaemia, which is estimated to afflict 2 billion people worldwide (WHO 2009). In grownups, IDA causes weariness, lethargy, and restlessness; however, long-term IDA in infants has far-reaching implications, including decreased mental development and predisposition to infections. Because of the frequent loss of blood, IDA is more common in women of childbearing age. Worm infections, malaria, and other infectious disorders such as HIV and tuberculosis aggravate IDA. In developing countries, approximately 250 million pre-school children and pregnant women in the world are facing Vitamin A deficiency (VAD) ; major cause of neonatal myopia and raises the risk of sickness & mortality following serious illnesses (Greenwald and McDonald 2001;WHO 2011).

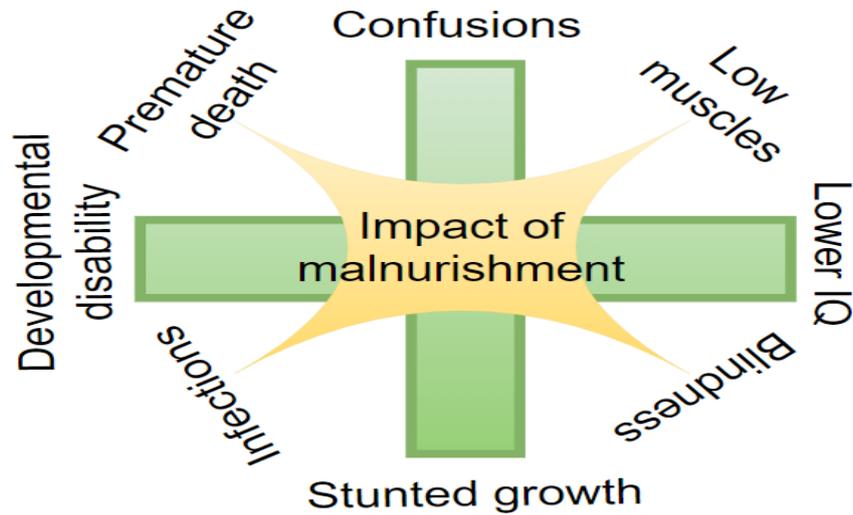


Figure 2: Impact of Malnutrition

Peas, chickpeas, lentils, common beans, and mung beans are the most prevalent pulse crops that are significant in dietary proteins, complex carbohydrates, vitamins, and minerals and are used in traditional diets in many regions of the world (Messina *et al.*, 1996; Duranti, 2006; Patterson *et al.*, 2009; Roy *et al.*, 2010; Diapari *et al.*, 2014; Ray *et al.*, 2014; Diapari *et al.*, 2015; Jha *et al.*, 2015; Jha *et al.*, 2015). They are readily available to all demographics on a routine basis and provide cheapest priced form of protein and minerals (WHO 1992; Bouis *et al.*, 2000; World Bank 2008).

2. Solving the micronutrient malnutrition problem

Because micronutrient deficiencies are a persistent and major public health issue all over the world, several strategies have indeed been established to address issue. In certain cases, intervention programs such as supplement, fortified foods, and dietary diversification have indeed been helpful in lowering hunger.

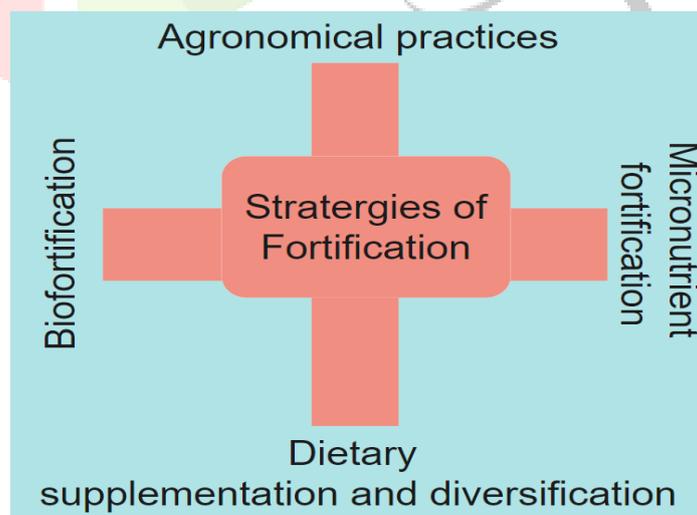


Figure 3: Approaches of fortification

2.1 Micronutrient Fortification

Micronutrient fortification is the practise of incorporating one or more micronutrients into commonly consumed items such as dairy, oils, cereals, and sauces. Dietary staples including wheat flour and edible oil, as well as food consumed by a specific demographic, such as supplementary diets for migrated individuals and young ones, can be fortified (Mass and Targeted fortification). The government usually designs, mandates, instigates, and regulates this sort of fortifications. It's really a good technique to deal with vitamin shortages in such a wider population. Customers must always be provided with information on fortification via the label (Dary and Hainsworth, 2008).

2.2 Agronomic Practices

Agriculture is the primary source of nutrients for humans, particularly for the population of poor countries (Graham *et al.*, 2001; Schneeman, 2001). Cereals such as wheat, maize, rice, and cassava are the predominant dietary sources in most nations, although they are deficient in key vitamins and minerals. Micronutrient content of staple crops can be increased through agronomic biofortification. This is typically accomplished by employing some of the micronutrients as fertilisers to boost plant absorption. These micronutrients are also required by the plant's metabolic activities. Agronomic biofortification is a quick and effective way to fix soil. It helps to preserve soil fertility by enhancing fertilisers with micronutrients for plants in order to increase the nutritional content of staple crops like rice, millet, sorghum, wheat, maize, cassava, and sweet potato (De Valença *et al.*, 2017).

2.3 Dietary Diversification and Supplementation

Dietary variety is the most long-term technique for overcoming micronutrient deficiency. An attempt is made in this to consume a variety of different healthful foods, such as fruits and vegetables and various grains. Affordability, dietary habits, accessibility, living style, and bioavailability are all elements that contribute to the success of this method (Malik and Maqbool, 2020).

2.4 Bio fortification

Biofortification is now one of the most cost-effective ways to combat global hunger. It focuses on increasing the micronutrient content and bioavailability of staple crops' edible parts (Jat *et al.*, 2017). Furthermore, bio fortified food has now become a niche item in western countries on the basis of functional ingredients, that are food products that, in addition to the basic nutrition, have the ability to strengthen people's health. Many customers' food preferences has switched to products enhanced with nutrients as they have become more aware of the favourable health impacts of such nutrients (Marques *et al.*, 2021).

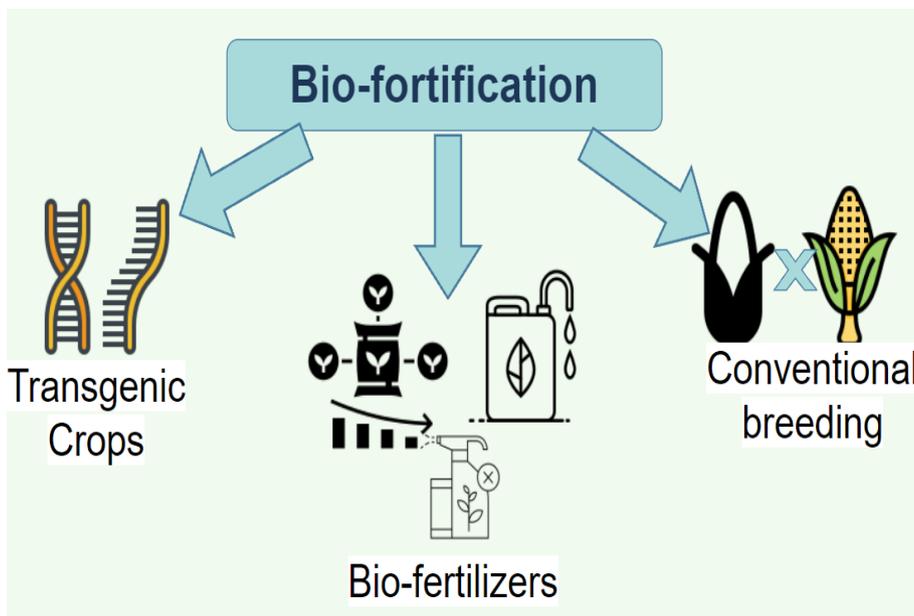


Figure 4: Methods of Bio-fortification

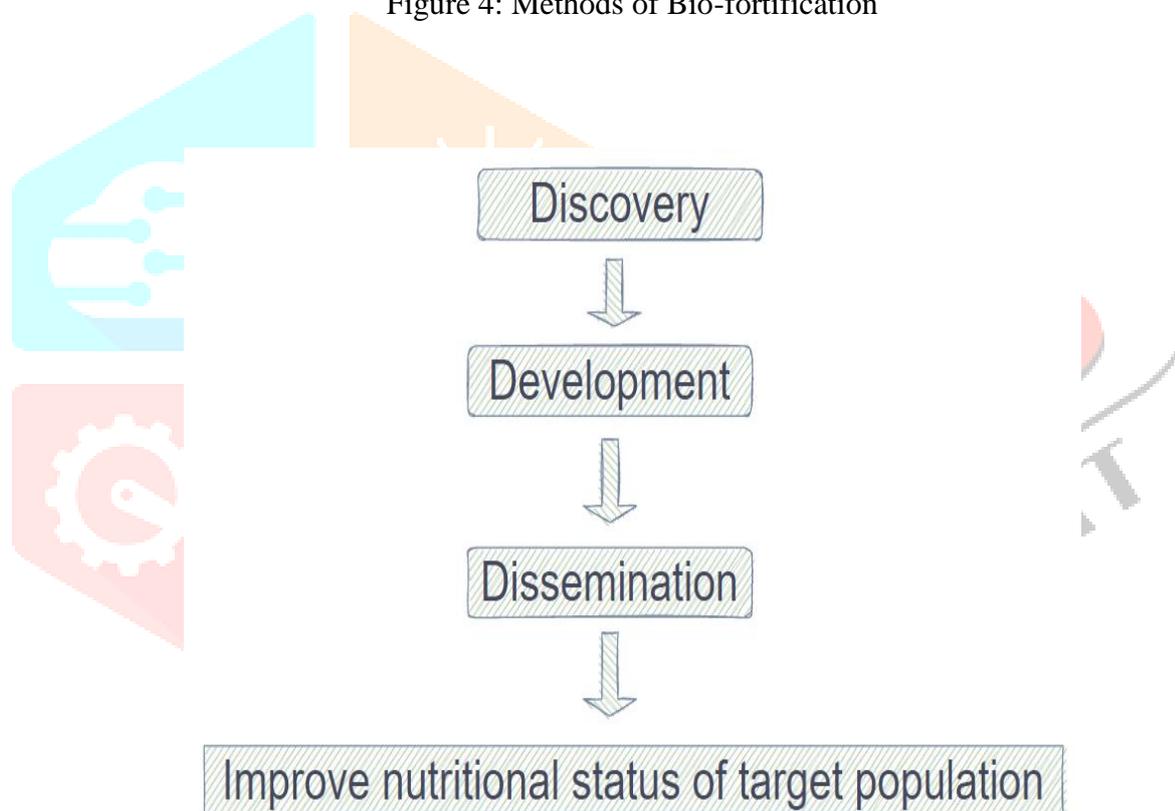


Figure 5: Pathway of Bio-fortification

2.4.1 Orthodox Breeding

Plant breeding in the traditional sense improves the vital nutrients in foods by improving cultivars and manipulating plant genomes within their natural genetic limitations. Plant breeding can help farmers establish new, profitable, and 'bio fortified' crop lines to combat hunger by using variations to develop new, productive, and 'bio fortified' crop lines. These methods are used to find cultivars that are high in the nutrients that are wanted. Then, by crossing, this trait is introduced into cultivated types, and individual plants are selected for the desired traits. Bio-fortified cultivars with high amounts of micronutrients such

as zinc or beta-carotene can be developed using a breeding method (Nestel *et al.*, 2006). While traditional breeding has attracted little interest from researchers than genetic ways to bio fortified crops, transgenic approaches have received more. (Marques *et al.*, 2021). There are a variety of non-transgenic approaches for breeding bio-fortified crops, including single-seed descent, marker-selected breeding, and genomic selection (Jiang, 2013).

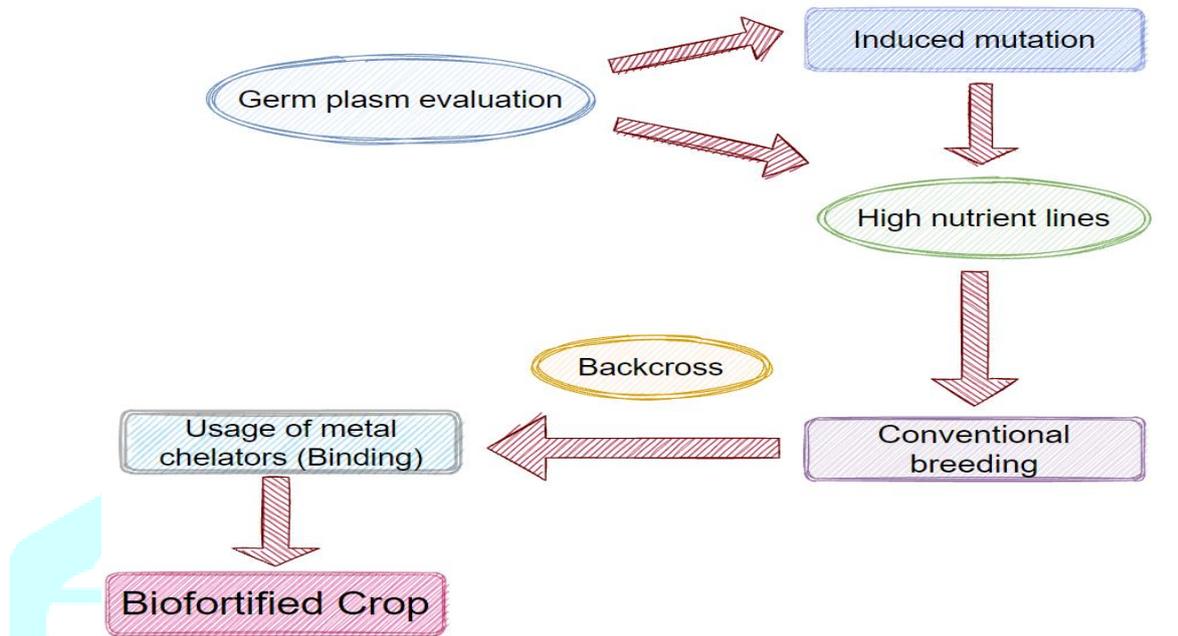


Figure 6: Steps involved in breeding approach of bio-fortification

2.4.2 Agronomic Approaches

Agronomic techniques to biofortification include the use of mineral fertilisers in the soil, foliar fertilisation, and soil inoculation with beneficial microorganisms (White and Broadley, 2009).

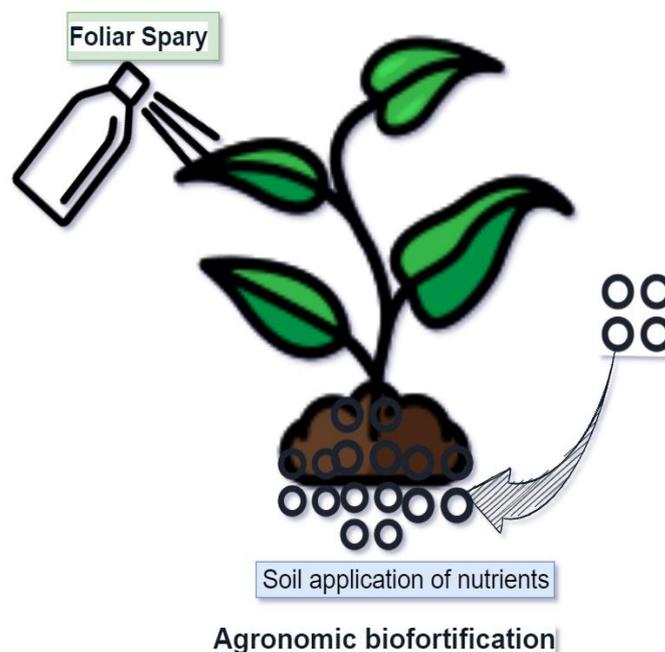


Figure 7: Schematic representation of agronomic approach of bio-fortification

2.4.2.1 Mineral Fertilizer

Minerals fertilisers are inorganic substances containing important elements that can then be administered towards the soil surface the micronutrients condition of the soil and, as a consequence, plant development can upsurge. Mineral fertilisers with better solubility and transportation of minerals should be used to improve the proportion of mineral deposits in edible plant tissues since phyto-availability of minerals in the soil is often inadequate (White and Broadley, 2009). This method can be used to deliver mineral elements to plants, but not organic nutrients such as vitamins, which the plant produces. Because Se, I, and Zn were mobile in both the soil and the plant, this method worked well (Dai *et al.*, 2004).

Regular applications of fertiliser for bio fortification are usually required, which can be damaging to the environment and limit the availability of other minerals (Hirschi, 2009). Furthermore, the soil composition in a particular geographic region, variances in mineral mobility, and the potential for anti-nutrient chemicals to reduce mineral bioavailability are all obstacles to the strategy's successful implementation (Frossard *et al.*, 2000)

2.4.2.2 Foliar Fertilization

The application of fertilisers directly to the leaves is known as foliar fertilisation. It might work if mineral elements aren't readily available in the soil or can't be translocated to edible tissues. In numerous experiments, pulse crops were biofortified with micronutrients such as Fe, Zn, and Se via foliar application, resulting in higher amounts of these elements in the harvested grain (Garg *et al.*, 2018).

2.4.2.3 Plant Growth Promoting Microorganisms

Rhizobia, mycorrhizal fungi, actinomycetes, and diazotrophic bacteria are beneficial soil microorganisms that form a symbiotic relationship with plant roots and protect plants through a variety of mechanisms, including nutrient mineralization, availability, and the production of plant growth hormones. Though they are naturally present in the soil, inoculation or agricultural management measures can help to increase their numbers. Plant growth-promoting (PGP) soil microorganisms such as *Enterobacter*, *Bacillus*, and *Pseudomonas sp.* can be used to boost micronutrient phytoavailability. These are mostly employed as seed inoculants, and they promote plant growth by producing growth hormones, antibiotics, chitinases, and siderophores, as well as inducing disease resistance and mineralized (Mahaffee and Kloepper, 1994). PGP microorganisms produce siderophore chemicals that chelate iron, solubilize phosphorus, and limit pathogen growth (Panhwar *et al.*, 2012). As a result, it contributes significantly to soil fertility and iron fortification. PGP bacteria are commonly found in soil, compost, and decomposing organic materials and they offer a cost-effective and risk-free way to boost crop productivity while also improving environmental and soil health (Gopalakrishnan *et al.*, 2016). Using microorganism inoculants and mycorrhizal association, numerous studies have showed enhanced concentrations of Fe, Se, and Zn. Furthermore, colonisation of *Pseudomonas sp.*, *Brevibacterium sp.*, *Bacillus sp.*, *Enterobacter sp.*, and *Acinetobacter sp.* in the roots and nodules of legumes such as chickpeas, soybeans, and peas has been reported to improve nitrogen

fixation, plant growth, and grain yield in legumes such as chickpeas, soybeans, and peas (Cavagnaro,2008; Gopalakrishnan *et al.*, 2015).

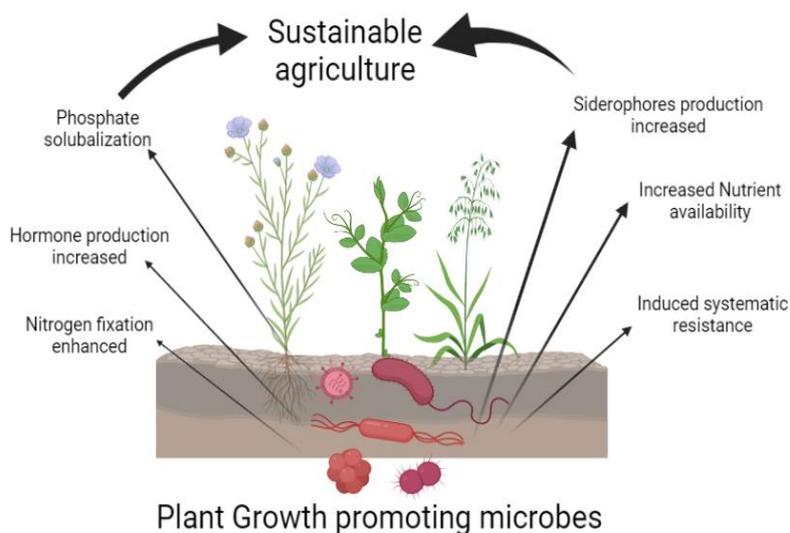


Figure 8: Role of Plant Growth promoting microbes in bio-fortification

2.4.3. Transgenic Genetic Engineering

Unless there is genetic heterogeneity in nutrient content between cultivars, breeders have nothing to work with. In this case, transgenic methods may be a viable option. Micronutrient genomics looks at the relationship between genetic sequences, nutrition, and health. Recent biofortification initiatives have depended on the ability to quickly discover and characterise gene function before designing and constructing plant metabolism using such genetic features (Hirschi, 2009). The recent availability of fully sequenced genomes in a variety of crops bolstered this strategy. Along with boosting the concentration of micronutrients, this method can also be used to target the removal of antinutrients or the addition of promoters that improve micronutrient bioavailability. This method used genes from bacteria and other organisms as well as genes linked with several metabolic pathways in plants. Transgenic crop development takes a significant initial investment, but it could be a long-term strategy with the potential to reach vast populations, particularly in underdeveloped nations. To address a micronutrient shortage, several crops have been successfully changed utilising a transgenic technique. In pulse crops, however, a genetic engineering method has been used to improve other nutritional profiles (Jha and Warkentin, 2020).

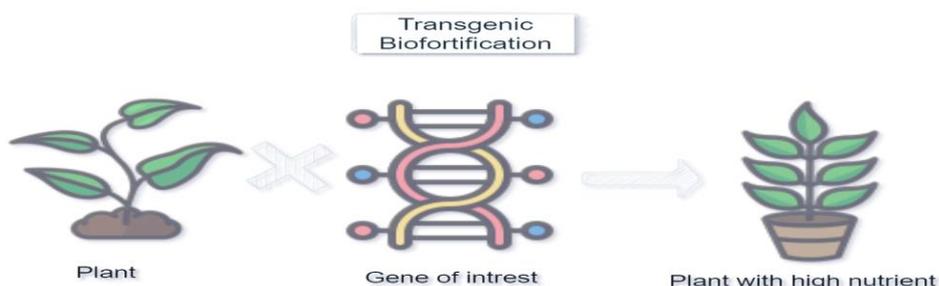


Figure 9: Steps involved in transgenic approach of bio-fortification

3. Bio fortified crop with enhanced nutrient to improve global health

Approximately 842 million people are undernourished around the world today, meaning they do not obtain enough food to eat. Furthermore, nearly 2 billion people worldwide suffer from "hidden hunger," a form of malnutrition caused by a lack of important micronutrients in their daily diet rather than a lack of calories. As a result, under-nutrition is responsible for over one-third of all child fatalities under the age of five worldwide, and one out of every four children is stunted as a result of poor nutrition. As the world's population reaches ten billion people and climate change takes hold, attaining food security will become even more difficult in the next decades. Crops can be biofortified in one of three ways: by adding the proper mineral or inorganic chemical to fertiliser, by traditional plant breeding, or by using biotechnology is more cost-effective, long-lasting, and realistic. The genes of interest are inserted directly into the plant genome with transgenic agricultural technology, and the resulting recombinant proteins that are expressed may not be possible with traditional plant breeding methods. A new branch of biotechnology based on the notion of genome editing has recently risen to prominence. Genome editing is concerned with the creation of precise incisions, mutations, and substitutions in plant and other eukaryotic cells using nuclease-based engineering systems such as the TALENS (transcription activator like effector nucleases) or the CRISPR (clustered regularly interspaced short palindromic repeats)/CRISPR-associated (Cas) systems. This technology will change the way we think about improving global nutrition by upgrading food crops. Genome editing has been carried out on crops such as barley, rice, tobacco, maize, and arabidopsis, and is still in the experimental stage (Zimmermann and Qaim, 2004;Paine *et al.*, 2005)

3.1 Biofortified Rice

Three million preschool-aged children suffer from vitamin A insufficiency each year. Half a million of them will go blind, and two-thirds will die soon after. Rice, more than any other staple grain, is the most potential genetically fortified crop because it feeds more than half of the world's population. Brown rice, the most nutritious form of processed rice, is made by hulling paddy gathered in the field. Iron, zinc, copper, calcium, phosphorus, and vitamins (B1-B9) and (E) are all present in unpolished brown rice; however, it lacks vitamins A, D, and C. White rice grains, on the other hand, are preferred by the ordinary consumer because of their lightness, softness, easy digestion, better eating properties, and quicker cooking time. Rice that has been polished (milled) has a lesser nutritional value than brown rice. Nonetheless, the quantities of mineral reduction may differ between rice cultivars and grain milling techniques. As a result, scientists are producing bio-fortified rice varieties that are nutritionally enriched even after processing and polishing (Hefferon, 2015).

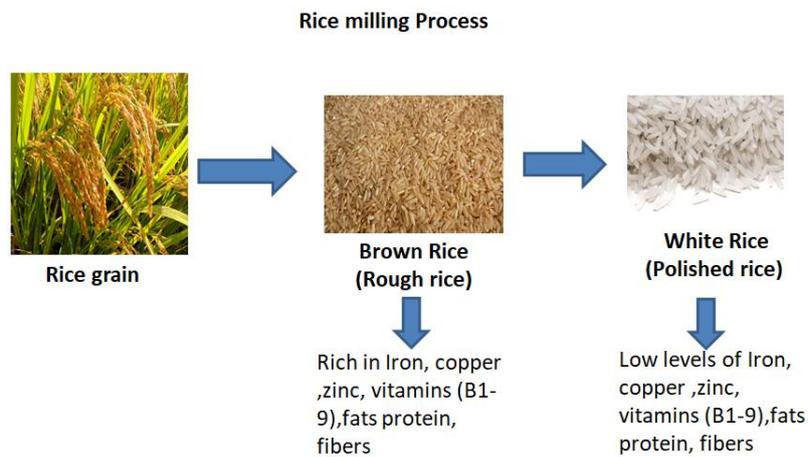


Figure 10 : Pictorial representation of rice milling process leading to loss of nutrients from rice grains

Bio fortification is thought to be a good way to boost the vitamin content of food crops like rice. It is also a viable and sustainable technique for alleviating micronutrient deficiencies in persons who mostly consume rice and have limited access to a diverse diet (or food markets) and adequate health care. Different ways have been achieved in rice bio fortification research projects for maintaining, increasing, and introducing new micronutrients in rice grain (Beyer, 2010; Harmon, 2013).

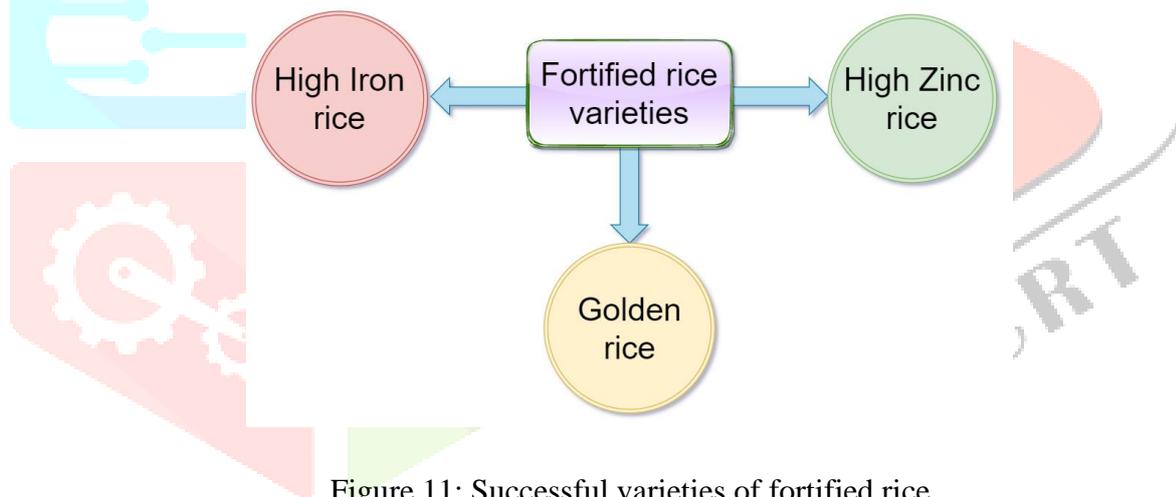


Figure 11: Successful varieties of fortified rice

3.1.1 Golden rice

Vitamin A can be derived from food; animal and plant items as preformed vitamin A or pro-vitamin A (Tang *et al.*, 2009). Vitamin A deficiency (VAD) is a worldwide problem that predominantly affects most parts of Africa and Asia, greatly impacting 250 million toddlers globally causing visual and decrease in immune approachability that results in several incidence of severe respiratory, gastro-intestinal infections. Certain nations, people are consuming vitamin A supplements (chemically synthesised) on a regular basis to prevent vitamin A deficiency (Potrykus, 2001; Al-Babili and Beyer, 2005; Schaub *et al.*, 2005). 70% of the average caloric intake is fulfilled by rice which is rich source of nutrients, and is consumed by more than half of the world's population.

In several developing nations, on an average person's diet is deficient in various micronutrients leading to various health issues hence the availability of bio fortified rice would be a long-term remedy. The

development of innovative molecular breeding tools has helped in development of various rapid bio fortified, biotic and abiotic stress tolerant rice varieties. One such technology is the transgenic method to the development of genetically modified (GM) rice. Recently, food-based interventions to boost the availability and consumption of pro vitamin A–rich foods have been proposed as a feasible and long-term solution to combat vitamin A deficiency worldwide (Ribaya-Mercado *et al.*, 2004).

In recent years, scientists have introduced the pro vitamin A carotenoids synthesis route into common foods, such as genetically altered "Golden rice" (GR) (Ye *et al.*, 2000). GR was created to help penurious and neglected people in developing countries avoid vitamin A deficiency. Because of the build-up of -carotene and the carotenoid biosynthesis pathway in rice, GR are carotenoid bio fortified rice with yellowish orange hue grain. Two enzymes, phytoene synthase (psy) and phytoene desaturase (crtl) , are introduced into the endosperm via an endosperm-specific glutelin (Gtl) promoter to establish a b-carotene biosynthetic pathway in the rice grains to make Golden Rice (Tang *et al.*, 2009).

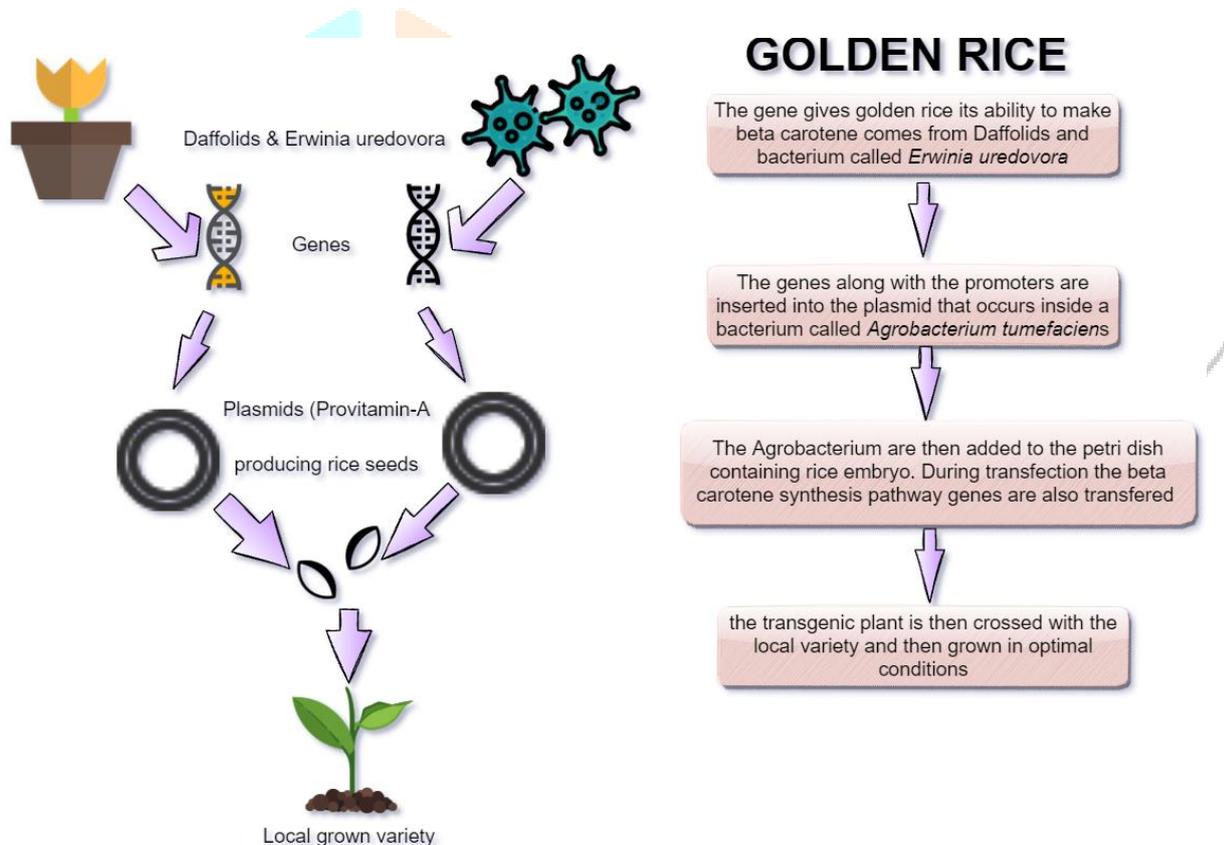


Figure 12: Schematic representation of preparation of Golden rice

The above mentioned findings Golden rice is the first genetically altered crop which could be a viable option to be the created expressly to combat malnutrition imply that for addressing Vitamin A insufficiency in rice-eating populations. Golden Rice has the distinct advantage of being able to reach isolated rural populations who do not have access to supplementation programmes. Rice has also been genetically modified to tackle other key causes of malnutrition, such as iron and folate deficiencies. Improved iron storage and transport proteins in plants, as well as the addition of a phytase to increase iron absorption in the gut, were used to solve these issues. RNAi silencing-based methods have also been used to create

transgenic rice that expresses critical amino acids such free lysine (Beyer *et al.*, 2002; Stein *et al.*, 2008; Swamy *et al.*, 2019).

3.1.2 Iron rich rice

Iron is among the most important minerals for human health. Post-harvest processing reduces the iron content of rice more than almost any other mineral. Paddy (raw rice) contains 38 parts per million of iron, which is reduced to 8.8 parts per million in brown rice after processing and 4.1 parts per million in milled rice (Trijatmiko *et al.*, 2016). Concerns about apparent iron loss in consumable rice grain prompted the development of iron bio-fortification for milled rice. Having enough iron in rice would help to maintain the health of children and pregnant women in developing countries. Iron deficiency causes IDA, which has serious health consequences for people, particularly children and women. IDA affects 32.9 percent of the global population, with sub-Saharan Africa and South Asia having a significantly higher risk of infection. It harms children's cognitive development, weakens their immune systems, and increases their morbidity risk. IDA can also have a negative impact on productivity, cause premature deliveries, and raise the chance of death in women. In such countries, developing high iron milled rice as part of a bio-fortification initiative could help combat IDA. Plant breeders have long sought to develop high iron rice cultivars with high yield, disease resistance, and superior seed vigour through conventional plant breeding and molecular approaches (Kassebaum *et al.*, 2014).

3.1.3 High Zinc Rice

Zinc is required for the control of Fe absorption in the intestine, and an adequate amount of zinc (along with iron) is required for IDA treatment. Zinc is required for physical growth and development, immune system function, reproductive health, sensory functions, and neurobehavioral development. Most importantly, zinc is required for the activation of over 300 enzymes and proteins because it is the only metal involved in all six classes of enzyme structure and function (i.e. zinc finger proteins). Zinc is required for the proper function of many transcription factors (regulators), zinc finger proteins, and enzymes that require Zn in various forms. Zinc deficiency affects 17.3 percent of the world's population, and it kills more than 400 million children under the age of five each year. Zinc deficiency in children can cause diarrhoea, limited weight gain, stunted growth, and anorexia (depending on their age). Neurobehavioral abnormalities are more common in infants, while skin changes and dwarfing are more common in toddlers and school children. Rice is the predominant source of zinc in Asian countries like Bangladesh, giving 49 percent of dietary zinc to children and 69 percent of dietary zinc to women. Zinc bio-fortification employs similar molecular breeding procedures to iron biofortification. In addition, zinc and iron uptake and homeostasis are intimately related in cereals. Members of the zinc and iron-regulated transporter protein (ZIP) family mediate iron and zinc uptake in rice (Arsenault *et al.*, 2010; Levenson, C.W. and Morris, 2011; Graham *et al.*, 2012; Prasad *et al.*, 2014; Hefferon, 2019).

Rice biofortification based on transgenic and RNAi-mediated antinutrient pathway silencing resulted in the production of some rice types with a 6.3-fold increase in iron (Wirth *et al.*, 2009), 2.0-fold increase in zinc (Johnson *et al.*, 2011), and 37 µg/g total carotenoid (Paine *et al.*, 2005) in biofortified rice grain. Increased detailed knowledge of rice genome sequencing data will lead to more value biofortified rice in the future, as scientific advancements in plant genetic modification are dynamic processes. Rice seed characteristics, plant development, and rice leaf stomatal density have all changed as a result of the recently established CRISPR/Cas9 technology. On the basis of GR, new biofortified rice events, such as the aSTARice (or Astaxanthin Rice), have been developed. Carotenoids and keto-carotenoids (such as astaxanthin and canthaxanthin) are important antioxidants found in aSTARice. The hunt for candidate genes suitable for rice biofortification programmes to combat hidden hunger is ongoing in the gene pool of rice varieties and other creatures (Majumder *et al.*, 2019).

3.2 Bio fortified wheat grain

Wheat, like so many other basic cereals, is low in two essential minerals: iron and zinc. Approximately 2 billion people around the world suffer from zinc and iron deficiencies, particularly in places where cereal-based diets are the predominant. While wheat flour is frequently fortified during manufacturing, biofortification, that comprises the cultivation of additional wheat cultivars with increased zinc and iron content in their grains, is a much more appealing and long-term solution (Borrill *et al.*, 2014).

To increase the iron content of wheat grain, there are four basic ways (White and Broadley, 2009). The first is to produce iron-rich lines, and the second is to establish iron-rich lines through transgenic manipulation. The use of iron-containing (or enhanced) fertilisers is the third stage, and inoculating microorganisms that encourage plant development is the fourth. (Shi *et al.*, 2020). Traditional plant breeding and transgenic modifications are both examples of genetic bio-fortification. Genetic biofortification is incredibly cost-effective once the crop lines are created because it requires very little ongoing input (Cu *et al.*, 2020). Traditional breeding, on the other hand, has failed to produce iron-rich wheat lines since yield has been shown to be inversely related to Fe content, and research has previously focused on yield instead of iron content (Connorton and Balk, 2019). Despite the fact that transgenic wheat has significantly increased grain iron content, it is still in its early phases of development, with only a few large planting regions (Li *et al.*, 2018). The use of iron-containing fertilisers and microorganisms to enhance plant development is known as agronomic bio-fortification. Several agronomic strategies are frequently used in cereal bio-fortification (Garg *et al.*, 2018). Although transgenic wheat has considerably boosted iron content in grains, it is still in its early stages of development, with only a few major regions of planting (Li *et al.*, 2018). Iron-containing fertilisers and microorganisms are used in agronomic biofortification to boost plant development. Agronomic approaches are frequently used in cereal biofortification (Ludwig and Slamet-Loedin, 2019). Moreover, different factors such as soil chemistry, temperature, rainfall, and wheat lines can all have an effect on bacteria's effect. As a result, new approaches to improving the efficacy of iron bio-fortification in wheat are still required (Shi *et al.*, 2020).

3.3 Bio fortified Maize

Maize is one of the top three cereal crops in Asia and is a major source of basic food and energy in Sub-Saharan Africa (SSA), Latin America, and the Caribbean. However, maize is deficient in several essential amino acids, vitamins, and minerals. Bio-fortified maize cultivars loaded with critical minerals and vitamins could be highly advantageous in rural areas where access to a diversified diet, dietary supplements, and enriched foods is limited. There has been tremendous progress in the creation, testing, and deployment of maize cultivars biofortified with quality protein maize (QPM), provitamin A, and kernel zinc. Maize scientists have developed improved cultivars with improved nutritional value, such as quality protein maize (QPM) rich in two essential amino acids, orange maize biofortified with provitamin A carotenoids, and high-Zn-enhanced maize. Higher amounts of lysine and tryptophan, kernel Zn, and provitamin A have been successfully improved in maize through conventional breeding (Prasanna *et al.*, 2001; TWumasi-Afriyie *et al.*, 2011; Pixley *et al.*, 2013; Prasanna *et al.*, 2020).

3.4 Bio fortified lentil

Plant-based diets are becoming increasingly popular around the world due to its ability to improve one's health by lowering blood pressure, BMI, cholesterol, diabetes, obesity, and cardiovascular disease. Lentil is one of the most popular plant-based foods because of its quick cooking capabilities and low cost of access to high-quality protein, vitamins, dietary fibre, and minerals including iron, zinc, and selenium. Protein, micronutrients, vitamins, and minerals such as iron, zinc, selenium, folates, and carotenoids are abundant in lentils (Podder *et al.*, 2020).

4. Advantages of bio-fortification

4.1 Effective outreach

Bio fortification has an advantage over many other methods for enhancing people's nutritional status because it is targeted to the population through staple foods. Many processed and fortified foods are out of reach for the poor, and incorporating them into daily diets through other means, such as free distribution, has various obstacles, including boosting nutrition awareness, introducing the new product, and guaranteeing proper use (which might be difficult project if the community is uneducated as it is in most of such cases). Such goods are time-limited endeavours, as they require a significant amount of effort, money, and labour to maintain.

4.2 Cost effective

Bio-fortified seeds include improvement in the variety of crop by introduction of some specific genotypes into their seed variety. These varieties are capable of producing nutritionally dense staples and are resistant to many environmental pressures.

4.3 Sustainable

Once the crop is introduced with new genome, its products and seeds will contain the same genotype and the cycle will continue without much further investment as compared to other methods of fortification or supplementations.

Few more additional advantages of crop bio-fortification are as follows:

- Fortified seed does not incur yield penalty. Moreover, it offers important indirect benefits like diseases resistance plants, which increases farm's productivity.
- Overcome malnutrition problem in a larger area.
- Increase in nutritional content of daily diets.
- Improvement of plant or crop quality and increment of variability in germplasm (Olson et al., 2021).

5. Limitations

The transgenic technique has overcome the restrictions of genetic variation in crops, which has been a key difficulty in traditional breeding operations, but general acceptance is still an issue. As a result, farmers and the general public must embrace and adopt bio-fortified crops with increased qualitative qualities. This could be due to the time it takes to identify, modify, and express a target gene in order to understand its effects. Agronomic Biofortification is a common approach, however its success rate is highly variable (Wissuwa and Ae, 2001; Lyons et al., 2005; Waters and Sankaran, 2011).

Other drawbacks include crop post-harvest handling while supplementing biofortification methods (Inaba and Macer, 2004; Lyons *et al.*, 2005). Furthermore, the presence of certain antinutrients in crops lowers the bioavailability of micronutrients (Sestili *et al.*, 2010; Watanabe *et al.*, 2005; Haas *et al.*, 2005).

6. Conclusion

Mineral and vitamin deficiencies are projected to become increasingly dangerous in the future, and biofortification strategies are emerging as a viable solution. Biofortification shows significant promise for alleviating the micronutrient deficiency problem in the developing countries because it is a low-cost, simple, and crop-based solution. Significant progress has been made in this area, and future strategic research and policy could help biofortification achieve considerable success in the coming years. The biofortification process takes advantage of all family members' daily consistent ingestion of staple foods, particularly women and children, who are most at risk of malnutrition. The biofortification method is aimed squarely at low-income households because to the abundance of dietary staples in their diets. Following an initial investment in manufacturing fortified agricultural seeds, subsequent costs are modest, and germplasm can be dispersed throughout the world. This is a major feature of plant breeding, which makes the current technique environmentally friendly and cost-effective. Even if international financing and government attention for micronutrient deficiency issues fail, consumption and production of nutritionally

improved varieties can be sustained once established. Biofortification is a cost-effective approach to provide nutrients to undernourished communities, primarily in rural areas. Commercial-food fortification, in combination with biofortification processes, is highly recommended. There is a considerable necessity to create a balanced food plan that meets the nutritional needs. Therefore, the most important solution to eliminate micronutrient malnutrition is to develop the process of biofortification to increase consumption of non-staple foods. But this will take a lot of time, an active government department for creating awareness and relatively a huge investment. To overcome nutritional disorders in less developed areas, a strong interdisciplinary approach between plant scientist and human nutritional diet holds a great impact.

Author Contribution Statement

N.S conceptualized and designed the article; V.V wrote the manuscript; N.S and P.T. critically read and revised the manuscript.

Declarations

Conflict of Interest: The authors declare no competing interests.

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